Doctoral dissertation

Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels based on the case of clams and crabs from the Vistula Lagoon

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(Bioakumulacja i transfer rtęci (Hg) w organizmach z niższych poziomów troficznych na przykładzie małży i krabów z Zalewu Wiślanego)

by
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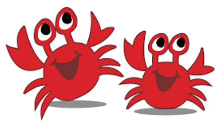

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Publications included in the dissertation

1. **<u>Wilman, B</u>**., Bełdowska, M., Rychter, A., Kornijów, R. 2023. Different pathways of accumulation and elimination of neurotoxicant Hg and its forms in the clam Atlantic rangia (*Rangia cuneata*). *Science of The Total Environment*, 858, 160018. doi: 10.1016/j.scitotenv-.2022.160018.

   Impact factor (IF) in a given year: 8.2, 5-year IF: 8.6, MEiN points: 200

2. **<u>Wilman, B</u>**., Bełdowska, M., Rychter, A., Popławska, A. 2023. Factors determining bioaccumulation of neurotoxicant Hg in the zebra mussels (*Dreissena polymorpha*): influence of biometric parameters, sex and storage of shell. *Marine Pollution Bulletin*, *197*, 115718. doi: 10.1016/j.marpolbul.2023.115718.

   Impact factor (IF) in a given year: 5.3, 5-year IF: 6.1, MEiN points: 100

3. **<u>Wilman, B</u>**., Normant-Saremba, M., Rychter, A., Bełdowska, M. 2024. Total body burden of neurotoxicant Hg in Chinese mitten crab (*Eriocheir sinensis*) Considerations of distribution and human risk assessment. *Marine Pollution Bulletin*, *199*, 116028. doi: 10.1016/j.marpolbul.2024.116028.

   Impact factor (IF) in a given year: 5.3, 5-year IF: 6.1, MEiN points: 100

4. **<u>Wilman, B</u>**., Bełdowska, M., Rychter, A., Popławska, A. 2024. Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body. *Marine Pollution Bulletin*, *209*, 117118. doi: 10.1016/j.marpolbul.2024.117118.

   Impact factor (IF) in a given year: 5.3, 5-year IF: 6.1, MEiN points: 100

Summarized metrics

Total IF in a given year: 24.1, total 5-year IF: 26.9, MEiN points: 500

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Appendix A/ Załącznik A: List of abbreviations/ Spis skrótów

The following list describes the meaning of the most important abbreviations and acronyms used throughout the dessertation :

BAF	Bioaccumulation factor/Współczynnik bioakumulacji
BSAF	Biota-sediment accumulation factor/Współczynnik akumulacji organizm-osad
FSF	Fine sediment fraction/Udział frakcji najdrobniejszej osadu (<0,063mm)
HgF1	Labile mercury fraction, mainly of mercury halides/Labilna frakcja rtęci, głównie halogenki rtęci
HgF2	Labile mercury fraction bound to humic substances and organic ligands/Labilna frakcja rteć w połączeniach z materią organiczną
HgF3	Mercury sulphide/Siarczek rtęci
HgF4	Semi-labile mercury fraction: sulfate and oxide/Semi-labilna frakcja rtęci: siarczan i tlenek rtęci
HgF5	Stable residual mercury fraction/Rezydualna frakcja rtęci
HgTOT	Total mercury/Rtęć całkowita
LOI	Lost in ignition/Strata przy prażeniu
OM	Organic matter/Materia organiczna
SPM	Susspended particulate matter/Materia zawieszona
W	Water content/Zawartość wody

Highlights

1) **To determine in which form mercury enters the crustacean and mollusc organism with the food, and in which form it is bioaccumulated in soft and hard tissues.**

2) **Recognition of the distribution of mercury in the internal organs of organisms from lower trophic levels, using the example of the largest Baltic crustacean, *Eriocheir sinensis*.**

3) **Recognition of mercury transfer pathways in invertebrate organisms based on environmental factors, biometric parameters and sex.**

4) **To relate the accumulation of Hg fraction in hard tissues to the type of biomineralization of clam shells and crab's exoskeleton.**

5) **Filling the research gap on the identification of different pathways of Hg elimination in organisms from lower trophic levels.**

6) **Determination of mercury concentrations in alien species, with a view to determining their role in Hg trophodynamics, which are becoming a new link in the trophic chain of the southern Baltic.**

Keywords

Mercury, Hg fractions, macrozoobenthos, alien species, bioaccumulation, elimination, trophic chain, *Dreissena polymorpha*, *Rangia cuneata*, *Eriocheir sinensis*, *Rhithropanopeus harrisii*

Summary

Mercury (Hg) is an element that has no positive function in living organisms. **It can have toxic effects even in trace amounts and in any chemical form: gaseous, alkaline and organometallic** (Castro-Gonzalez and Mendez-Armenta, 2008; Hosseini et al., 2013). This is due to the characteristic properties of this element: volatility, reactivity and high toxicity - especially of its organic forms (Kabata-Pendias and Mukherjee, 2007). The widely reported incidents of Hg poisoning at Minamata Bay in Japan have largely triggered a global, urgent need for research on mercury and the threat of the toxin to the natural environment, the aquatic food chain and human health (D'Itri, 1993; Malm et al., 1995). It has been proven that uncontrolled emissions of Hg especially from anthropogenic sources have contributed to increased levels of Hg in the atmosphere and on land, and most importantly in aquatic ecosystems which has led to more global problems related to the health and life of humans, as well as other living organisms (Risher and Amler, 2005; Ekino et al, 2007; Dikme et al, 2013; Bosch et al, 2016; Ye et al, 2016). The mercury issue is mainly considered in the context of marine and ocean pollution, as **fish and seafood are globally the main source of mercury to the human body** (Cheng et al., 2011; Mania et al., 2012). For this reason, the presence of Hg in seafood is of public health interest (Burger and Gochfeld, 2012; Gibb and O'Leary, 2014; Mozaffarian, 2009).

The ability of Hg to accumulate and excrete from organisms largely depends on its chemical form (Bosch et al., 2016). Organic mercury, mainly methylmercury (MeHg), undergoes biomagnification downstream in the trophic chain. It is (next to dimethylmercury) the most toxic form of Hg (Branco et al., 2007; Kojadinovic et al., 2006). Research on mercury in the sea has focused mainly on abiotic samples (water and sediments) and on organisms at higher levels of the trophic pyramid. **Poorly recognized are the transformations of mercury in the first links of the trophic network, including benthic organisms**. This is an important cognitive gap, since at this stage the metal bioaccumulates most from the surrounding environment (Scheuhammer et al., 2007). Now that metal inputs to aquatic ecosystems are restricted due to restrictions, remobilization of Hg from sediments is becoming increasingly important, where mercury is remobilized into the marine environment through a series of biotic and abiotic processes. **Thus, the metal can bioaccumulate in benthic organisms** and biomagnify in downstream links of the trophic web, thus posing the greatest threat to marine mammals, waterbirds and humans (Jackson, 1998). Work on mercury ingress and distribution in organs is also quite well described in the world literature however in organisms from higher trophic levels i.e. fish, birds and mammals (Scheuhammer et al, 2007; Bustamante et al, 2016;

Falkowska et al, 2017; Grajewska et al, 2019; Manceau et al, 2021). **Studies in organisms from lower trophic levels, such as crabs and clams, mainly focus on determining the concentration of total mercury (THg) in soft tissue and the effect of seasonality on its variability** (Amirad et al, 2006; Chiarelli and Roccheri, 2014; Jedruch et al, 2019). Processes concerning the pathways of mercury elimination from the body along with the epidermal product (feathers or hair) are also quite well described in birds or marine mammals (Falkowska et al, 2017; Grajewska et al, 2019; Manceau et al, 2021). **The mechanisms of mercury transport on the soft body-shell pathway or by intra-organ transfer to the exoskeleton are not fully recognized**. To a large extent, this is due to the small size of benthic organisms and their individual organs or tissues, i.e. the difficulty in obtaining a representative amount of material for chemical analysis.

Most of the scientific literature to date has focused on the biomagnification and bioaccumulation of total mercury or one of its most toxic forms, namely methylmercury, in the trophic network of marine ecosystems (Wren and MacCrimmon, 1986; Baeyens et al. 2003; Schmitt et al. 2011; Hong et al. 2016; Guntés et al. 2021). **There is a research gap on what forms of mercury are supplied to organisms from lower trophic levels through the food web. It is also important to study all forms of mercury, especially the labile forms - the most toxic and bioavailable to soft tissues**. It is the form of the metal that largely determines its distribution and transformations in the environment, as well as in organisms.

The area of the Baltic Sea and its estuaries (including the Vistula Lagoon) is under intense pressure from various types of anthropogenic impacts, with the introduction of non-indigenous species (NIS) considered a key factor affecting the dynamics of its ecosystem (Snoeijs-Leijonmalm and Andrén, 2017). Due to the low level of biodiversity in the Baltic Sea, a significant increase in the number of successful introductions of non-native species into the Baltic Sea has been observed in recent decades (Leppäkoski and Olenin, 2000). **One of the most abundant groups of NIS in the Baltic Sea are zoobenthic organisms**, the planktonic larval form of which has mainly been imported with the ballast water of ships (Warzocha et al., 2016).

Previous studies indicate that non-native species often have a wide environmental tolerance (temperature and salinity) or a broad environmental niche (Marvier et al., 2004). Contrary to this earlier theory, recent work in the Baltic region has shown that non-native species do not necessarily expand their niche, but rather remain more adapted in it compared to native species (Herkül et al., 2016). **In the Vistula Lagoon region, clusters of stable populations have been formed by two non-native bivalve species: *Rangia cuneata* and**

***Dreissena polymorpha*, and an alien species of crab: Harris mud crab *Rhithropanopeus harrisii*, becoming one of the dominant taxa in the group of zoobenthic organisms**. **Another alien species of crab: Chinese mitten crab *Eriocheir sinensis* is becoming increasingly common in estuaries, lagoons and bays of northern Poland**, as a result of its migration to search for resources in the area (Ojaveer et al., 2007; Ewers et al., 2023). Consequently, the listed species become a new link in the trophic chain of this ecosystem, providing food for waterbird fish, or mammals (Knoll, 2004; Hegele-Drywa et al., 2014; Puntila, 2016). **As a result, the role of NIS in the trophodynamics of mercury in the trophic network of the southern Baltic Sea potentially, becomes crucial** due to their increasing abundance and spread.

Taking into account the state of knowledge on the issue to date, including literature studies and the results of previous research, **the research hypotheses reviewed in this study were formulated as follows**:

- **i.** *The level of total mercury concentration and its toxicity to organisms in crabs and clams are related to the habitat characteristics within their identified cluster of occurrence and their diet;*
- **ii.** *Physiological state (season), biometric parameters and sex are the main determinants of mercury uptake and elimination in crabs and clams;*
- **iii.** *The exoskeleton may be an important site for the accumulation and temporary inactivation of mercury via the transfer pathway: soft body-shell in bivalves and elimination via the soft tissue-exoskeleton pathway in crabs.*

The essence of the present study was to fill the cognitive gap, present in the research regarding the recognition of mercury entry and elimination pathways in organisms from lower trophic levels, and to determine mercury concentrations in alien species, which were so far an unrecognized source of mercury in the trophic network in the studied ecosystem**. Hence, the following research objectives were set**:

- **i.** Determination of the main pathways of mercury entry in species of alien bivalves: *Rangia cuneata* and *Dreissena polymorpha* and crabs *Rhithropanopeus harrisii* and *Eriocheir sinensis* from the Vistula Lagoon as a factor determining its concentration level and fractional contribution;
- **ii.** Recognizing the distribution of total mercury and its labile and stable forms in the body of crabs and mussels;

iii. Determination of factors affecting the variability of the body burden of crabs and mussels with mercury neurotoxin;
iv. Learning about the role of the exoskeleton in the elimination of mercury in invertebrates using the crab and clam as examples;
v. To describe the role of alien species in the southern Baltic ecosystem in relation to the trophodynamics of mercury in the study area.

The scope of the study was limited to the Polish area of the Vistula Lagoon, whose hydrological condition is determined mainly by freshwater inflow from rivers. The main inflow of pollutants (including mercury) is the Russian Pregoła River; inflow from Polish rivers comes from the Bauda, Elbląg, Pasłęka, Nogat and Szkarpawa rivers (Ezhova et al., 2005; Uścinowicz and Zachowicz 1996). Currently, the Vistula Lagoon is also contaminated with the residues of various pesticides and fertilizers used in agriculture in the past. Wastewater from mechanical-biological treatment plants in Tolkmicko, Frombork, Braniewo, Stegna and Krynica Morska continuously flows directly into the lagoon. The Vistula Lagoon was therefore included in the Natura 2000 environmental protection program and placed on the list of Baltic Protected Areas introduced by HELCOM (Szefer, 2002). The Lagoon is part of the Gulf of Gdansk cut off from the waters of the Baltic Sea by the dike of the Vistula Spit. The only strait (at the time of the study) connecting the lagoon with the bay is the Pilawa Strait (located in the Russian Federation). These conditions favored the accumulation of organic matter and the retention of incoming pollutants, including Hg in the bottom sediments. Hence, organisms, especially zoobenthos, are particularly vulnerable to the accumulation of pollutants in their tissues.

Mussel **organisms** (**publication 1, publication 2**) **were collected** during a cruise by the research unit of the Marine Fisheries Institute in Gdynia - ŁM MIR 2, in the western part of the Vistula Lagoon in April, August and October 2019 and April 2020 (Fig.1). On the other hand, **American crab specimens were taken during the cruise** in April, August and October 2020 (**Publication 4**). In addition, during the summer season of 2021, *R.cuneata* clams were collected from 7 survey stations, in the western part of the Vistula Lagoon, in order to determine the spatial variability of the studied xenobiotic Hg (**publication 1**) as part of a cruise under the direction of Prof. Korniyov of the Marine Fisheries Institute in Gdynia (Fig.1).

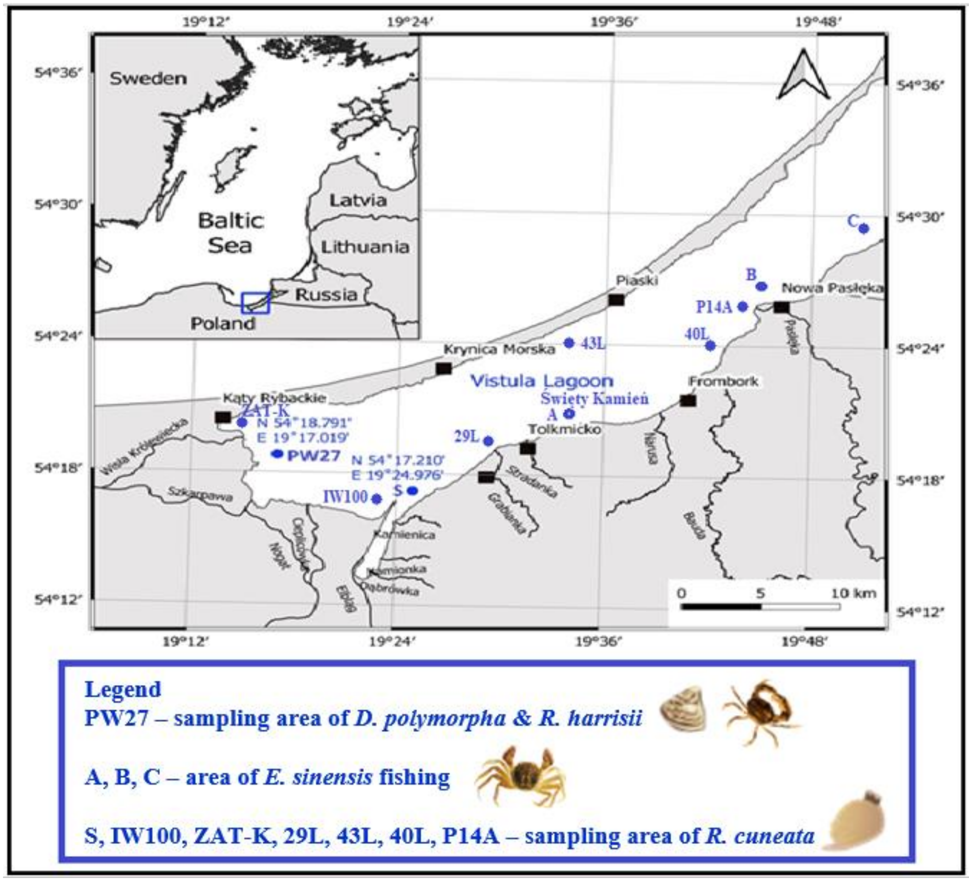


Figure 1 Study area

Biological material (clams and crabs) at stations S and PW27 was collected with a hand dredge (with dimensions: 36x21 cm, trawl length 60 cm and mesh size 3x3 mm) from a depth of 2.5 m and then sieved through a sieve with a mesh size of 0.25 mm. Individuals were hand-picked from the sieve and placed in aerated thermoses with flood water. After transporting the samples to the laboratory, the clam organisms were placed in an aquarium in filtered water for 24 hours for purification. The biological material was frozen at -20°C until further analysis. In addition, dietary components of clams and American crabs and elements of their habitat were also collected at each research station. Superdense water to filter out suspended particulate matter (SPM) was collected with a bathometer from the bottom layer. Plankton were collected from the water column using a 10 µm grid. The surface layer of bottom sediments (top 10 cm) was also collected for analysis. Water samples from SPM and plankton were filtered through roasted Whatman GF/F filters after transport to the laboratory (**publication 1, publication 2, publication 4**). The collected material was stored at -20°C. During sampling, *in situ* measurements of water were also taken. Salinity [PSU], water pH, water temperature [°C] and visibility in the water column using Secchi disk were measured.

Individuals *of Eriocheir sinensis* (**publication 3**) **were collected** in October 2019 in the Vistula Lagoon in the area of St. Stone, which is located between Tolkmicko and Frombork - area A (Fig.1). The crabs were collected from the beetles, put out by local fishermen in cooperation with the stationary inspector Romuald Jachimowicz of the Frombork District Fisheries Inspectorate and Prof. Dr. Monika Normant-Saremba. Immediately after being removed from the beetles, the crabs were frozen at -20^{o}C for further analysis. In October 2019, additional crabs were collected from fisheries in the Vistula Lagoon in the area of the southeastern part of the Vistula Lagoon, between Tolkmicko (marked as A) and the border with Russia (marked as C) (Fig.1). The third collection site (marked B) was located between Frombork and the border with Russia. In addition, the material of *E.sinensis speci*mens collected in October 2011, 2017 and 2020 caught in the area of the St. Stone fishery - area A (Fig.1) was analyzed.

The next step was to determine the biometric parameters of the organisms and the sex of the test specimens (**Publication 1 - 4**). For this purpose, **dissection was performed on the organisms**, where in the case of bivalves the soft body was separated from the shell (**publication 1, publication 2**). In the case of the American crab, the soft body, legs and carapace were isolated (**publication 4**). The woolly soft-shell crab is the largest representative of Baltic invertebrates (Normant et al, 2000; Czerniejewski and Beldowska, 2003), so to determine the distribution of mercury and its forms in individual organs, soft tissues (leg muscles - FWL, pincer muscles - FC; gonads - GO, hepatopancreas - HP, gills - GI) and exoskeleton (dactylus, carapace, merus, pincer and mittens - cuticular outgrowths) were dissected (**publication 3**). A total of **1546 soft tissue and 1546 exoskeleton samples**, 18 surface sediment samples, 27 SPM seeps and 27 plankton seeps **were prepared for further chemical analysis**.

Total mercury (**HgTOT**) **and mercury fractions** were analyzed on a DMA 80 analyzer by thermal desorption using an automatic DMA mercury analyzer (Milestone, Italy) (**Publication 1 - 4**). The use of a 5-step thermal desorption method allowed the separation of five groups of compounds with similar properties:

- **HgF1** - (Hg-L1, $Hg_{labile1a}$, $Hg_{adsorbed}$.) - are the most labile Hg compounds ($HgCl_2$, $HgBr_{(2)}$, $HgI_{(2)}$, $Hg(CN)_2$). They mainly form loose surface compounds with the substrate and can be partially absorbed from the gastrointestinal tract, which is related to the solubility of these compounds in water (Broussard et al., 2002; Aksentor and Sattarov, 2020).

- **HgF2** - (Hg-L1, $Hg_{labile1b}$, $Hg_{(absorbed1)}$)) - Hg compounds combined with humic substances, MeHg, as well as other Hg compounds formed with ligands with strong complexing properties (($(CH_3COO)_2Hg$, $Hg(NO_3)_{(2)}$-$H_{(2)}O$, $Hg(SCN)_{(2)}$), $Hg(ClO_{4)(2)}$-$xH_{(2)}O$). These Hg compounds are easily transported inside the body's cells - they are absorbed more fully than inorganic salts because they are more soluble in lipids and bind to sulfhydryl groups (Broussard et al., 2002). They are also subject to bioaccumulation and biomagnification in the trophic chain (Jedruch et al., 2018; Cavoura et al., 2019).
- **HgF3** - (Hg-S1, HgS) - insoluble HgS, which is one of the most stable forms of Hg and least toxic to organisms. However, it can be converted to an organic form with the involvement of sulfate-reducing bacteria and by fungi (Lugo et al., 2020).
- **HgF4** - (Hg-L2, $Hg_{labile2}$, $Hg_{(adsorbed2)}$)) mainly semi-volatile compounds: HgO, $HgSO_4$ and HgF_2, potentially available to organisms (Aksentor and Sattarov, 2020).
- **HgF5** - (Hg-S2, $Hg_{residual}$) forms that were unavailable to the environment, associated with the residual fraction embedded in minerals.

Publications 1 and 2 used the nomenclature of **Hg_{labile}** and **Hg_{stable}** according to the methodology described in the work of Beldowska et al, (2018) and Jedruch et al, (2018). During the laboratory work, there was also a modification of the mercury fractionation method, as a result of which the nomenclature for the different fractions for different matrices was normalized to one: **HgF1, Hg2, HgF3, HgF4 and HgF5** (Wilman et al., 2023) and was used in **publications 3 and 4**.

The moisture content [W], organic matter content [LOI] and the proportion of the finest fraction in surface sediment samples [FSF] were also determined according to the method described in the article by Saniewska et al, (2014).

Determination of the mercury accumulation factor (BAF and BSAF) allowed us to achieve the first objective: <u>**Determination of the main routes of mercury entry in alien species of bivalves: *Rangia cuneata* and *Dreissena polymorpha* and crabs *Rhithropanopeus harrisii* and *Eriocheir sinensis* from the Vistula Lagoon as a factor determining its concentration level and fractional contribution**</u>. In **publications 1 and 2**, BAF coefficients were determined on the path of soft body - plankton, soft body - suspension - and BSAF: soft body - sediment. In the case of the bivalve *D. polymorpha* **(publication 2),** the bioaccumulation factor indicates that plankton is the main vector of Hg into the bivalve body (Fig.2). The present results showed that the fraction of mercury that was most accumulated with food was HgF1, i.e. halogenated mercury compounds that constitute a high percentage in plankton or microalgae. *Dreissena polymorpha* is an obligate filterer with the ability to filter a large volume of water, obtaining the necessary nutrients along with plankton or detritus (Beldowska et al., 2018, Wilman et al., 2023). Consequently, more labile mercury compounds are delivered to the body and bioaccumulate. In the case of the BSAF factor, we found that the sediment is not a source of Hg to the mussel body. The resuspension of Hg bound to fine sediment particles and its accumulation into the body as a result of water filtration may be of least importance here, since the variegated crayfish is the only one of the freshwater bivalves that attaches itself to the bottom with byssus, and therefore prefers a hard substrate of coarse sediment nature, which has a low concentration of Hg (Fig.2).

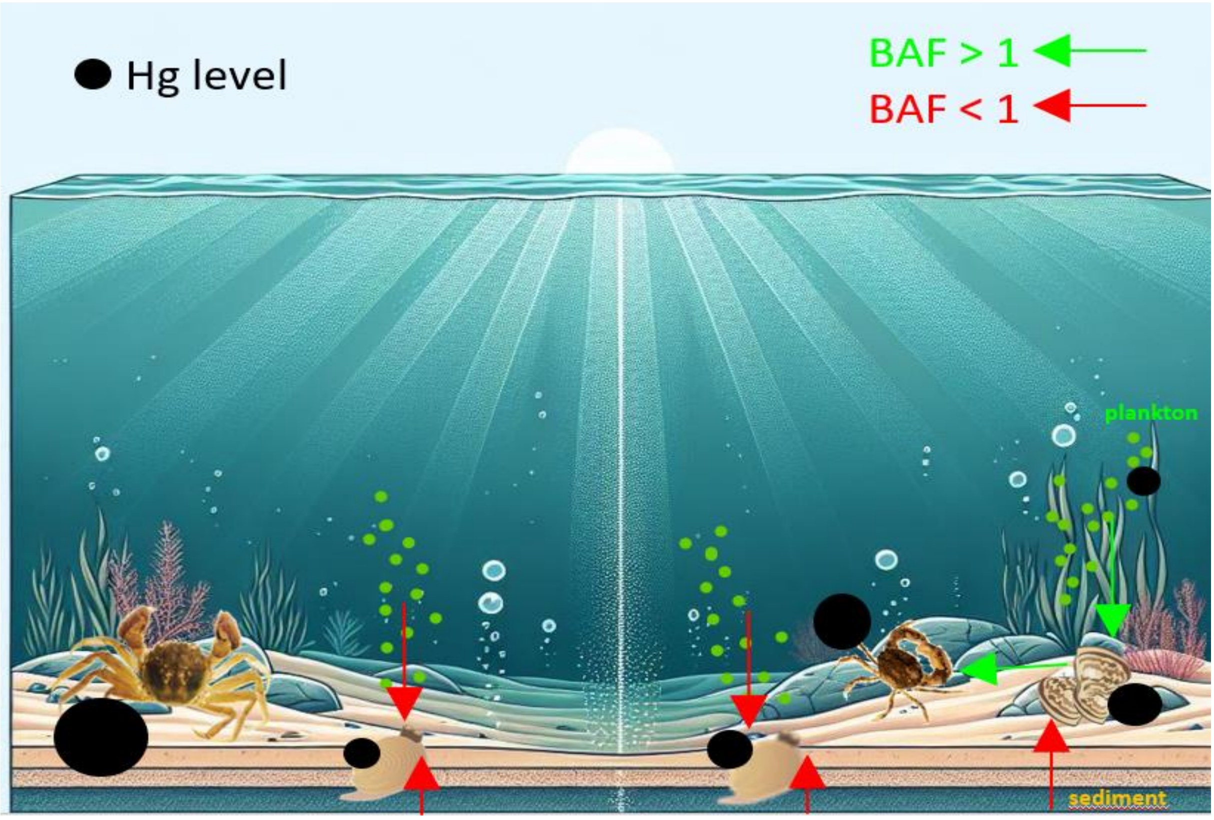


Figure 2 Xenobiotic Hg concentration and bioaccumulation factor (BAF) in alien species of crabs and clams from the Vistula Lagoon

Calculated bioaccumulation coefficients for the *R. cuneata* organism **(publication 1)** showed limited accumulation of Hg in the body of *R. cuneata* from marine suspended solids and plankton (Fig.2). *R. cuneata* is not an obligate filterer. In addition to filtering water from suspended particles, it feeds on organic matter and detritus from sediments. This is confirmed by studies of the digestive tract of this mussel species showed a significant proportion of detritus or sand (80%) compared to microalgae or vascular plant remains (Darmell, 1998). Therefore, the type of sediment in the area of the clam's habitat in which it can burrow and the proportion of each form of mercury in the substrate are important. Analysis of Hg concentrations and fractions in *R. cuneata* showed spatial variability in the Vistula Lagoon. Verification of the habitat (sediment) in terms of Hg concentration and organic matter (MO) content and the proportion of the finest fraction (FSF) showed the opposite trend: in areas where Hg concentrations in *R. cuneata* were highest, MO and FSF were the least enriched in HgTOT. In contrast, in areas where the lowest Hg concentrations in *R. cuneata* were determined, organic matter and sediments were most contaminated with mercury. Higher concentrations of HgTOT in the clam body were determined in the areas closest to the shore (ZAT-K, 43L and 40L (Fig.1)), where elevated concentrations of labile mercury ($HgSO_4$ and HgO (HgF4)) were observed in the sediments. Statistical analyses showed that the source of accumulated Hg in this case was the sediment, where Hg occurred mainly in labile, more bioavailable forms for the body. At stations located in the mouth of the Elblag River (SUC-R2) and in the central part of the Vistula Lagoon (IW100, 29L and P14A), clams accumulated mercury from the sediment less efficiently at deeper stations, as Hg occurred here mainly in a stable form (**Publication 1**).

The BAF coefficient estimated for *R. harrisii* from the Vistula Lagoon **(publication 4)** indicates bioaccumulation of Hg from food (Fig.2). The American crab formed its occurrence classifier in the clam occurrence classifier, among others: *Dreissena polymorpha*. It probably uses this area as a refugia (a shelter place in shells plus a food base). Hence, it is likely that the higher proportion of mercury of trophic (animal) origin is reflected in the percentage of Hg fraction in crabs from the Vistula Lagoon, in contrast to the Puck Bay, where BAF < 1 (Wilman et al., 2019), and the crab's food was mainly plant food accounting for pond 50% of the crab's diet (Hegele-Drywa and Normant, 2009).

Due to the fact that the woolly-tailed crab is the most mobile representative of zoobenthos in the study area, with a typically aquatic-terrestrial lifestyle**,** it is difficult to demonstrate a direct source of mercury into the body of *E. sinensis* **(Publication 3).** The main routes of metal entry into the crab's body are the digestive system and gills (Soegiantoetal., 1999). During exposure to toxic metals, the gills and hepatopancreas act mainly as transient tissue storage for accumulated metals. In the woolly soft-shell crab, the results confirmed on the transfer of Hg from the gills and liver-pancreas into the crab's body. Halogenated and semi-labile forms of mercury (HgF1, HgF4) were adsorbed on the surface of the gills from water, while in the hepatopancreas, a fraction of mercury mainly of trophic origin (HgF2) was determined (Fig.3).

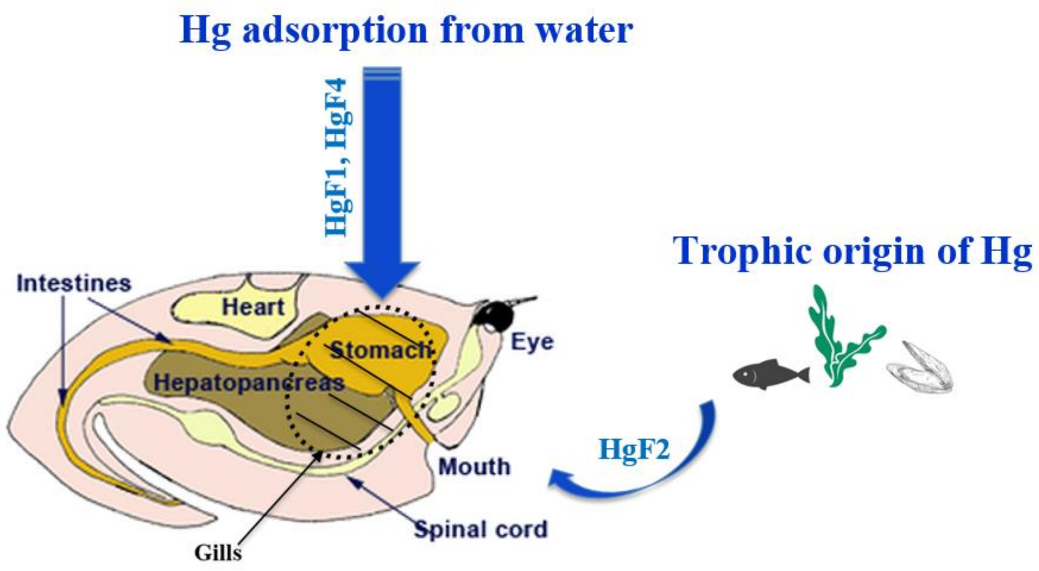


Figure 3 Routes of mercury entry into the body of the Chinese mitten crab

The realization of the first objective and the literature study of the biochemical properties of mercury and compounds involved in shell/ shell biomineralization, allowed us to realize the second objective of the work: **Recognizing the distribution of total mercury, as well as its labile and stable forms, in the body of crabs and bivalves**. Differences in dietary preferences and the form of Hg supplied with food in the clam species studied were reflected in the bioaccumulation of mercury and its fractions in the body (**publication 1 and 2**). The concentration of HgTOT in the body of *D. polymorpha* was statistically significantly higher than that of the xenobiotic in the body of *R. cuneata*. The proportion of halogenated forms of HgF1 in the corpuscle of *D. polymorpha* accounted for 40% of the proportion of HgTOT, while in the corpuscle of *R. cuneata* it accounted for 5%. In the case of the HgF2 fraction, the opposite trend was observed, where in the body of D. polymorpha HgF2 accounted for 55% of the share, while in *R. cuneata* 93%. In the case of bivalve shells, a statistically significantly higher concentration of Hg was also determined in the shell of *D. polymorpha* than *in R. cuneata*. Although the chemical composition of the shells of *R. cuneata* and *D. polymorpha* in the Vistula Lagoon had the highest proportion of proteins, averaging 40%, carbohydrates 30%, lipids and mineral matter together 30% (Dabrowska et al. 2017), the concentration of Hg was at a low level, most likely due to the saturation of the shell with calcium carbonate, which is used in the process of calcification - shell growth. Which was most evident in the species *R. cuneata*, which has a massive shell, compared to the small and thin shell of *D. polymorpha*. However, when it came to the percentage of Hg fractions, their percentages were at a similar level in both species. In bivalve shells, a high proportion of the semi-labile fraction of mercury compounds HgF4 and the stable fraction HgS were determined in contrast to the soft body. A recent study by the team of Immel et al, (2024) showed that this is most likely due to the fact that this matrix, although generally poorly glycosylated, contains a set of potentially calcium-binding proteins, among others, sulfur amino acids such as cysteine or methionine, containing a $^{-}$SH thiol group with which mercury can form a stable HgS complex, and a set of acidic sulfate proteins where the $SO_{(4}^{)(2-)}$ anion can bind to the $Hg^{(2+)}$cation.

Considering the American crab, the median concentration of total mercury was statistically significantly different in different parts of its body **(publication 4)**. The highest values of median HgTOT concentration were found in the soft body (viscera: gonads, gills, heart, digestive system). Almost twice the median HgTOT concentration was obtained in the crotal leg muscles. In the carapace, the median HgTOT concentration was almost four times lower compared to the crotch legs muscles. The highest percentage of mercury fraction was determined for HgF2 in all tissues of the studied organisms. This indicates a process of bioaccumulation of this form of mercury in the body of the American crab. This may be related to the fact that organic forms of Hg (belonging to the group of compounds of the HgF2 fraction) can bind to cysteine groups in the body (Gupta et al., 2018). Considering the HgF1 fraction, its highest proportion (10%) was determined for the soft body thus confirming the accumulation of HgF1 with the consumption of microalgae by young crabs. The stable form of mercury HgS was determined only in the carapace of the crab and was the mercury fraction directly correlated with HgF1. This may indicate, as has been observed in bivalves, that inorganic forms of mercury are redistributed into the carapace along with other inorganic compounds involved in the biomineralization process during carapace growth (Schofield et al., 2003).

Considering the woolly soft-shell crab, cluster analysis categorized the results of HgTOT concentrations in the soft tissues of *E. sinensis* into two groups: (i) those with the highest total mercury concentrations - the crab's muscles, and (ii) internal organs: gills, liver-pancreas and gonads, which had Hg concentrations an order of magnitude lower compared to muscles **(publication 3)**. The reason, influencing this differentiation, is the biochemical composition of individual tissues. The muscles of the crab *E. sinensis* are typically proteinaceous structures with low fat content compared to organs, which are the target group for Hg toxicity. The presence of multiple functional groups in the muscles and internal organs influenced the fact that mercury was present there in 100% labile forms, which are more bioavailable and toxic to the body, either forming biological Hg ligands or being better fat-soluble. In the case of the exoskeleton, three groups were determined, with statistically significant differences in mercury concentration: dactylus and carapace < merus and pincers <

cuticular outgrowths (Fig.4). The lowest mercury concentration in dactylus was mainly associated with a different type of carapace mineralization and function. Schofield et al, (2003) showed that as the calcium concentration level decreases, it is chlorine and bromine that take its place as the dominant inorganic element just in the dactylus, which can be confirmed by the mercury fractionation results, as the proportion of halogenated forms of Hg was highest there than in the rest of the exoskeleton. Because it is the most labile form of Hg and has a lower affinity for mercury toxicity target groups, it bioaccumulates more poorly, which resulted in the lowest Hg concentrations compared to the other exoskeleton elements, where the organism uses N-chitin protein in addition to calcium carbonate in the mineralization of the carapace.

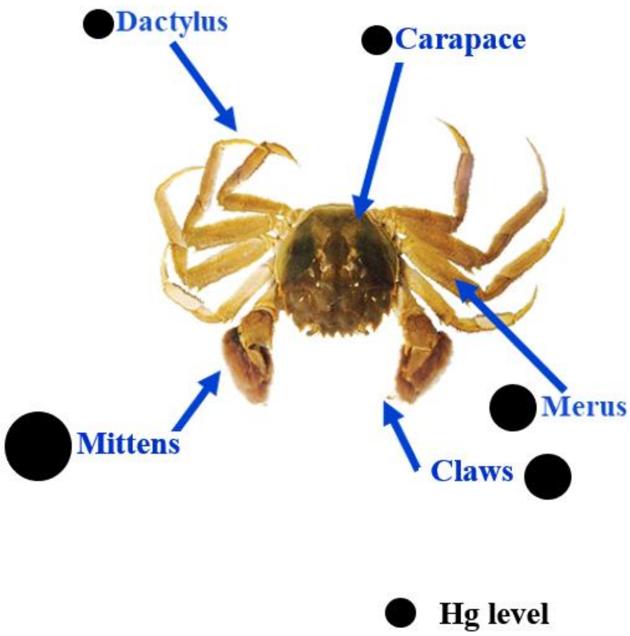


Figure 4 Concentration of Hg in tested parts of the exoskeleton of the Chinese mitten crab (*Eriocheir sinensis*) from the Vistula Lagoon

In addition to the biochemical properties of mercury compounds, which determine the body's burden of Hg neurotoxin, their concentration can also be determined by environmental factors and physiological processes in animals. Therefore, another goal of the dissertation was: **<u>To determine the factors affecting the variability of the body burden of mercury neurotoxin in crabs and mussels</u>**. Gender did not determine the level of Hg concentration in both species of clams and American crabs (**publication 1,2,4**). Only in the case of *R. cuneata* did juvenile forms have higher Hg concentrations compared to adults (**publication 1**). The main determinants of mercury uptake processes were water temperature and biometric parameters (**publication 1 and 2**) (Fig.5).

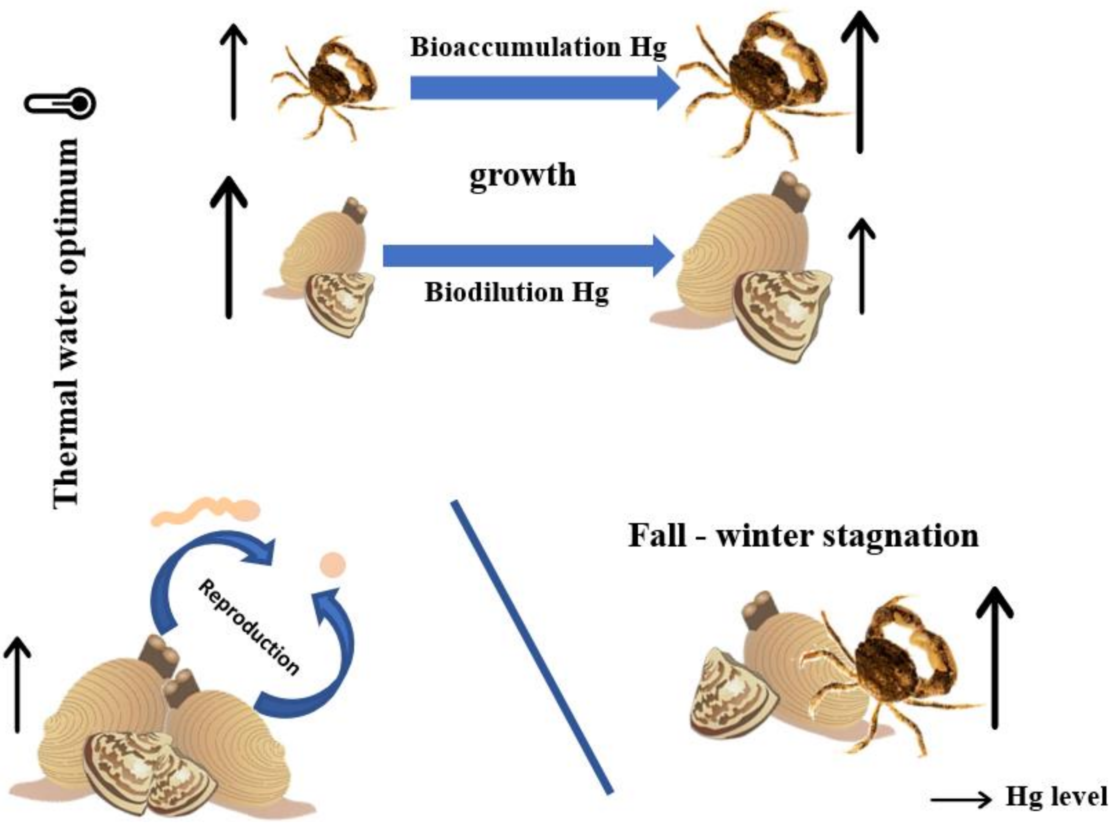


Figure 5 Effect of biometric parameters and water temperature on the concentrations of xenobiotic Hg in the body of alien species of clams and crabs from the Vistula Lagoon

In summer, of all the measurement seasons, the lowest mercury concentrations in clams were observed. This was the only season where the thermal optimum for reproduction was met; consequently, the clams were able to purge themselves of the xenobiotic through reproduction. In addition, in warmer water, clams show accelerated metabolism and increased energy consumption for growth and respiration (**Publication 1 and 2**). This was very evident in the species *D. polymorpha* (**publication 2**). Then the bioaccumulation rate for HgF1 including plankton was the highest of all those analyzed. During this season, the percentage of HgF1 in HgTOT by size class doubled: from 34% to 68%. In contrast, during the spring-autumn-winter stagnation period, when the water temperature is lower, the concentration of Hg in the bivalve body was higher (**Publication 1 and 2**). This was probably due to a slowdown in metabolism and accumulation of unfavorable metabolites, including toxic forms of mercury, in the body. During the summer season, as a result of increased energy expenditure associated with reproductive and growth processes, the clams were characterized by deteriorated physiological condition. The condition factor was inversely correlated with both the size of the individuals and the Hg concentration, which may indicate the detoxification process of the body from mercury taking place during this period due to intensive anabolic processes (growth and reproduction) (**Publication 1 and 2**).

The opposite trend was observed in the American crab (**publication 4**) compared to clams (Fig.5). Considering seasonal variation, the lowest Hg concentrations were determined in samples from spring, where individuals had the highest condition factor. An inverse relationship was observed in the summer and autumn seasons, where crabs in worse condition were characterized by higher HgTOT concentrations. It is likely that other processes determining Hg accumulation in tissues may take place during the growth phase of crabs. During the summer season, bioaccumulation of mercury was observed in croc leg muscles in successive size classes, which was mainly related to the fraction of mercury of trophic origin. With the concomitant bioaccumulation of Hg in the carapace can and the increase in the proportion of halogenated forms and Hg sulfide in subsequent size classes. The results presented here confirm that their growth rate is higher in the summer season, where at the same time in the process of growth (moulting), there is a bio-dilution of Hg due to the formation of a new, larger exoskeleton. In the autumn season, the opposite trend was observed: a decrease in the concentration of Hg in the crotch leg muscles in successive size classes and bioaccumulation of Hg in the carapace, and the muscle mass in the autumn season was on average 4 times higher than in the individuals of the summer season. This may indicate that they were gaining weight in preparation for winter stagnation, and Hg dilution was observed in the muscle mass of the legs. The twofold higher concentration of Hg in the soft corpuscles in autumn compared to summer may indicate that the body partially efficiently redistributes Hg compounds to the carapace during the period of increased Hg accumulation in the body. Hence, an increase in Hg concentrations was observed in successive size classes in the carapace of the American crab in autumn.

Biometric parameters such as size, weight of crabs and condition (K index) did not determine mercury concentrations in whole individuals of *E. sinensis* (**publication 3**). However, in the present study, differences in xenobiotic concentrations were observed, which were related to the sexual dimorphism of the crabs. Males were characterized by a higher hepatopancreas burden of xenobiotic Hg, including stable mercury. The increase in the proportion of stable forms in male crabs, may indicate that larger, older males are more efficient in detoxifying the body from Hg, which may be due to their longer exposure to Hg and developed adaptive mechanisms. In their study, Clifford et al. (2005) observed a decrease in methylmercury concentrations in the hepatopancreas of *E.sinensis* with the size of the individual, indicating more efficient demethylation of Hg as crabs age in larger males. *Eriocheir* *sinensis* males also showed higher mercury concentrations in the gonads. The spermatophore, which is small, is composed mainly of proteins, which distinguishes it from the lipid- and protein-rich oocytes of females. Proteins are a target group for toxic forms of mercury - capable of forming bonds with biological ligands (Shao et al., 2018). Thus, elimination of some mercury through gamete release could be an effective detoxification mechanism, especially in males. However, since *E. sinensis* usually dies after reproduction (Veilleux and Lafontaine, 2007; Dittel and Epifanio, 2009), the potential of this detoxification mechanism may be limited to the last stage of the life cycle.

The problem concerning the distribution of mercury in organs and its elimination is fairly well understood in the world literature only in organisms from higher trophic levels. Studies in organisms from lower levels, e.g., in invertebrates including bivalves and crabs, mainly focus on determining the concentration of mercury in soft tissue and the effect of seasonality and size of organisms on mercury concentration levels. The mechanisms of mercury transfer, both from soft tissues to shells and from internal organs to carapace for the forms of mercury studied are unknown. Therefore, in pursuit of the next research objective, an attempt was made: **To learn about the role of the exoskeleton in the process of mercury elimination in invertebrates, using the example of crabs and bivalves**. The processes of clam detoxification from Hg discussed in publications 1 and 2 seem to be efficient, so that the clam shell was characterized by low Hg concentrations. Biometric parameters indirectly influenced the Hg concentration in the shell. However, given that shell mass accounts for more than 90% of the clam's body mass, a different trend was observed (**publication 1 and 2**). In the case of *D. polymorpha*, regardless of the season, the mass of accumulated mercury in the shell mass was higher than in the whole body, regardless of the form in which it was present, which also confirms the body burden factor of mercury. Which may indicate that the transfer of Hg from the corpuscle to the shell in this mussel species is an important process of its elimination and temporary inactivation, thus protecting one's body from bioaccumulation of the xenobiotic (**publication 2**). In the case of *R. cuneata*, no such tendency was observed during the spring-autumn-winter period of clam stagnation. This relationship was observed only in summer, which may indicate that, in addition to reproduction in this bivalve species, the transfer of Hg from the corpuscles to the shell during the growth process in the growing season is also an important pathway for the elimination of mercury from the organism (**publication 1**).

In the American crab (**publication 4**), the mass of accumulated total mercury and its labile and stable forms was greater in the carapace than in the soft tissue and crotch legs. This was particularly evident during the preparatory period for winter stagnation, when Hg transfer from the body to the carapace was observed. Consequently, the mass of accumulated mercury in the carapace and the loading factor was also highest during this period. These crabs undergo moulting several times throughout their lives, which also allows them to rid their bodies of a certain pool of accumulated contaminants during the growth process. In the case of the woolly soft-shelled crab (**Publication 3**), the carapace is the largest part of the carapace and is shed during growth. The low concentration of mercury in this structure was probably due to the metal bio-dilution effect occurring during the rebuilding of the carapace after the last moulting. This is confirmed by the inverse correlation between Hg concentration and carapace width. Unlike in the case of merus - the smaller elements of the legs that are also discarded during moulting - the bio-dilution effect was also clearly visible in the pincers. This was especially true for males, which are characterized by larger and more massive pincers than females. Interpretation of the highest Hg concentration in the cuticular outgrowths - *mittens* of *E.sinensis* is difficult due to the lack of studies, for example, on their chemistry or their role in the organism, beyond their ornamental function. Potentially, mittens in the crab may represent sites of accumulation and temporary inactivation of Hg, analogous to hair in mammals or feathers in birds, since cuticular outgrowths are shed along with the carapace during moulting. By way of moulting in birds or hair replacement in mammals, a new Hg accumulation center is formed at the site of new cuticular outgrowths, which is then incorporated into these structures. Hence, the incorporation of Hg into outgrowths by the crab may be important in the process of its elimination from the body. As the process of moulting during the life of the woolly soft-shelled crab, occurs more frequently than reproduction, the transfer of Hg from the body to the carapace may gain importance (Fig.6). Statistical analyses showed that mercury was redistributed to the merus, carapace and pincer. As a result, this crab may eliminate a certain pool of contaminants in the process of moulting, i.e. growth alongside the detoxification process from labile more toxic forms to stable, HgS in the liver-pancreas (Fig.6).

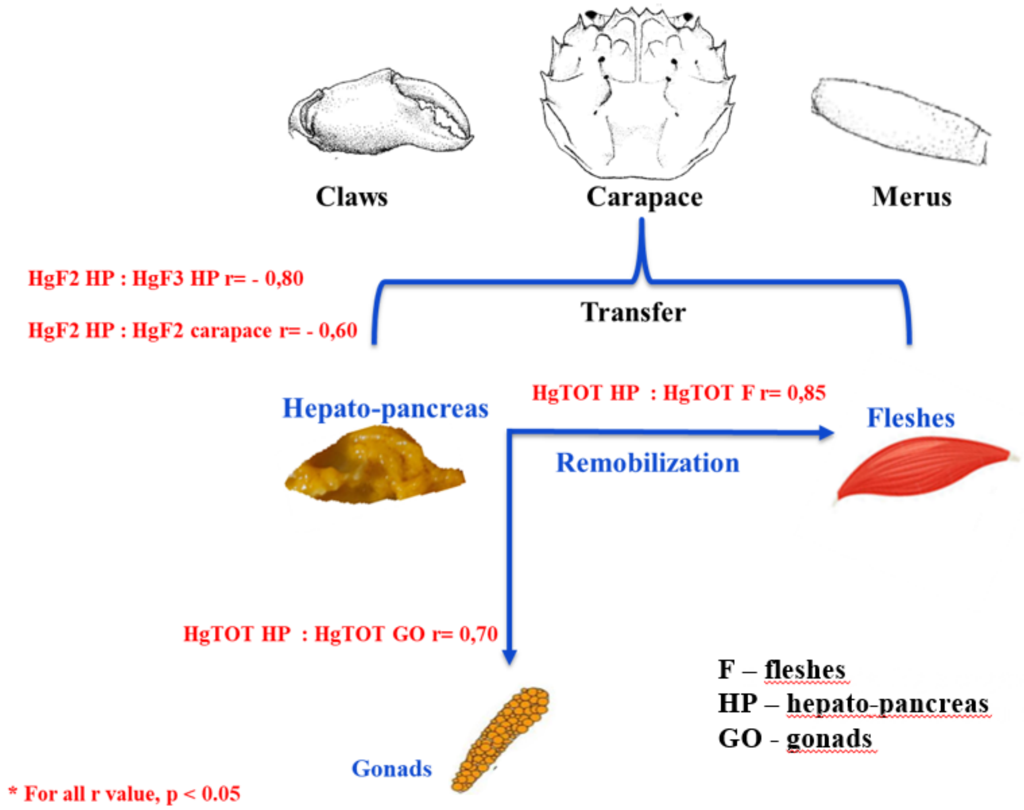


Figure 6 Transfer of mercury and its labile fraction HgF2 in the crab *Eriocheir sinensis*

Despite numerous papers on the biology and ecology of bivalves: *Dreissena polymorph* and *Rangia cuneata*, and the crabs *Rithropanopeus harrisii* and *Eriocheir sinensis*, there is a lack of literature on the role of these species as new components in the trophic network in the southern Baltic region. There has also been a lack of scientific reports on the determination of mercury concentration levels in surveyed bivalves and crabs in the area. These organisms are gradually expanding their ranges of occurrence in the southern Baltic. In the Baltic estuaries, they are becoming an important part of the diet of organisms from higher levels of the trophic network, especially birds or bottom-feeding fish (flounder, eel, bream, roach), commercially fished and consumed by humans. Hence, it is important to determine mercury concentration levels in non-native species as a potential new link (source of Hg) in the ecosystem. Therefore, the final objective was: **<u>To describe the role of non-native species in the southern Baltic ecosystem in relation to mercury trophodynamics in the study area</u>.** The concentration of total mercury in American crab and clams was at a low level, and this value was much lower than the maximum allowable concentration of Hg in fish and seafood for human consumption (**Publication 1,2 and 4**). The median concentration of HgTOT in the studied mussel species from the Vistula Lagoon was lower compared to mussels from the Puck Bay: *Limecola baltica* or *Mytillus trossulus* (Fig.7) (Jedruch et al., 2018; 2019). Also, the concentration of Hg in

American crab from the Vistula Lagoon was lower compared to studies conducted on the same species in the Puck Bay (Wilman et al., 2019) (Fig.7). In addition, the results obtained in the present study showed that more mercury is accumulated by clams and American crabs in the shell and carapace, respectively, than in the body. This may affect the transfer of toxic mercury in the trophic web, since the bivalve body is mainly eaten, for example, by woolly crabs. Birds or benthic fish, which ingest organisms whole, partially excrete exoskeleton or shell parts during the defecation process or with spat. As a result, a lower Hg load can be incorporated or biomagnified in the trophic network, in the region where the organisms become an important link in the food chain. Hg concentrations in sediment and suspended solids from the Vistula Lagoon were higher than in the Puck Bay, reinforcing the importance of Hg detoxification in the organisms of the studied species of alien bivalves and American crabs (Jędruch et al., 2017; Kwasigroch et al., 2021).

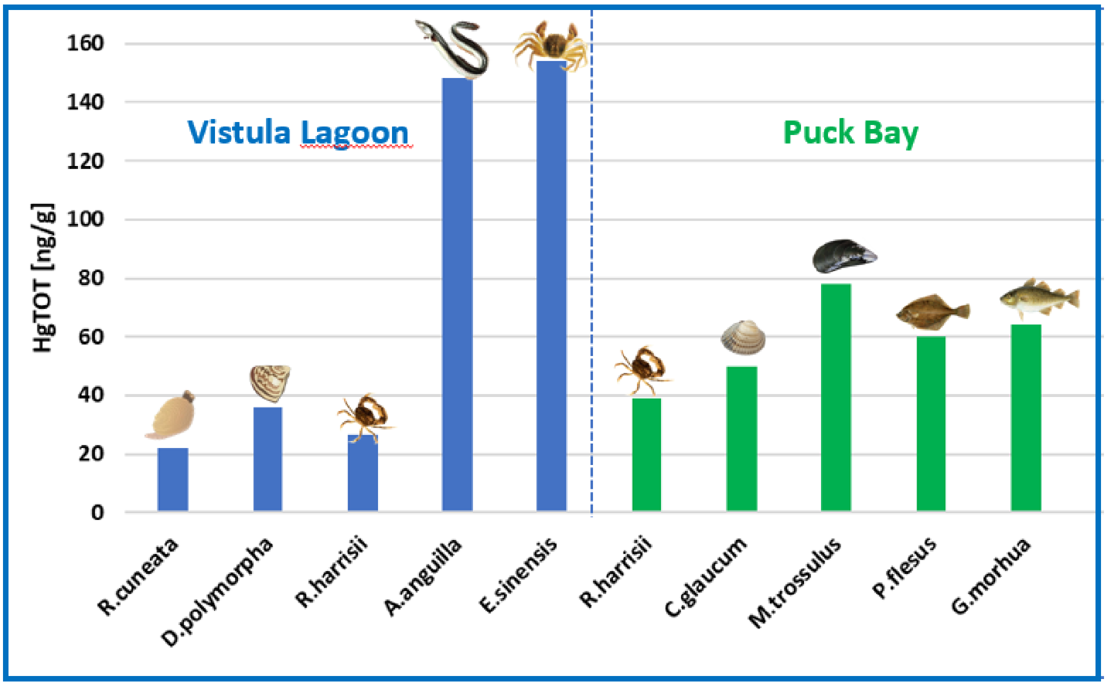


Figure 7 Comparison of Hg concentrations in non-native to native species in the southern Baltic region

Isotopic studies of $\delta^{13}C$, $\delta^{15}N$ in the woolly crab, due to its similar diet to fish, show a similar trophic level of these organisms (Li et al., 2023). Mercury concentration in the muscles of *E.sinensis* (**publication 3**) was higher than in the muscles of plankton-eating fish (sprat and herring) and in the muscles of predatory fish (cod, eel or flounder) in the study area (Fig.7) (Polak-Juszczak, 2018). The lower concentration of mercury in the muscles of fish, at comparable body size, may be due to a bio-dilution effect - fish build more muscle mass than crabs, which leads to dilution of the metal in the tissues. Crab meat contains very high amounts of minerals and amino acids and is a high-energy food (Chen et al., 2007; Normant et al., 2002), and according to the public, its meat is considered tastier than that of other crabs such as *A. ascatus* (Fladung, 2000). Consumption of 1.4 kg of *E.sinensis* crab meat from the Vistula Lagoon would deliver an acceptable weekly dose of the xenobiotic to the human body. By comparison, the same dose of Hg would be delivered by consuming 2.6 kg of fish muscle from the southern Baltic Sea. However, in the study area, the woolly soft-shell crab is not commercially fished or consumed by humans and has few natural enemies or competitors. In addition, due to insufficient salinity, it does not reproduce in the southern Baltic region, where it migrates to the western Baltic Sea after reaching sexual maturity. Hence, in the network of food relationships between species in the studied ecosystem, it may be a second-order consumer, achieving higher concentrations of Hg in the trophic chain during the period when it occurs in the study area.

The results obtained within the framework of this dissertation made it possible to verify the hypotheses:

i. *The level of total mercury concentration and its toxicity to organisms in crabs and clams are related to the habitat characteristics within the identified cluster of their occurrence and their diet;*

Hypothesis 1 was partially verified positively:

- The pathways of mercury entry into the body of the American crab *R. harrisii* and the clam *D. polymorpha* were related to the ecological niche occupied by the two alien species, which formed common occurrence clusters in the Vistula Lagoon (**Publication 2 and 4**).
- Alien species found in the Vistula Lagoon, despite higher sediment mercury loads, showed lower Hg concentrations compared to native species from the nearby Gulf of Gdansk, where sediment metal concentrations were lower (**Publication 1, 2 and 4**).
- The unique ability of *Dreissena polymorpha* to filter a large volume of water, allows the extraction of a large amount of nutrients as well as labile mercury **(publication 2**), in addition, its presence affects the toxicity of Hg accumulated in the tissues of the American crab (**publication 4**).

- In the alien species of the bivalve *R. cuneata*, **Hypothesis 1** was verified negatively, which is most likely due to the occurrence of this species for the shortest period of time in the Vistula Lagoon and continuous adaptation in the new environment (**publication 1**).

ii. *Physiological state (season), biometric parameters and sex are the main determinants of mercury uptake and elimination in crabs and clams;*

Regarding **Hypothesis 2**, it has been partially verified positively:

- Sex did not determine the concentration of HgTOT and its fractions in the studied organisms. The organisms cleared the xenobiotic most effectively during the growing season, and this process was intensified by their reproduction (**publication 1 - 4**).
- The growth of the clam body contributed to the bio-dilution and consequent decrease in the concentration of metal in the soft body, (**Publication 1 and 2**).
- In the American crab, the opposite trend was found in relation to Hg in the muscles, and compared to bivalves, a bioaccumulation of mercury was observed here as the crabs grew, which is related to a change in behavior to more predatory in older individuals (**publication 4**).
- Biometric parameters did not affect the concentration of xenobiotic Hg in the body of woolly soft-shelled crabs caught in the Vistula Lagoon. This is probably due to the presence in the study area of only adults preparing to migrate for reproduction. The differences in the concentration of xenobiotic Hg in the crabs were due to the sexual dimorphism characteristics of *E. sinensis* (**Publication 3**).

iii. *The exoskeleton may be an important site for the accumulation and temporary inactivation of mercury via the transfer pathway: soft body-shell in bivalves and elimination via the soft tissue-exoskeleton pathway in crabs;*

Regarding **Hypothesis 3**, it has been verified in the affirmative:

- The mass of total accumulated mercury as well as its fractions in the shells of *R. cuneata* and *D. polymorpha* and the carapace of the American crab was higher than in the corpus, as a result of the transfer of the metal in the form of labile forms and HgS (**publication 1, 2 and 4**).
- The distribution of Hg in the crab's organs was related to the trophic origin of mercury and its transfer from the hepatopancreas and muscle to the carapace, while halogen and

semilabile forms of Hg from the filtration process were redistributed to the exoskeleton (**publication 3**).

- Elimination of Hg from the muscles and from the liver-pancreas into the carapace was one of the important detoxification processes of the crab's body. Thus, crabs in the process of growth (moulting) can remove a certain pool of toxic Hg, thus protecting their body from the neurotoxin (**publication 3,4**). In contrast, the shell in bivalves can provide a target site for the accumulation and temporary inactivation of the metal (**publications 1 and 2**). Consequently, a smaller Hg load is biomagnified in the body.

Further development of research

The present study is a major addition to the world literature on the processes by which mercury neurotoxin occurs in organisms from the initial links of the trophic chain, since the problem concerning the distribution of mercury in organs and its elimination are fairly well understood in the world literature in organisms from higher trophic levels (fish, birds, marine mammals). This was made possible by extending previous studies conducted in benthic fauna to include the exoskeleton of organisms and the Hg fractionation method. Mercury in any form is toxic. However, toxicity varies depending on the form of mercury, which determines its physical and chemical properties. Complementing the above-described problems with the environmental aspect - seasonality, biometric parameters or gender is an important aspect in the context of assessing the potential risk to human health from the consumption of seafood, which, along with fish, is an important source of Hg to the human body.

Evaluation of alien species in the context of their biomagnification potential for mercury in the trophic network was difficult, due to the fact that within the niche in which they occurred they were the dominant or even the only taxon. Therefore, the results obtained showed further research perspectives that should be complemented by native species or higher-order consumers, among others, fish, as well as carbon and nitrogen isotopic analysis of $\delta^{13}C$, $\delta^{15}N$ to fully determine the role of alien species in the trophodynamics of Hg in the trophic network. This problem is currently being further developed in the NCN project OPUS-23 (Primary Producers as a Key Carrier of Historical and Modern Mercury in the Estuarine Trophic Network) under the leadership of Prof. Magdalena Beldowska. However, based on the results of Hg in the organisms studied, it can be assumed that the appearance and spread of alien species in the southern Baltic may have a positive impact on the ecosystem. Especially since on a geological scale, the Baltic Sea is a young sea, from still forming ecosystem and moderate biodiversity. Mercury concentrations in alien species were at low levels with the exception of the woolly soft-shell crab. Assuming that in the future these organisms, due to their increasing prevalence, will become an increasingly common component of the fish diet in the southern Baltic region, a lower load of toxic mercury will be introduced into the marine trophic chain, including humans. This is important because Hg has no positive function in living organisms and is toxic even at low doses.

Also, isotopic analysis of mercury $\delta^{202}Hg$ in individual tissues of organisms could be a great addition to the process of elimination of mercury from the body to the carapace or shell, and indicate its origin in the organism $\Delta^{199}Hg$, $\Delta^{200}Hg$, $\Delta^{201}Hg$ (Manceau et al., 2025). Using this method would have made it possible to numerically indicate the effectiveness of the processes described in this dissertation, which in this dissertation, were based on the biology and ecology of the organisms studied.

Nowatorski aspekt pracy

1) **Określenie w jakiej formie rtęć przedostaję się do organizmu skorupiaków i mięczaków wraz z pokarmem, a następnie w jakiej formie jest bioakumulowana w tkankach miękkich i twardych.**

2) **Poznanie dystrybucji rtęci w narządach wewnętrznych organizmów z niższych poziomów troficznych na przykładzie największego bałtyckiego skorupiaka *Eriocheir sinensis*.**

3) **Rozpoznanie szlaków transferu rtęci w organizmie bezkręgowców w oparciu o czynniki środowiskowe, parametry biometryczne i płeć.**

4) **Powiązanie akumulacji frakcji Hg w tkankach twardych z typem biomineralizacji muszli małży i egzoszkieletu krabów.**

5) **Uzupełnienie luki badawczej dotyczącej określenia różnych dróg eliminacji Hg w organizmach z niższych poziomów troficznych.**

6) **Oznaczenie stężenia rtęci w gatunkach obcych, w aspekcie określenia ich roli w trofodynamice Hg, które stają się nowym ogniwem w sieci troficznej południowego Bałtyku.**

Słowa kluczowe

rtęć, frakcje Hg, makrozoobentos, gatunki obce, bioakumulacja, eliminacja, sieć troficzna, *Dreissena polymorpha*, *Rangia cuneata*, *Eriocheir sinensis*, *Rhithropanopeus harrisii*

Streszczenie

Rtęć (Hg) jest pierwiastkiem, który nie pełni żadnej pozytywnej funkcji w organizmach żywych. **Może mieć toksyczny wpływ nawet w śladowych ilościach i w każdej postaci chemicznej: gazowej, alkalicznej i metaloorganicznej** (Castro-Gonzalez i Mendez-Armenta, 2008; Hosseini i in., 2013). Wynika to z charakterystycznych właściwości tego pierwiastka: lotności, reaktywności i wysokiej toksyczności - zwłaszcza jego form organicznych (Kabata-Pendias i Mukherjee, 2007). Szeroko opisane przypadki zatrucia Hg nad Zatoką Minamata w Japonii w dużej mierze wywołały globalną, pilną potrzebę badań nad rtęcią i zagrożeniem związanym z występowaniem toksyny w odniesieniu do środowiska przyrodniczego, wodnego łańcucha pokarmowego i zdrowia człowieka (D'Itri, 1993; Malm i in., 1995). Udowodniono, że niekontrolowana emisja Hg zwłaszcza ze źródeł antropogenicznych przyczyniała się do wzrostu poziomu Hg w atmosferze i na lądzie, a przede wszystkim w ekosystemach wodnych co w większym stopniu doprowadzała do globalnych problemów związanych ze zdrowiem i życiem ludzi, a także innych organizmów żywych (Bosch i in., 2016; Ye i in., 2016). Kwestia rtęci jest rozpatrywana głównie w kontekście zanieczyszczenia mórz i oceanów, ponieważ to właśnie **ryby i owoce morza są globalnie głównym źródłem rtęci do organizmu człowieka** (Cheng i in., 2011; Mania i in., 2012). Z tego powodu obecność Hg w produktach pochodzenia morskiego budzi zainteresowanie w aspekcie zdrowia publicznego (Burger i Gochfeld, 2012; Gibb i O'Leary, 2014; Mozaffarian, 2009).

Zdolność Hg do akumulacji i wydalania z organizmów w dużej mierze zależy od jej formy chemicznej (Bosch i in., 2016). Rtęć organiczna, głównie metylortęć (MeHg), ulega biomagnifikacji w kolejnych ogniwach łańcucha troficznego. Jest ona (obok dimetylortęci) najbardziej toksyczną formę Hg (Ekino i in., 2007; Branco i in., 2007; Kojadinovic i in., 2006). Badania nad rtęcią w morzu skupiają się głównie na próbkach abiotycznych (woda i osady) oraz na organizmach z wyższych szczebli piramidy troficznej. **Słabo rozpoznane są przemiany rtęci w pierwszych ogniwach sieci troficznej, między innymi w organizmach bentosowych**. Jest to istotna luka poznawcza, ponieważ na tym etapie metal ten ulega w największym stopniu bioakumulacji z otaczającego środowiska (Scheuhammer i in., 2007). Obecnie, gdy dopływ metali do ekosystemów wodnych jest ograniczony wskutek wprowadzonych restrykcji, to remobilizacja Hg z osadów staje się coraz ważniejsza, gdzie w wyniku szeregu procesów biotycznych i abiotycznych rtęć ulega remobilizacji do środowiska morskiego. **W ten sposób metal ten może ulegać bioakumulacji w organizmach bentosowych** i biomagnifikacji w kolejnych ogniwach sieci troficznego, stanowiąc tym samym największe zagrożenie dla ssaków morskich, ptaków wodnych i ludzi (Jackson, 1998). Prace

dotyczące wnikania rtęci oraz jej dystrybucji w narządach są również dość dobrze opisane w literaturze światowej jednak w organizmach z wyższych poziomów troficznych tj. ryby, ptaki i ssaki (Scheuhammer i in., 2007; Bustamante i in., 2016; Falkowska i in., 2017; Grajewska i in., 2019; Manceau i in., 2021). **Badania w organizmach z niższych poziomów troficznych takich jak np. kraby czy małże, skupiają się głównie na określeniu stężenia rtęci całkowitej (THg) w tkance miękkiej oraz wpływie sezonowości na jej zmienność** (Chiarelli i Roccheri, 2014; Jędruch i in., 2019). Procesy dotyczące dróg eliminacji rtęci z organizmu wraz z wytworem naskórka (pióra czy sierść) są również dość dobrze opisane w przypadku ptaków czy ssaków morskich (Falkowska i in., 2017; Grajewska i in., 2019; Manceau i in., 2021). **Mechanizmy transportu rtęci na drodze ciałko miękkie – muszla czy na drodze transferu wewnątrz-narządowego do egzoszkieletu nie są do końca rozpoznane**. W dużym stopniu jest to związane z niewielką wielkością organizmów bentosowych i ich poszczególnych narządów czy tkanek, czyli trudnością w uzyskaniu reprezentatywnej ilości materiału do analiz chemicznych.

Większość literatury naukowej do tej pory koncentrowała się na biomagnifikacji i bioakumulacji rtęci całkowitej lub jej jednej z najbardziej toksycznej formy, a mianowicie metylortęci w sieci troficznej ekosystemów morskich (Wren i MacCrimmon, 1986; Baeyens i in. 2003; Schmitt i in. 2011; Hong i in. 2016; Gentés i in. 2021). **Istnieje luka badawcza na temat, jakie formy rtęci są dostarczane organizmom z niższych poziomów troficznych poprzez sieć pokarmową. Ważne jest również zbadanie wszystkich form rtęci, zwłaszcza tych labilnych - najbardziej toksycznych i biodostępnych dla tkanek miękkich**. To właśnie forma metalu w dużym stopniu decyduje o jego dystrybucji i przemianom zachodzącymi w środowisku przyrodniczym, a także w organizmach.

Obszar Morza Bałtyckiego oraz jego estuariów (w tym Zalew Wiślany) znajduje się pod intensywną presją różnego rodzaju oddziaływań antropogenicznych, przy czym introdukcja gatunków nierodzimych (NIS) jest uważane za kluczowy czynnik wpływający na dynamikę jego ekosystemu (Snoeijs-Leijonmalm i Andrén, 2017). Ze względu na niski poziom różnorodności biologicznej w Morzu Bałtyckim w ostatnich dziesięcioleciach zaobserwowano znaczny wzrost liczby udanych introdukcji gatunków nierodzimych do Morza Bałtyckiego (Leppäkoski i Olenin, 2000). **Jedną z najliczniejszych grup NIS w rejonie Morza Bałtyckiego stanowią organizmy zoobentosowe**, których planktoniczna postać larwalna głównie została zawleczona wraz z wodami balastowymi statków (Warzocha i in., 2016).

Wcześniejsze badania wskazują, że gatunki nierodzime często mają szeroką tolerancję środowiskową (temperatura i zasolenie) lub szeroką niszę środowiskową (Marvier i in., 2004).

W przeciwieństwie do tej wcześniejszej teorii, ostatnie prace w rejonie Bałtyku wykazały, że gatunki nierodzime niekoniecznie muszą rozszerzać swoją niszę, ale raczej pozostają w niej bardziej przystosowane w porównaniu z rodzimymi gatunkami (Herkül i in., 2016). **W rejonie Zalewu Wiślanego klastry stabilnych populacji utworzyły dwa gatunki obce małży: *Rangia cuneata* i *Dreissena polymorpha* oraz gatunek obcy kraba: krabik amerykański *Rhithropanopeus harrisii*, stając się jednymi z dominujących taksonów w grupie organizmów zoobentosowych**. **Kolejny gatunek obcy kraba: krab wełnistoszczypcy *Eriocheir sinensis* staje się coraz bardziej powszechny w ujściach rzek, lagunach i zatokach północnej Polski**, w wyniku jego migracji w celu poszukiwania zasobów w tym rejonie (Ojaveer i in., 2007; Ewers i in., 2023). W konsekwencji wymienione gatunki stają się nowym ogniwem w łańcuchu troficznym tego ekosystemu, stanowiąc pokarm dla ryb ptaków wodnych, czy ssaków (Knoll, 2004; Hegele-Drywa i in., 2014; Puntila, 2016). **W wyniku tego rola NIS w trofodynamice rtęci w sieci troficznej południowego Bałtyku potencjalnie, staje się kluczowa** ze względu na ich coraz liczniejsze występowanie oraz rozprzestrzenianie się.

Uwzględniając dotychczasowy stan wiedzy na temat zagadnienia, w tym studia literaturowe oraz wyniki wcześniej przeprowadzonych badań, **hipotezy badawcze poddane weryfikacji w niniejszej pracy, zostały sformułowane następująco**:

- **i.** *Poziom stężenia rtęci całkowitej oraz jej toksyczność dla organizmów w krabach i małżach są związane z charakterystyką habitatu w obrębie zidentyfikowanego klastra ich występowania oraz ze sposobem ich odżywiania;*
- **ii.** *Stan fizjologiczny (pora roku), parametry biometryczne oraz płeć są głównymi czynnikami determinującymi proces przyswajania i eliminacji rtęci w badanychkrabach i małżach;*
- **iii.** *Egzoszkielet może stanowić istotne miejsce akumulacji i czasowej dezaktywacji rtęci na drodze transferu: ciałko miękkie – muszla w małżach oraz eliminacji na drodze tkanki miękkie – egzoszkielet w krabie.*

Istotą niniejszej pracy było wypełnienie luki poznawczej, obecnej w badaniach dotyczącej rozpoznania dróg wnikania i eliminacji rtęci w organizmach z niższych poziomów troficznych oraz określenie stężenia rtęci w gatunkach obcych, które stanowiły dotąd nierozpoznane źródło rtęci w sieci troficznej w badanym ekosystemie**. Stąd też wyznaczono następujące cele badawcze**:

i. Określenie głównych dróg wnikania rtęci do organizmu w gatunkach obcych małży: *Rangia cuneata* i *Dreissena polymorpha* oraz krabów: *Rhithropanopeus harrisii* i *Eriocheir sinensis* z Zalewu Wiślanego jako czynnika determinującego jej poziom stężenia i udział frakcji;

ii. Rozpoznanie dystrybucji rtęci całkowitej oraz jej labilnych i stabilnych form w organizmie krabów i małży;

iii. Określenie czynników wpływających na zmienność obciążenia organizmu krabów i małży neurotoksyną rtęci;

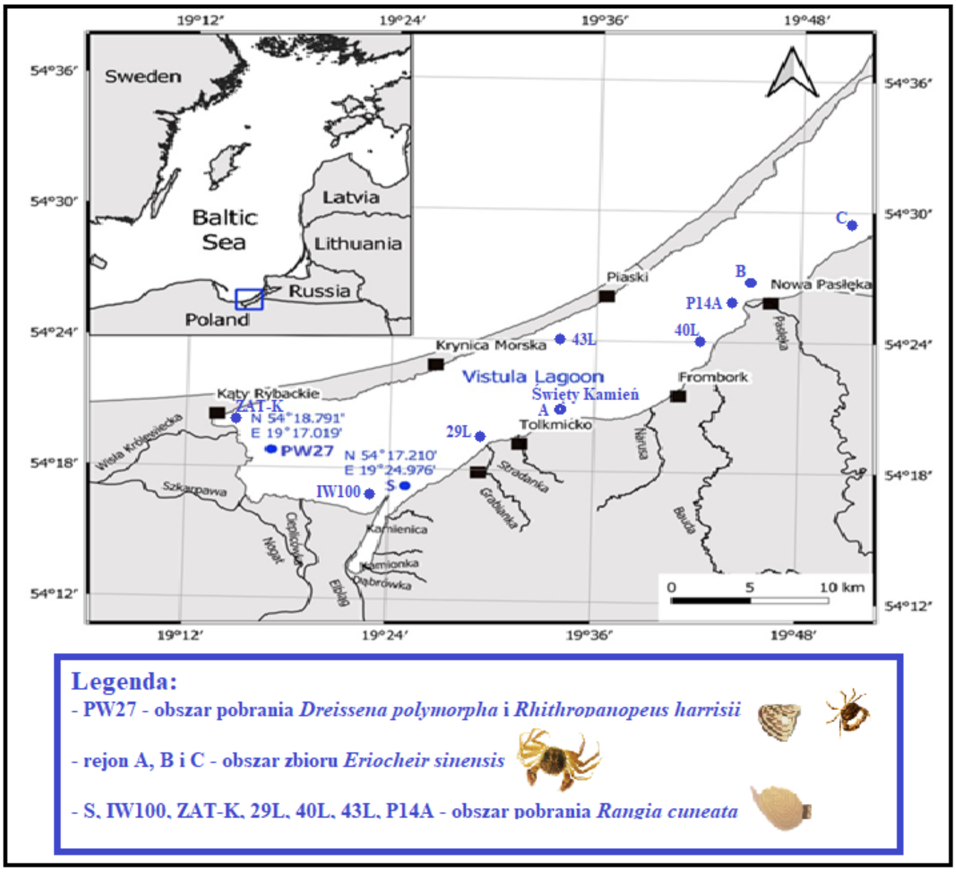
iv. Poznanie roli egzoszkieletu w procesie eliminacji rtęci w bezkręgowcach na przykładzie kraba i małży;

v. Opisanie roli gatunków obcych w ekosystemie południowego Bałtyku w odniesieniu do trofodynamiki rtęci w badanym rejonie.

Zakres badań został ograniczony do polskiego obszaru Zalewu Wiślanego, którego stan hydrologiczny jest określany głównie przez dopływ słodkiej wody z rzek. Głównym dopływem zanieczyszczeń (w tym rtęci) jest rosyjska rzeka Pregoła; dopływ z polskich rzek pochodzi z Baudy, Elbląg, Pasłęki, Nogatu i Szkarpawy (Ezhova i in., 2005; Miotk-Szpiganowicz i in., 2007). Obecnie Zalew Wiślany jest również zanieczyszczony pozostałościami różnych środków ochrony roślin i nawozów stosowanych w przeszłości w rolnictwie. Ścieki z mechaniczno-biologicznych oczyszczalni w Tolkmicku, Fromborku, Braniewie, Stegnie i Krynicy Morskiej w sposób ciągły trafiają bezpośrednio do zalewu. Zalew Wiślany został zatem objęty programem ochrony środowiska naturalnego Natura 2000 i umieszczony na liście Bałtyckich Obszarów Chronionych wprowadzonej przez HELCOM (Szefer, 2002). Zalew jest częścią Zatoki Gdańskiej odciętą od wód morza Bałtyckiego przez wał Mierzei Wiślanej. Jedyną cieśnina (w okresie prowadzonych badań) łączącą zalew z zatoką jest Cieśnina Piławska (znajdująca się na terenie Federacji Rosyjskiej). Uwarunkowania te sprzyjały gromadzeniu materii organicznej oraz zatrzymywaniu dopływających do niego zanieczyszczeń, w tym Hg w osadach dennych. Stąd organizmy, a zwłaszcza zoobentos jest szczególnie narażony na akumulację zanieczyszczeń w swoich tkankach.

Organizmy małży (**publikacja 1, publikacja 2**) **były pobierane** podczas rejsu jednostką badawczą Morskiego Instytutu Rybackiego w Gdyni – ŁM MIR 2, w zachodniej części Zalewu Wiślanego w kwietniu, sierpniu i październiku 2019 roku oraz kwietniu 2020 roku (Rys.1). Natomiast **osobniki krabika amerykańskiego zostały pobrane podczas rejsu** w kwietniu, sierpniu i październiku 2020 roku (**publikacja 4**). Dodatkowo, w sezonie letnim

2021, małże *R.cuneata* zostały zebrane z 7 stacji pomiarowych, w zachodniej części Zalewu Wiślanego, w celu określenia zmienności przestrzennej badanego ksenobiotyku Hg (**publikacja 1**) w ramach rejsu pod kierownictwem prof. Kornijowa z Morskiego Instytutu Rybackiego w Gdyni (Rys.1).



Rysunek 1 Obszar badań

Materiał biologiczny (małże i krabiki) na stacji S i PW27 był pobierany za pomocą dragi ręcznej (o wymiarach: 36x21 cm, długością włoka 60 cm i wielkością oczek siatki 3x3 mm) z głębokości 2,5 m, a następnie przesiewany przez sito o wielkości oczek 0,25 mm. Osobniki były wybierane ręcznie z sita i umieszczane w napowietrzanych termosach z wodą zalewową. Po przewiezieniu próbek do laboratorium, organizmy małży zostały umieszczone na dobę w akwarium w przefiltrowanej wodzie w celu puryfikacji. Do czasu dalszych analiz materiał biologiczny został zamrożony w temperaturze -20^{o}C. Ponadto na każdej stacji badawczej zebrano również składniki diety małży i krabika amerykańskiego oraz elementy ich środowiska życia. Wodę naddeną w celu odfiltrowania zawiesiny cząstek stałych (SPM) zebrano za pomocą batometru z warstwy przydennej. Plankton zebrano z kolumny wody za pomocą siatki 10 µm. Do analizy zebrano również powierzchniową warstwę osadów dennych (górne 10 cm). Próbki wody z SPM oraz planktonu po przetransportowaniu do laboratorium przefiltrowano przez wyprażone filtry Whatman GF/F (**publikacja 1, publikacja 2, publikacja 4**). Zebrany materiał przechowywano w temperaturze -20°C. Podczas próbkowania zostały wykonane również pomiary *in situ* wody. Zmierzono zasolenie [PSU], pH wody, temperaturę wody [°C] oraz widoczność w kolumnie wody przy użyciu Secchiego disk**.**

Osobniki *Eriocheir sinensis* (publikacja 3) zostały pobrane w październiku 2019 roku w Zalewie Wiślanym w rejonie Świętego Kamienia, który znajduje się pomiędzy Tolkmickiem, a Fromborkiem – rejon A (Rys.1). Kraby zostały zebrane z żaków, wystawionych przez lokalnych rybaków przy współpracy z st. Inspektorem Romualdem Jachimowiczem z Okręgowego Inspektoratu Rybołówstwa we Fromborku oraz prof. dr hab. Moniką Normant-Sarembą. Bezpośrednio po wyjęciu z żaków kraby zamrażano w temperaturze -20°C w celu dalszych analiz. W październiku 2019 roku zostały pobrane dodatkowo kraby z łowisk w Zalewie Wiślanym w rejonie południowo-wschodniej części Zalewu Wiślanego, pomiędzy Tolkmickiem (oznaczone jako A), a granicą z Rosją (oznaczone jako C) (Rys.1). Trzecie miejsce zbioru (oznaczone jako B) znajdowało się pomiędzy Fromborkiem, a granicą z Rosją. Dodatkowo przeanalizowano materiał osobników *E.sinensis* pobranych w października 2011, 2017 oraz 2020 roku odłowionego w rejonie łowiska Św. Kamień – rejon A (Rys.1).

Kolejnym krokiem było oznaczenie parametrów biometrycznych organizmów oraz płci badanych osobników (**publikacja 1 – 4**). W tym celu **dokonano sekcji na organizmach**, gdzie w przypadku małży ciałko miękkie zostało oddzielone od muszli (**publikacja 1, publikacja 2**). W przypadku krabika amerykańskiego wyizolowano ciałko miękkie, odnóża oraz karapaks (**publikacja 4**). Krab wełnistoszczypcy jest największym przedstawicielem bezkręgowców bałtyckich (Normant i in., 2000; Czerniejewski i Bełdowska, 2003), dlatego do określenia dystrybucji rtęci i jej form w poszczególnych narządach wypreparowano tkanki miękkie (mięśnie odnóży krocznych - FWL, mięśnie szczypcowe – FC; gonady - GO, wątrobo-trzustka – HP, skrzela – GI) oraz egzoszkielet (dactylus, karapaks, merus, szczypce i mittens – wyrostki kutykularne) (**publikacja 3**). W sumie **do dalszych analiz chemicznych przygotowano 1546 tkanek miękkich oraz 1546 próbek egzoszkieletu**, 18 próbek osadów powierzchniowych, 27 sączków z SPM i 27 sączków z planktonem.

Analizę rtęci całkowitej (HgTOT) oraz frakcji rtęci przeprowadzono na analizatorze DMA 80, metodą desorpcji termicznej przy użyciu automatycznego analizatora rtęci DMA (Milestone, Włochy) (**publikacja 1 – 4**). Zastosowanie 5-etapowej metody desorpcji termicznej pozwoliło na rozdzielenie pięciu grup związków o podobnych właściwościach:

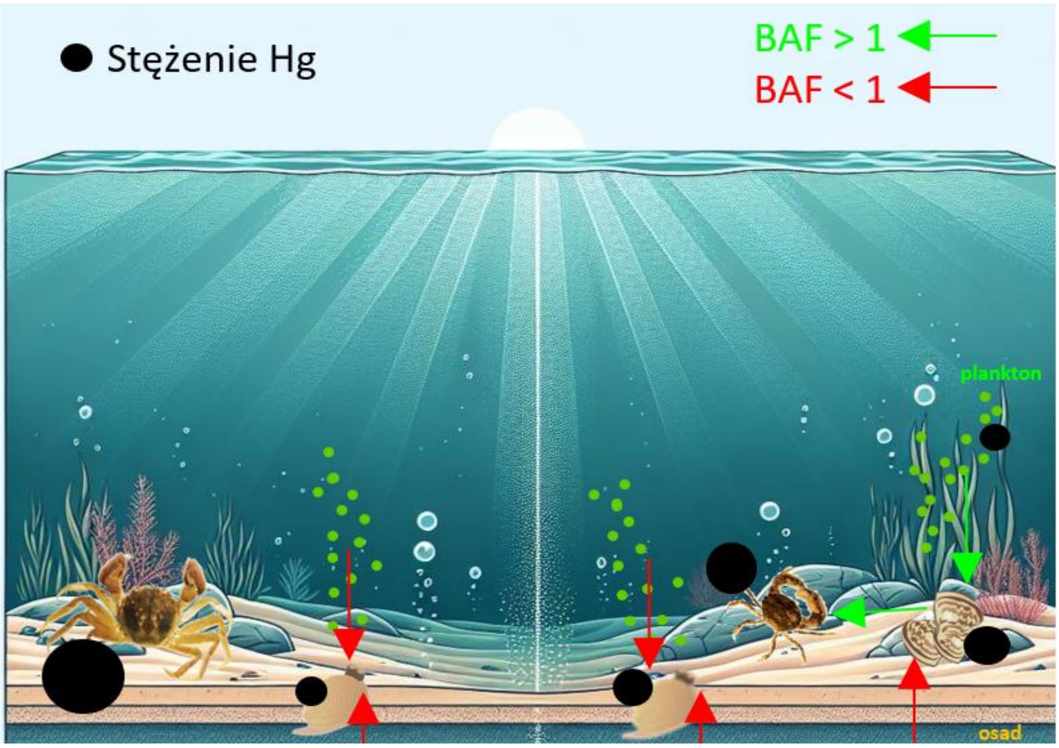
- **HgF1** – (Hg-L1, $Hg_{labile1a}$, $Hg_{adsorbed}$.) - to najbardziej labilne związki Hg ($HgCl_2$, $HgBr_2$, HgI_2, $Hg(CN)_2$). Tworzą one głównie luźne związki powierzchniowe z podłożem i mogą być częściowo wchłaniane z przewodu pokarmowego, co jest związane z rozpuszczalnością tych związków w wodzie (Broussard i in., 2002; Aksentor i Sattarov, 2020).

- **HgF2** – (Hg-L1, $Hg_{labile1b}$, $Hg_{absorbed1}$) - związki Hg połączone z substancjami humusowymi, MeHg, a także inne związki Hg utworzone z ligandami o silnych właściwościach kompleksujących ($(CH_3COO)_2Hg$, $Hg(NO_3)_2 \cdot H_2O$, $Hg(SCN)_2$, $Hg(ClO_4)_2 \cdot xH_2O$). Te związki Hg są łatwo transportowane wewnątrz komórek organizmu - są wchłaniane pełniej niż sole nieorganiczne, ponieważ są bardziej rozpuszczalne w lipidach i wiążą się z grupami sulfhydrylowymi (Broussard i in., 2002). Ulegają również bioakumulacji i biomagnifikacji w łańcuchu troficznym (Jędruch i in., 2018; Cavoura i in., 2019).
- **HgF3** – (Hg-S1, HgS) - nierozpuszczalny HgS, będący jedną z najbardziej stabilnych form Hg i najmniej toksyczny dla organizmów. Może on jednak zostać przekształcony w formę organiczną przy udziale bakterii redukujących siarczany i przez grzyby (Lugo i in., 2020).
- **HgF4** – (Hg-L2, $Hg_{labile2}$, $Hg_{adsorbed2}$) głównie związki półlotne: HgO, $HgSO_4$ i HgF_2, potencjalnie dostępne dla organizmów (Aksentor i Sattarov, 2020).
- **HgF5** – (Hg-S2, $Hg_{residual}$) formy, które były niedostępne dla środowiska, związane z frakcją resztkową osadzoną w minerałach.

W **publikacji 1 i 2** wykorzystano nomenklaturę **$Hg_{labilna}$** i **$Hg_{stabilna}$** zgodnie z metodyką opisaną w pracy Bełdowska i in., (2018) oraz Jędruch i in., (2018). W czasie trwania prac laboratoryjnych nastąpiła również modyfikacja metody frakcjonowania rtęci, wskutek której nomenklatura dla poszczególnych frakcji dla różnych matryc została znormalizowana do jednej: **HgF1, Hg2, HgF3, HgF4 i HgF5** (Wilman i in., 2023) i została zastosowana w **publikacji 3 i 4**.

Określono również wilgotność [W], zawartość materii organicznej [LOI] i udział najdrobniejszej frakcji w próbkach osadów powierzchniowych [FSF] zgodnie z metodą opisaną w artykule Saniewska i in., (2014).

Wyznaczenie współczynnika akumulacji rtęci (BAF i BSAF) pozwoliło na realizację celu pierwszego: **<u>Określenie głównych dróg wnikania rtęci do organizmu w obcych gatunkach małży: *Rangia cuneata* i *Dreissena polymorpha* oraz krabów: *Rhithropanopeus harrisii* i *Eriocheir sinensis* z Zalewu Wiślanego jako czynnika determinującego jej poziom stężenia i udział frakcji</u>**. W **publikacji 1 oraz 2** wyznaczono współczynniki BAF na drodze ciałko miękkie - plankton, ciałko miękkie - zawiesina –oraz BSAF: ciałko miękkie - osad. W przypadku małży *D.polymorpha* **(publikacja 2)** współczynnik bioakumulacji wskazuje na to, że plankton jest głównym wektorem Hg do organizmu małży (Rys.2). Niniejsze wyniki wykazały, że frakcją rtęci, która w największym stopniu była akumulowana wraz z pokarmem była HgF1, czyli halogenowe związki rtęci stanowiące wysoki udział procentowy w planktonie czy mikroglonach. *Dreissena polymorpha* jest obligatoryjnym filtratorem o zdolności do filtrowania dużej objętości wody, pozyskując niezbędne składniki odżywcze wraz z planktonem czy detrytusem (Bełdowska i in., 2018, Wilman i in., 2023). W konsekwencji więcej labilnych związków rtęci jest dostarczanych do organizmu i podlega bioakumulacji. W przypadku współczynnika BSAF, okazało się, że osad nie jest źródłem Hg do organizmu małży. Resuspensja Hg związanej z drobnymi cząstkami osadów i jej akumulacja do organizmu w wyniku filtracji wody może mieć tutaj najmniejsze znaczenie, gdyż racicznica zmienna, jako nieliczna z małży słodkowodnych przytwierdza się do dna przy pomocy bisiora, w związku z tym preferuje twarde podłoże o charakterze osadów gruboziarnistych, które charakteryzuje się niskim stężeniem Hg (Rys.2).

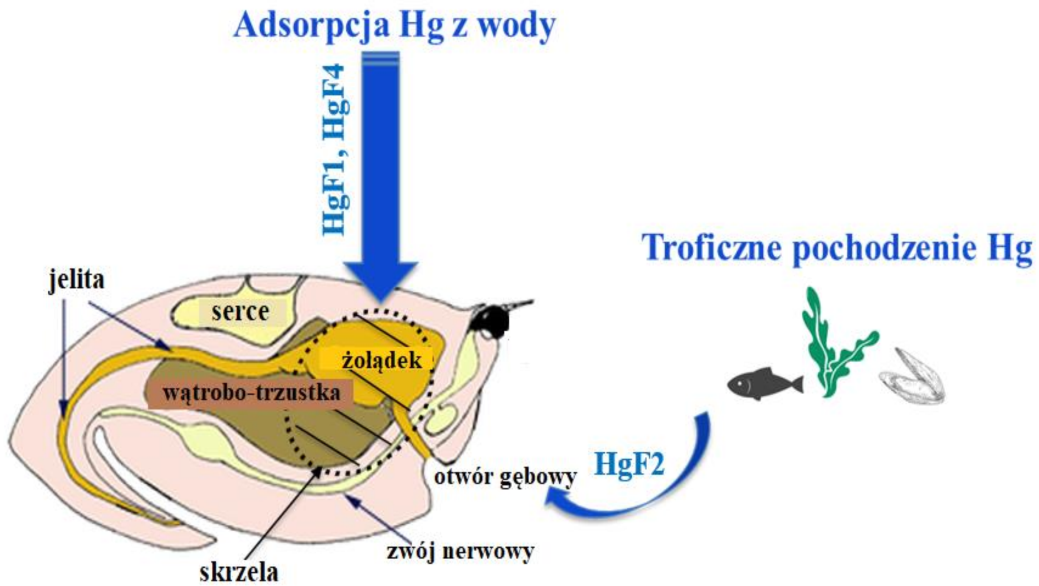


Rysunek 2 Stężenie ksenobiotyku Hg oraz współczynnik bioakumulacji (BAF) w gatunkach obcych krabów i małży z Zalewu Wiślanego

Wyliczony współczynniki bioakumulacji dla organizmu *R. cuneata* **(publikacja 1)** wykazały na ograniczoną akumulację Hg w ciele *R. cuneata* z zawiesiny morskiej i planktonu (rys.2). *R. cuneata* nie jest obligatoryjnym filtratorem. Oprócz filtrowania wody z zawieszonych cząstek, żywi się materią organiczną i detrytusem z osadów. Potwierdzają to badania przewodu pokarmowego tego gatunku małży wykazywały znaczny udział detrytusu czy piasku (80%) w porównaniu do mikroglonów czy szczątek roślin naczyniowych (Darnell, 1998). W związku z tym istotny jest typ osadu w rejonie habitatu małży, w którym może się zakopywać oraz udział poszczególnych form rtęci w podłożu. Analiza stężenia Hg i jej frakcji w *R. cuneata* wykazała zmienność przestrzenną w Zalewie Wiślanym. Weryfikacja siedliska (osadu) pod względem stężenia Hg i zawartości materii organicznej (MO) oraz udziału najdrobniejszej frakcji (FSF) wykazała odwrotną tendencję: na obszarach, gdzie stężenia Hg w *R. cuneata* były najwyższe, MO i FSF były w najmniejszym stopniu wzbogacone w HgTOT. Z kolei na obszarach, gdzie oznaczono najniższe stężenia Hg w *R. cuneata*, materia organiczna i osady były najbardziej zanieczyszczone rtęcią. Wyższe stężenia HgTOT w ciałku małży oznaczono w obszarach położonych najbliżej brzegu (ZAT-K, 43L i 40L (Rys.1)), gdzie obserwowano podwyższone stężenie labilnej rtęci ($HgSO_4$ i HgO (HgF4)) w osadach. Analizy statystyczne wykazały, że źródłem zakumulowanej Hg był w tym przypadku osad, gdzie Hg występowała głównie w labilnych, bardziej biodostępnych dla organizmu formach. Na stacjach zlokalizowanych w ujściu rzeki Elbląg (SUC-R2) oraz w centralnej części Zalewu Wiślanego (IW100, 29L i P14A), na głębszych stacjach małże akumulowały rtęć z osadu mniej efektywnie, ponieważ Hg występowała tu głównie w stabilnej formie (**publikacja 1**).

Współczynnik BAF oszacowany dla *R. harrisii* z Zalewu Wiślanego **(publikacja 4)** wskazuję na bioakumulację Hg z pokarmu (Rys.2). Krabik amerykański swój klaser występowania utworzył m.in. w klaserze występowania małży: *Dreissena polymorpha*. Prawdopodobnie wykorzystuje ten obszar jako refugia (miejsce schronu w muszlach plus baza pokarmowa). Stąd prawdopodobnie wyższy udział rtęci pochodzenia troficznego (zwierzęcego) znajduje swoje odzwierciedlenie w udziale procentowym frakcji Hg w krabikach z Zalewu Wiślanego, w przeciwieństwie do Zatoki Puckiej, gdzie BAF < 1 (Wilman i in., 2019), a pokarmem krabika był głównie pokarm roślinny stanowiący pond 50% diety kraba (Hegele-Drywa i Normant, 2009).

Ze względu na to, iż krab wełnistoszczypcy jest najbardziej mobilnym przedstawicielem zoobentosu w badanym rejonie, o typowo wodno-lądowym trybie życia**,** trudno jest wykazać bezpośrednie źródło rtęci do organizmu *E. sinensis* **(publikacja 3).** Główną drogą wnikania metali do organizmu kraba są układ pokarmowy i skrzela. Podczas narażenia na toksyczne metale, skrzela i wątrobo-trzustka działają głównie jako przejściowy magazyn tkankowy dla zakumulowanych metali. W krabie wełnistoszczypcym wyniki badań potwierdziły na transfer Hg ze skrzeli i wątrobo-trzustki do organizmu kraba. Halogenowe i semi-labilne formy rtęci (HgF1, HgF4) były adsorbowane na powierzchni skrzeli z wody, natomiast w wątrobo-trzustce oznaczono frakcję rtęci głównie pochodzenia troficznego (HgF2) (Rys.3).

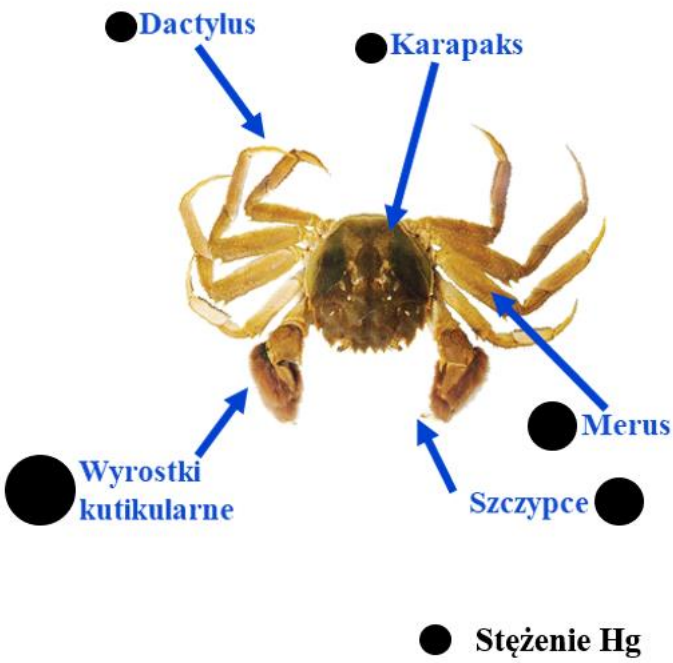


Rysunek 3 Drogi wnikania rtęci do organizmu kraba wełnistoszczypcego.

Realizacja celu pierwszego oraz studia literaturowe dotyczące biochemicznych właściwości rtęci i związków biorących udział w biomineralizacji muszli/pancerza, pozwoliły na realizację celu drugiego pracy: **Rozpoznanie dystrybucji rtęci całkowitej oraz jej labilnych i stabilnych form, w organizmie krabów i małży**. Różnice w preferencjach pokarmowych i forma Hg dostarczana wraz z pożywieniem w badanych gatunkach małży miała odzwierciedlenie w bioakumulacji rtęci i jej frakcji w organizmie (**publikacja 1 i 2**). Stężenie HgTOT w ciałku *D. polymorpha* było istotnie statystycznie wyższe od stężenia ksenobiotyku w organizmie *R. cuneata*. Udział halogenowych form HgF1 w ciałku *D. polymorpha* stanowił 40% udziału w HgTOT natomiast w ciałku *R. cuneata* 5%. W przypadku frakcji HgF2 obserwowano odwrotną tendencję, gdzie w organizmie racicznicy HgF2 stanowiła 55% udziału, natomiast w *R. cuneata* 93%. W przypadku muszli małży oznaczono również istotnie statystycznie większe stężenie Hg w muszli *D. polymorpha* niż *w R. cuneata*. Chociaż w składzie chemicznym muszli *R. cuneata* i *D. polymorpha* w Zalewie Wiślanym największy udział miały białka, średnio 40%, węglowodany 30%, lipidy i materia mineralna łącznie 30% (Dąbrowska i in., 2017) to stężenie Hg było na niskim poziomie, najprawdopodobniej ze względu na wysycenie muszli węglanem wapnia, który jest wykorzystywany w procesie kalcyfikacji - przyrostu muszli. Co najbardziej było widoczne w gatunku *R. cuneata*, który posiada masywną muszlę, w porównaniu do niewielkiej i cienkiej muszli *D. polymorpha*. Jednak w przypadku udziału procentowego frakcji Hg ich udział procentowy był na podobnym poziomie w obu gatunkach. W muszlach małży oznaczono wysoki udział frakcji semi-labilnych związków rtęci HgF4 oraz stabilnej frakcji HgS w przeciwieństwie do ciałka miękkiego. Badania zespołu Immel i in., (2016) wykazały, że jest to najprawdopodobniej spowodowane tym, że matryca ta, choć ogólnie słabo glikozylowana, zawiera zestaw potencjalnie wiążących wapń białek m.in. aminokwasy siarkowe takie jak cysteina czy metionina, zawierające grupę tiolową ^{-}SH, z którą rtęć może tworzyć stabilny kompleks HgS i zestaw kwaśnych białek siarczanowych, gdzie anion SO_4^{2-} może wiązać się z kationem Hg^{2+}.

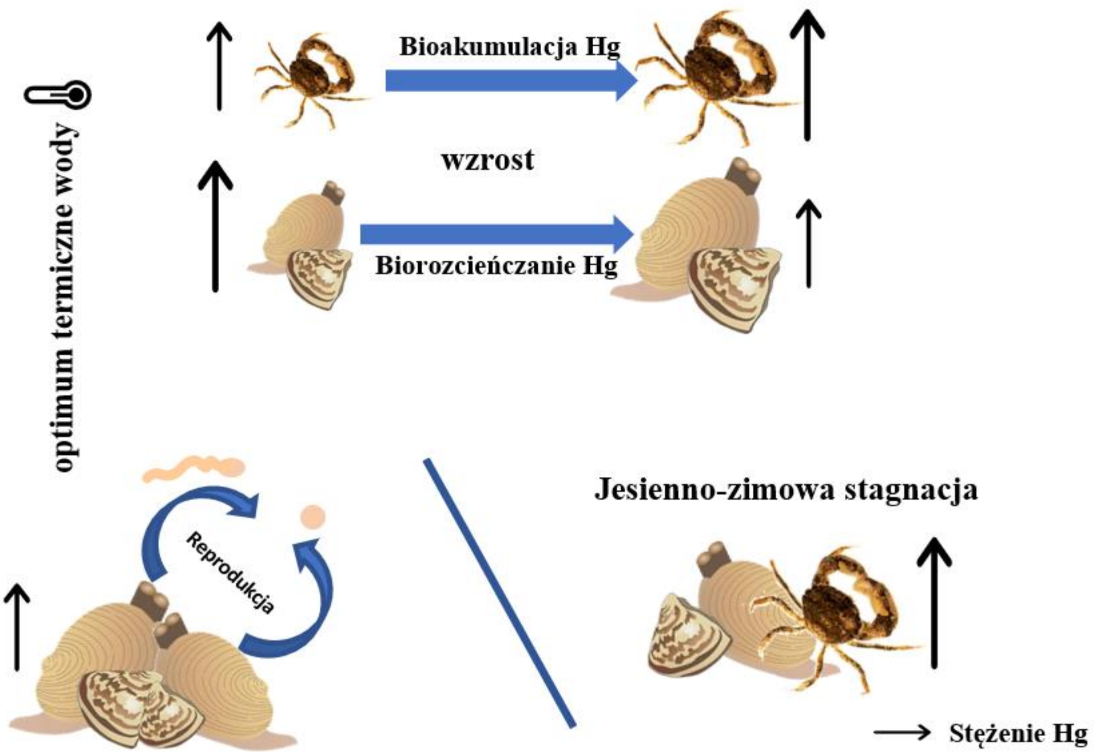
Biorąc pod uwagę krabika amerykańskiego, mediana stężenia rtęci całkowitej różniła się istotnie statystycznie w poszczególnych częściach jego organizmu **(publikacja 4)**. Najwyższe wartości mediany stężenia HgTOT stwierdzono w ciałku miękkim (wnętrznościach: gonady, skrzela, serce, układ pokarmowy). Prawie dwukrotnie mniejszą medianę stężenia HgTOT uzyskano w mięśniach odnóżach krocznych. W karapaksie mediana stężenia HgTOT była prawie czterokrotnie niższa w porównaniu do mięśni odnóży krocznych. Najwyższy procent frakcji rtęci oznaczono w przypadku HgF2 we wszystkich tkankach badanych organizmów. Wskazuje to na proces bioakumulacji tej formy rtęci w organizmie krabika amerykańskiego. Może to być związane z faktem, że organiczne formy Hg (należąca do grupy związków frakcji HgF2) mogą wiązać się z grupami cysteinowymi w organizmie (Gupta i in., 2018). Biorąc pod uwagę frakcję HgF1, to jej najwyższy udział (10%) oznaczono w przypadku ciała miękkiego potwierdzając tym samym akumulację HgF1 wraz z konsumpcją mikroglonów przez młode kraby. Stabilną formę rtęci HgS oznaczono tylko w karapaksie krabika i była to frakcja rtęci bezpośrednio skorelowana z HgF1. Może to wskazywać, podobnie jak zaobserwowano u małży, że nieorganiczne formy rtęci są redystrybuowane do pancerza wraz z innymi związkami nieorganicznymi, uczestniczącymi w procesie biomineralizacji w trakcie wzrostu pancerza (Schofield i in., 2003).

Biorąc pod uwagę kraba wełnistoszczypcego, analiza skupień skategoryzowała wyniki stężenia HgTOT w tkankach miękkich *E. sinensis* w dwóch grupach: (i) o najwyższej wartości stężenia rtęci całkowitej - mięśnie kraba oraz (ii) narządy wewnętrzne: skrzela, wątrobo-trzustka i gonady, które charakteryzowały się stężeniem Hg o rząd wielkości niższym w porównaniu do mięśni **(publikacja 3)**. Przyczyną, wpływającą na to zróżnicowanie jest skład biochemiczny poszczególnych tkanek. Mięśnie kraba *E. sinensis* są strukturami typowo białkowymi, o małej zawartości tłuszczu w porównaniu do narządów, które stanowią grupę targetową toksyczności Hg. Obecność wielu grup funkcyjnych w mięśniach i narządach wewnętrznych wpływała na to, iż rtęć występowała tam w 100% w formach labilnych, które są bardziej biodostępne i toksyczne dla organizmu, tworząc biologiczne ligandy Hg bądź są lepiej rozpuszczalne w tłuszczach. W przypadku ezgoszkieletu wyznaczono trzy grupy, różniące się istotnie statystycznie stężeniem rtęci: dactylus i karapaks < merus i szczypce < wyrostki kutikularne (Rys.4). Najniższe stężenie rtęci w dactylus w głównym stopniu związane było z innym typem mineralizacji pancerza i jego funkcją. Schofield i in., (2003) wykazali, że w miarę spadku poziomu stężenia wapnia, to chlor i brom zajmują jego miejsce jako dominujący pierwiastek nieorganiczny właśnie w dactylus, co potwierdzać mogą wyniki frakcjonowania rtęci, gdyż udział halogenowych form Hg był tam najwyższy niż w pozostałych elementach egzoszkieletu. Ze względu na to, iż jest to najbardziej labilna forma Hg i ma mniejsze powinowactwo do grup targetowych toksyczności rtęci, słabiej ulega bioakumulacji, co skutkowało najniższym stężeniem Hg w porównaniu do pozostałych elementów egzoszkieletu, gdzie obok węglanu wapnia w procesie mineralizacji pancerza, organizm ten wykorzystuje białko N-chitynowe.



Rysunek 4 Stężenie Hg w badanych częściach egzoszkieletu kraba wełnistoszczypcego (*Eriocheir sinensis*) z Zalewu Wiślanego

Oprócz biochemicznych właściwości związków rtęci, które determinują obciążenie organizmu neurotoksyną Hg, ich stężenie może być również determinowane czynnikami środowiskowymi oraz procesami fizjologicznymi zwierząt. Dlatego kolejnym celem dysertacji było: **Określenie czynników wpływających na zmienność obciążenia organizmu krabów i małży neurotoksyną rtęci**. Płeć nie determinowała poziomu stężenia Hg w obu gatunkach małży oraz krabiku amerykańskim (**publikacja 1,2,4**). Jedynie w przypadku *R. cuneata* formy juwenilne charakteryzowały się wyższym stężeniem Hg w stosunku do osobników dorosłych (**publikacja 1**). Głównymi czynnikami determinującymi procesy przyswajania rtęci były temperatura wody oraz parametry biometryczne (**publikacja 1 i 2**) (Rys.5).



Rysunek 5 Wpływ parametrów biometrycznych oraz temperatury wody na koncentracje ksenobiotyku Hg w organizmie gatunków obcych małży i kraba z Zalewu Wiślanego

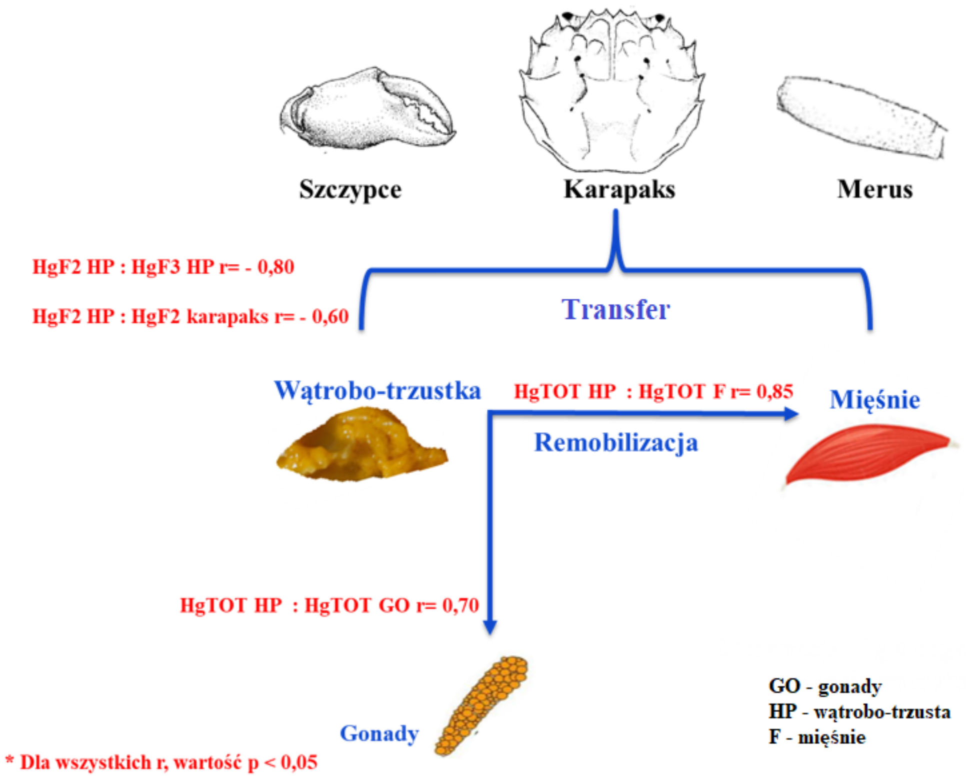
Latem spośród wszystkich sezonów pomiarowych obserwowano najniższe stężenie rtęci w małżach. Był to jedyny sezon, gdzie zostało spełnione optimum termiczne do rozrodu, w konsekwencji małże mogły oczyścić się z ksenobiotyku w wyniku reprodukcji. Dodatkowo, w cieplejszej wodzie małże wykazują przyspieszony metabolizm oraz zwiększone zużycie energii na wzrost i respirację (**publikacja 1 i 2**). Bardzo dobrze było to widoczne w gatunku *D. polymorpha* (**publikacja 2**). Wtedy współczynnik bioakumulacji dla HgF1 z uwzględnieniem planktonu był najwyższy spośród wszystkich analizowanych. W tym sezonie procentowy udział HgF1 w HgTOT w poszczególnych klasach wielkości podwoił się: z 34% do 68%. Natomiast w okresie wiosenno-jesienno-zimowej stagnacji, kiedy temperatura wody jest niższa, stężenie Hg w ciałku małży było wyższe (**publikacja 1 i 2**). Prawdopodobnie wynikało to ze spowolnienia metabolizmu oraz kumulacji niekorzystnych metabolitów, w tym toksycznych form rtęci, w organizmie. W sezonie letnim, w wyniku zwiększonego wydatku energetycznego związanego z procesami reprodukcji i wzrostu, małże charakteryzowały się pogorszoną kondycją fizjologiczną. Współczynnik kondycji był odwrotnie proporcjonalnie skorelowany zarówno z rozmiarem osobników, jak i ze stężeniem Hg, co może wskazywać na zachodzący w tym okresie proces detoksykacji organizmu z rtęci w związku z intensywnymi procesami anabolicznymi (wzrostem i rozrodem) (**publikacja 1 i 2**).

W krabiku amerykańskim (**publikacja 4**) obserwowano odwrotną tendencję w porównaniu do małży (Rys.5). Biorąc pod uwagę zmienność sezonową, najniższe stężenie Hg oznaczono w próbkach z wiosny, w której osobniki charakteryzowały najwyższym współczynnikiem kondycji. Odwrotną zależność obserwowano w sezonie letnim i jesiennym, gdzie kraby w gorszej kondycji charakteryzowały się wyższym stężeniem HgTOT. Prawdopodobnie w fazie wzrostu krabów mogą zachodzić inne procesy determinujące akumulację Hg w tkankach. W sezonie letnim obserwowano bioakumulację rtęci w mięśniach odnóży krocznych w kolejnych klasach wielkości, która była głównie związana z frakcją rtęci pochodzenia troficznego. Przy jednoczesnym biorozcieńczaniu Hg w puszcze pancerza oraz wzroście udziału form halogenowych i siarczku Hg w kolejnych klasach wielkości. Prezentowane wyniki potwierdzają, że w okresie letnim ich tempo wzrostu jest większe, gdzie jednocześnie w procesie wzrostu (linienia), dochodzi do biorozcieńczenia Hg wskutek tworzenia nowego, większego egzoszkieletu. W sezonie jesiennym obserwowano odwrotną tendencję: spadek stężenia Hg w mięśniach odnóży krocznych w kolejnych klasach wielkości i bioakumulację Hg w karapaksie, a masa mięśni w okresie jesieni była średnio 4 razy większa niż w osobnikach z sezonu letniego. Może to wskazywać na to, iż przygotowując się do zimowej stagnacji, przybierały na masie i obserwowano rozcieńczanie Hg w masie mięśniowej odnóży. Dwukrotnie wyższe stężenie Hg w ciałku miękkim jesienią w porównaniu do lata, może wskazywać na to, iż organizm w okresie wzmożonej kumulacji Hg w organizmie częściowo efektywnie redystrybuuje związki Hg do pancerza. Stąd obserwowano wzrost stężenia Hg w kolejnych klasach wielkości w karapaksie krabika amerykańskiego jesienią.

Parametry biometryczne takie jak wielkość, masa krabów oraz kondycja (K index) nie determinowały stężenia rtęci w całych osobnikach *E. sinensis* (**publikacja 3**). Jednak w niniejszych badaniach zaobserwowano różnice w stężeniu ksenobiotyku, które związane były z dymorfizmem płciowym krabów. Samce charakteryzowały się większym obciążeniem wątrobo-trzustki ksenobiotykiem Hg, w tym rtęci stabilnej. Wzrost udziału form stabilnych w samcach kraba, może wskazywać na to, iż większe, starsze samce efektywniej radzą sobie z detoksykacją organizmu z Hg, co może wynikać z ich dłuższej ekspozycji na Hg i rozwiniętych mechanizmów adaptacyjnych. Clifford et al. (2005) w swoich badaniach obserwowali spadek stężenia metylortęci w wątrobo-trzustce *E. sinensis* wraz z wielkością osobnika, wskazując na efektywniejszą demetylację Hg wraz z wiekiem krabów u większych samców. Samce *Eriocheir sinensis* wykazywały również wyższe stężenia rtęci w gonadach. Niewielki spermatofor, zbudowany jest w głównie z białek, co odróżnia go od bogatych w lipidy i białka oocytów samic. Białka stanowią grupę docelową dla toksycznych form rtęci – zdolnej do tworzenia wiązań z biologicznymi ligandami (Shao i in., 2018). Zatem eliminacja części rtęci poprzez uwalnianie gamet mogłaby stanowić efektywny mechanizm detoksykacji, szczególnie u samców. Jednakże, ze względu na fakt, iż *E. sinensis* zazwyczaj ginie po zakończeniu rozrodu (Veilleux i Lafontaine, 2007; Dittel i Epifanio, 2009), potencjał tego mechanizmu detoksykacyjnego może być ograniczony do ostatniego etapu cyklu życiowego.

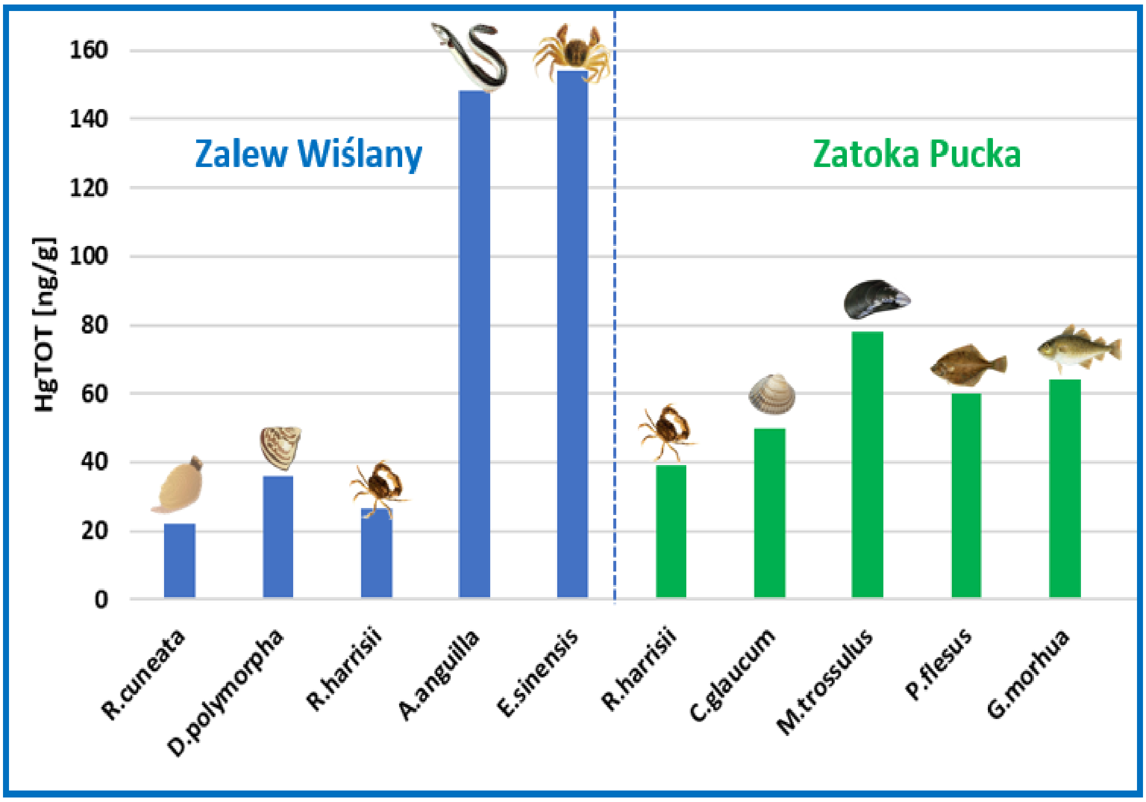
Problem dotyczący dystrybucji rtęci w narządach oraz jej eliminacji jest dość dobrze poznany w literaturze światowej jedynie w organizmach z wyższych poziomów troficznych. Badania w organizmach z niższych poziomów np. u bezkręgowców w tym małżach i krabach, skupiają się głównie na określeniu stężenia rtęci w tkane miękkiej oraz wpływie sezonowości i wielkości organizmów na poziom stężenia rtęci. Mechanizmy transferu rtęci, zarówno z miękkich tkanek do muszli, jak i z narządów wewnętrznych do pancerza dla badanych form rtęci są nieznane. Dlatego w realizacji kolejnego celu badawczego podjęto próbę: **Poznania roli egzoszkieletu w procesie eliminacji rtęci w bezkręgowcach na przykładzie kraba i małży**. Omawiane w publikacji 1 i 2 procesy detoksykacji małży z Hg wydają się być wydajne, przez co muszla małży charakteryzowała się niskim stężeniem Hg. Parametry biometryczne pośrednio wpływały na stężenie Hg w muszli. Jednak biorąc pod uwagę fakt, że masa muszli stanowi ponad 90% masy organizmu małży zaobserwowano inną tendencję (**publikacja 1 i 2**). W przypadku *D. polymorpha* niezależnie od sezonu masa zakumulowanej rtęci w masie muszli była wyższa niż w całym ciałku, niezależnie od formy w jakiej występowała, co również potwierdza współczynnik obciążenia organizmu rtęcią. Co może wskazywać na to, że transferowanie Hg z ciałka do muszli w tym gatunku małża jest istotnym procesem jej eliminacji i czasowej dezaktywacji, chroniąc w ten sposób swój organizm przed bioakumulacją ksenobiotyku (**publikacja 2**). W przypadku *R. cuneata* w okresie wiosenno-jesienno-zimowej stagnacji małży nie obserwowano takiej tendencji. Zależność tę obserwowano jedynie latem, co może świadczyć o tym, że obok reprodukcji w tym gatunku małża, transferowanie Hg z ciałka do muszli w procesie wzrostu w sezonie wegetacyjny jest również istotną drogą eliminacji rtęci z organizmu (**publikacja 1**).

W krabiku amerykańskim (**publikacja 4**) masa zakumulowanej rtęci całkowitej oraz jej labilnych i stabilnych form była większa w karapaksie, niż w tkance miękkiej i odnóżach krocznych. Było to szczególnie widoczne podczas okresu przygotowawczego do zimowej stagnacji, kiedy to zaobserwowano transfer Hg z ciała do pancerza. W związku z tym masa nagromadzonej rtęci w pancerzu i współczynnik obciążenia był również najwyższy w tym okresie. Kraby te w ciągu całego swojego życia przechodzą kilkukrotne linienie, co umożliwia im również pozbywać się pewnej puli zakumulowanych zanieczyszczeń z organizmu w procesie wzrostu. W przypadku kraba wełnistoszczypcego (**publikacja 3**) karapaks stanowi największy element pancerza i jest zrzucany w trakcie wzrostu. Niskie stężenie rtęci w tej strukturze prawdopodobnie wynikało z efektu biorozcieńczania metalu, zachodzącego podczas odbudowy pancerza po ostatnim linieniu. Potwierdza to odwrotna korelacja między stężeniem Hg a szerokością karapaksu. Inaczej niż w przypadku merusa – mniejszych elementów odnóży, które również są odrzucane podczas linienia – efekt biorozcieńczania był wyraźnie widoczny także w szczypcach. Szczególnie dotyczyło to samców, które charakteryzują się większymi i masywniejszymi szczypcami niż samice. Interpretacja najwyższego stężenia Hg w wyrostkach kutikularnych - mitten *E. sinensis* jest trudna ze względu na brak badań np. dotyczących chemizmu czy ich roli w organizmie, poza funkcją ozdobną. Potencjalnie, mittens u kraba mogą stanowić miejsca akumulacji i czasowej dezaktywacji Hg, analogicznie jak sierść u ssaków czy pióra u ptaków, ponieważ wyrostki kutikularne są zrzucane wraz z pancerzem podczas linienia. Na drodze pierzenia u ptaków czy wymiany sierści u ssaków w miejscu tworzenia się nowych wytworów skórnych, powstaje nowe centrum akumulacji Hg, która następnie jest wbudowywana w te struktury. Stąd wbudowywanie Hg w wyrostki przez kraba może mieć znaczenie w procesie jej eliminacji z organizmu. W związku z tym, że proces linienia w ciągu życia kraba wełnistoszczypcego, występuję częściej niż rozród na znaczeniu może zyskiwać transfer Hg z ciała do pancerza (Rys.6). Analizy statystyczne wykazały, że rtęć redystrybuowana była do merus, karapaksu i szczypiec. W wyniku tego, krab ten może eliminować pewną pulę zanieczyszczeń w procesie linienia, czyli wzrostu obok procesu detoksykacji z labilnych bardziej toksycznych form do stabilnych, HgS w wątrobo-trzustce (Rys.6).



Rysunek 6 Transfer rtęci i jej labilnej frakcji HgF2 w organizmie kraba *Eriocheir sinensis*

Pomimo licznych prac na temat biologii i ekologii małży: *Dreissena polymorph* i *Rangia cuneata* oraz krabów: *Rithropanopeus harrisii* i *Eriocheir sinensis*, brakuje literatury na temat roli tych gatunków, jako nowych komponentów w sieci troficznej w regionie południowego Bałtyku. Brakowało również doniesień naukowych na temat oznaczania poziomu stężenie rtęci w badanych małżach i krabach w tym obszarze. Organizmy te stopniowo poszerzają swoje zasięgi występowania w południowym Bałtyku. W estuariach Bałtyku stają się one ważnym elementem diety organizmów z wyższych poziomów sieci troficznej, zwłaszcza ptaków czy ryb żerujących przy dnie (flądra, węgorz, leszcz, płoć), poławianych komercyjnie i konsumowanych przez ludzi. Stąd ważne jest określenie poziomu stężenia rtęci w gatunkach nierodzimych jako potencjalnie nowego ogniwa (źródła Hg) w ekosystemie. Dlatego ostatnim celem było: **<u>Opisanie roli gatunków obcych w ekosystemie południowego Bałtyku w odniesieniu do trofodynamiki rtęci w badanym rejonie</u>.** Stężenie rtęci całkowitej w krabiku amerykańskim i małżach było na niskim poziomie, a wartość ta była znacznie niższa niż maksymalne dopuszczalne stężenie Hg w rybach i owocach morza przeznaczonych do spożycia przez ludzi (**publikacja 1,2 i 4**). Mediana stężenia HgTOT w badanych gatunkach małży z Zalewu Wiślanego była niższa w porównaniu do małży z Zatoki Puckiej: *Limecola baltica* czy *Mytillus trossulus* (Rys.7) (Jędruch i in., 2018; 2019). Również stężenie Hg w krabiku amerykańskim z Zalewu Wiślanego było niższe w porównaniu do badań prowadzonych na tym samym gatunku w Zatoce Puckiej (Wilman i in., 2019) (Rys.7). Ponadto, wyniki uzyskane w niniejszej pracy wykazały, że więcej rtęci małże i krabik amerykański akumulują odpowiednio w muszli i pancerzu, niż w ciałku. Może mieć to wpływ na transfer toksycznej rtęci w sieci troficznej, ponieważ zjadane jest głównie ciałko małży np. przez kraby wełnistoszczypce. Ptaki lub ryby bentosożerne, które połykają organizmy w całości, częściowo wydalają elementy egzoszkieletu czy muszli podczas procesu defekacji lub z wyplówkami. W konsekwencji mniejszy ładunek Hg może być włączony do obiegu bądź biomagnifikowany w sieci troficznej, w rejonie, gdzie organizmy te stają się istotnym ogniwem w łańcuchu pokarmowym. Stężenie Hg w osadzie i zawiesinie z Zalewu Wiślanego było wyższe niż w Zatoce Puckiej, co wzmacnia znaczenie detoksykacji Hg w organizmach badanych gatunków obcych małży i krabika amerykańskiego (Jędruch i in., 2017; Kwasigroch i in., 2021).



Rysunek 7 Porównanie stężenia Hg w gatunkach obcych do gatunków rodzimych w rejonie południowego Bałtyku

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Badania izotopowe $\delta^{13}C$, $\delta^{15}N$ w krabie wełnistoszczypcym, ze względu na podobną dietę do ryb, wykazują podobny poziom troficzny tych organizmów (Li i in., 2023). Stężenie rtęci w mięśniach *E. sinensis* (**publikacja 3**) było wyższe niż w mięśniach ryb planktonożernych (szprot i śledź) oraz w mięśniach drapieżnych ryb (dorsz, węgorz czy stornia) w badanym rejonie (Rys.7) (Polak-Juszczak, 2018). Niższe stężenie rtęci w mięśniach ryb, przy porównywalnej wielkości ciała, może wynikać z efektu biorozcieńczania – ryby budują większą masę mięśniową niż kraby, co prowadzi do rozcieńczenia metalu w tkankach. Mięso kraba zawiera bardzo duże ilości minerałów i aminokwasów i jest wysokoenergetycznym pożywieniem (Chen i in., 2007; Normant i in., 2002), a według opinii publicznej jego mięso jest uważane za smaczniejsze niż mięso innych krabów np. *A. ascatus* (Fladung, 2000). Spożycie 1,4 kg mięsa krabowego *E.sinensis* z Zalewu Wiślanego dostarczyłoby dopuszczalną, tygodniową dawkę ksenobiotyku do organizmu człowiek. Dla porównania, tę samą dawkę Hg dostarczyłoby spożycie 2,6 kg mięśni ryb z południowego Bałtyku. Jednak w rejonie badań, krab wełnistoszczypcy nie jest poławiany komercyjnie i spożywany przez człowieka oraz posiada niewielu naturalnych wrogów, czy konkurentów. Dodatkowo, ze względu na zbyt niskie zasolenie, nie rozradza się w rejonie południowego Bałtyku, gdzie po osiągnięciu dojrzałości płciowej, migruje do zachodniej części Morza Bałtyckiego. Stąd w sieci zależności pokarmowych między gatunkami w badanym ekosystemie może być konsumentem II rzędu, osiągając wyższe stężenie Hg w łańcuchu troficznym w okresie, kiedy występuje w badanym obszarze.

Wyniki uzyskane w ramach niniejszej dysertacji umożliwiły weryfikację postawionych hipotez:

i. *Poziom stężenia rtęci całkowitej oraz jej toksyczność dla organizmów w krabach i małżach są związane z charakterystyką habitatu w obrębie zidentyfikowanego klastra ich występowania oraz ze sposobem ich odżywiania;*

Hipoteza 1 została zweryfikowana częściowo pozytywnie:

- Drogi wnikania rtęci do organizmu krabika amerkańskiego *R. harrisii* i małży *D. polymorpha* były powiązane z niszą ekologiczną zajmowaną przez oba obce gatunki, które tworzyły wspólne skupiska występowania w Zalewie Wiślanym (**publikacja 2 i 4**).
- Wyjątkowa zdolność *Dreissena polymorpha* do filtrowania dużej objętości wody, umożliwia pozyskiwanie dużej ilości substancji odżywczych jak również labilnej rtęci

(**publikacja 2**), dodatkowo jego obecność wpływa na toksyczność Hg akumulowanej w tkankach krabika amerykańskiego (**publikacja 4**).

- W gatunku obcym małży *R. cuneata* **Hipoteza 1** została zweryfikowana negatywnie, co jest najprawdopodobniej związane z występowaniem tego gatunku najkrócej w skali czasu w Zalewie Wiślanym i ciągłą adaptacją w nowym środowisku (**publikacja 1**).
- Gatunki obce występujące w Zalewie Wiślanym (poza krabem wełnistoszczypcym), mimo większego obciążenia osadów rtęcią, wykazywały niższe stężenia Hg w porównaniu do gatunków rodzimych z pobliskiej Zatoki Gdańskiej, gdzie stężenie metalu w osadach było niższe (**publikacja 1, 2 i 4**), co wskazało na negatywne zweryfikowanie **Hipotezy 1.**

ii. *Stan fizjologiczny (pora roku), parametry biometryczne oraz płeć są głównymi czynnikami determinującymi proces przyswajania i eliminacji rtęci w badanych krabach i małżach;*

Odnosząc się do **Hipotezy 2** została ona zweryfikowana częściowo pozytywnie:

- Najefektywniej organizmy oczyszczały się z ksenobiotyku w okresie wegetacyjnym, a proces ten był intensyfikowany ich reprodukcją (**publikacja 1, 2 i 4**).
- Wzrost organizmu małży przyczyniał się do biorozcieńczenia i w konsekwencji spadku stężenia metalu w ciałku miękkim, (**publikacja 1 i 2**).
- W krabiku amerykańskim stwierdzono odwrotną tendencję w stosunku do stężenia Hg w mięśniach, w porównaniu do małży. Obserwowano tu bioakumulację rtęci wraz ze wzrostem krabów, co jest związane ze zmianą behawioru na bardziej drapieżny w starszych osobnikach (**publikacja 4**).
- Aspekt płci w **Hipotezie 2,** został zweryfikowany negatywnie, ponieważ płeć nie determinowała stężenia rtęci całkowitej oraz jej frakcji w badanych organizmach (**publikacja 1-4**).
- Parametry biometryczne nie wpływały na stężenie ksenobiotyku Hg w organizmie kraba wełnistoszczypcego odłowionego w Zalewie Wiślanym, co zweryfikowało negatywnie **Hipotezę 2** w tym gatunku. Prawdopodobnie jest to związane z występowanie w obszarze badań tylko osobników dorosłych, przygotowujących się do migracji w celu rozrodu. Różnice w koncentracji ksenobiotyku Hg w krabach wynikały z cech dymorfizmu płciowego *E. sinensis* (**publikacja 3**).

iii. *Egzoszkielet może stanowić istotne miejsce akumulacji i czasowej dezaktywacji rtęci na drodze transferu: ciałko miękkie – muszla w małżach oraz eliminacji na drodze tkanki miękkie – egzoszkielet w krabie;*

Odnosząc się do **Hipotezy 3** została ona zweryfikowana pozytywnie:

- Masa całkowitej zakumulowanej rtęci jak i jej frakcji w muszlach *R. cuneata* i *D. polymorpha* i pancerzu krabika amerykańskiego była wyższa niż w ciałku, w wyniku transferu metalu w postaci labilnych form i HgS (**publikacja 1, 2 i 4**).
- Dystrybucja Hg w narządach kraba była związana z pochodzeniem troficznym rtęci i jej transferem z wątrobo-trzustki i mięśni do pancerza, natomiast halogenowe i semilabilne formy Hg z procesu filtracji były redystrybuowane do egzoszkieletu (**publikacja 3**).
- Eliminacja Hg z mięśni oraz z wątrobo-trzustki do pancerza była jednym z istotniejszych procesów detoksykacji organizmu kraba. W ten sposób kraby w procesie wzrostu (linienie) mogą usuwać pewną pulę toksycznej Hg, dzięki czemu chronią swój organizm przed neurotoksyną (**publikacja 3,4**). Natomiast muszla w małżach może stanowić miejsce docelowej akumulacji i czasowej dezaktywacji metalu (**publikacja 1 i 2**). W konsekwencji mniejszy ładunek Hg ulega bioakumulacji w organizmie i biomagnifikacji w sieci troficznej.

Dalszy rozwój badań

Niniejsze badania stanowią duże uzupełnienie w literaturze światowej, dotyczące procesów jakim zachodzi neurotoksyna rtęci w organizmach z początkowych ogniw łańcucha troficznego, gdyż problem dotyczący dystrybucji rtęci w narządach oraz jej eliminacji są dość dobrze poznane w literaturze światowej w organizmach z wyższych poziomów troficznych (rybach, ptakach, ssakach morskich). Było to możliwe dzięki poszerzeniu dotychczasowych badań prowadzonych w faunie dennej o egzoszkielet organizmów oraz metodę frakcjonowania Hg. Rtęć w każdej formie jest toksyczna. Jednakże, toksyczność różni się w zależności od formy rtęci, która określa jej właściwości fizyczne i chemiczne. Uzupełnienie wyżej opisywanych problemów o aspekt środowiskowy - sezonowość, parametry biometryczne czy płeć jest istotnym aspektem w kontekście oceny potencjalnego ryzyka dla zdrowia człowieka, wynikającego z konsumpcji owoców morza, które obok ryb, stanowią istotne źródło Hg do organizmu ludzi.

Ocena gatunków obcych w kontekście ich potencjału biomagnifikacyjnego rtęci w sieci troficznej była trudna, ze względu na fakt, iż w obrębie niszy, w której występowały były one taksonem dominującym lub wręcz jedynym. Dlatego uzyskane wyniki wykazały dalsze perspektywy badawcze, które powinny być uzupełnione o gatunki rodzime czy konsumentów wyższego rzędu m.in. ryby, a także o analizę izotopową węgla i azotu $\delta^{13}C$, $\delta^{15}N$ by w pełni określić rolę gatunków obcych w trofodynamice Hg w sieci troficznej. Problem ten jest obecnie dalej rozwijany w projekcie NCN OPUS-23 (Producenci pierwotni jako kluczowy nośnik historycznej i współczesnej rtęci w estuaryjnej sieci troficznej) pod kierownictwem prof. Magdaleny Bełdowskiej. Jednak na podstawie uzyskanych wyników Hg w badanych organizmach można przypuszczać, że pojawienie się i rozprzestrzenianie gatunków obcych w południowym Bałtyku może mieć pozytywny wpływ na ekosystem. Zwłaszcza iż w skali geologicznej, Morze Bałtyckie jest młodym morzem, ze wciąż kształtującym się ekosystemem i umiarkowanej bioróżnorodności. Stężenie rtęci w gatunkach obcych było na niskim poziomie z wyjątkiem kraba wełnistoszczypcego. Zakładając, że w przyszłości organizmy te, ze względu na coraz częstsze występowanie, będą coraz powszechniejszym składnikiem diety ryb w rejonie południowego Bałtyku, mniejszy ładunek toksycznej rtęci będzie wprowadzany do morskiego łańcuch troficznego, w tym do człowieka. Jest to istotne, gdyż Hg nie pełni żadnej pozytywnej funkcji w organizmach żywych i jest toksyczna nawet w niskich dawkach.

Również analiza izotopowa rtęci $\delta^{202}Hg$ w poszczególnych tkankach organizmów mogłaby być dużym uzupełnieniem procesu eliminacji rtęci z ciała do pancerza czy muszli, a także wskazać na jej pochodzenia w organizmie $\Delta^{199}Hg$, $\Delta^{200}Hg$, $\Delta^{201}Hg$ (Manceau i in., 2025). Użycie tej metody pozwoliłoby w sposób liczbowy wskazać na efektywność opisywanych procesów, które w niniejszej dysertacji, były oparte o biologię i ekologię badanych organizmów.

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Different pathways of accumulation and elimination of neurotoxicant Hg and its forms in the clam Atlantic rangia (*Rangia cuneata*)

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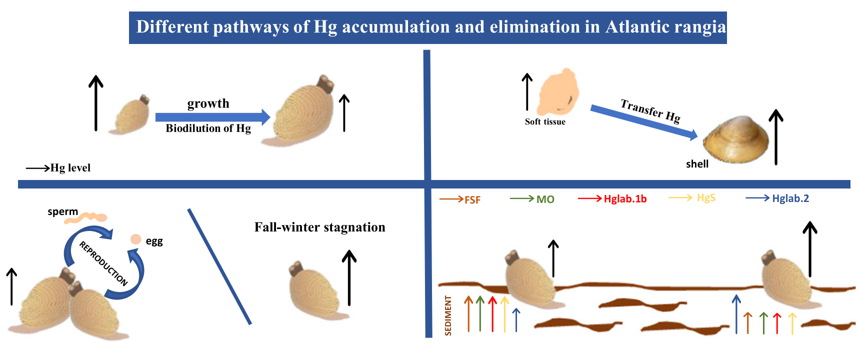
HIGHLIGHTS

- The rate of detoxification of the clam body from mercury was dependent on season.
- The breeding season was the most effective process in clams from Hg detoxication.
- The mass of accumulated mercury was higher in shell mass than in body mass in summer.
- Transfer of Hg from the body to the shell depended on the forms Hg, mostly HgS.
- The level of Hg in clams depended on the Hg forms, which was present in the sediment.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) is one of the most hazardous environmental pollutants, negatively affecting the ecosystem. The pathways of Hg elimination are well recognized in organisms from higher trophic levels compared to invertebrates such as clams. The aim of this study was to identify pathways of Hg accumulation in an alien species clams: *Rangia cuneata*, which represented an unrecognized source of Hg into the trophic chain of the southern Baltic Sea. An important aspect of this study was to determine Hg detoxification processes based on physiological state and biometric parameters of the atlatntic rangia. Special consideration was given to the role of shell in this process and the form of Hg in which it occurred. The study was also considered in terms of geographical changes in the Hg concentration in clams and the factors involved.

Sex did not determine the concentration of Hg and its fraction in clams soft tissue and shell. Clams detoxified xenobiotic effectively in summer when their metabolism was accelerated. As a result, clams grew faster in warmer water than they accumulated Hg. In addition, this process was intensified by their reproduction. The mass of accumulated mercury was higher in the shell mass than in the body mass in summer. Transfer of Hg from the body to the shell depended on the forms Hg, mostly HgS. Geographical changes in the mercury concentration in clams was related to the form of Hg in the sediment. In areas where were more fines sediment fraction and organic matter accumulated in the sediment, mercury was present in a less bioavailable form, which caused that clams had lower Hg concentrations in their body. With assumption that in the future, due to its increasingly frequent occurrence, atlatntic rangia will become more common component of fish diet, a smaller load of toxic mercury will be introduced to the marine trophic chain.

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1. Introduction

Mercury (Hg) is a highly toxic metal. It adversely affects both the adult human being and the fetus. It contributes to neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease and autism (Bakir et al., 1973: Olivieri et al., 2002). Hg biomagnifies in the trophic web, posing the greatest threat to higher organisms such as large predatory fish, water birds, marine mammals and humans. Currently, fish and seafood are the main source of mercury into the human body, which is a major concern in areas of the world where they are an important component of the diet (Mania et al., 2012). The problem regarding the distribution of Hg in organs and its elimination from the body are well understood in the world literature in organisms from higher trophic levels such as fish, birds and mammals (Falkowska et al., 2017; Grajewska et al., 2019). Studies in organisms from lower trophic levels, e.g. in invertebrates such as clams, focused on determining Hg concentrations in tissues and the effect of seasonality on mercury concentration levels (Jędruch et al., 2019; Jędruch and Bełdowska, 2020). This is likely due to the size of the organisms and the difficulty in obtaining a representative mass of organ material for chemical analysis. One of important link in the trophic chain of the Vistula catchment (southern Baltic), incorporating Hg accumulated in sediments into the circulation, becomes the alien clams species *Rangia cuneata*, which is native to the Gulf of Mexico (Atlantic Ocean). It entered the Atlantic Ocean with ballast water in the 1960s, and in the southern Baltic region probably as a result of dredger activity dredging the Kaliningrad sea channel (Warzocha et al., 2016). It is estimated to have appeared in the Baltic Sea catchment between 2007 and 2008 and is the one of the largest clams species in this region. The largest colonies of this mussel are found in the Vistula Lagoon, and it is colonising new habitats. A combination of low salinity, high turbidity and a soft substrate of sand, mud and vegetation appears to be the most favorable habitat for *R. cuneata* (Tarver, 1972). The low salinity and lack of competition from other species for food has provided the Vistula Lagoon with suitable conditions for the development and colonization of *R.cuneata*. Atlantic rangia is a non-selective filter-feeder, turning large quantities of plant, detritus and phytoplankton into clam biomass (Darnell, 1958) but the species also appears to obtain organic matter and phosphate from the sediment by direct ingestion or by feeding on bacteria associated with these materials (Tenore et al., 1968). Atlantic rangia is becoming an important food source for water birds, demersal fish or crabs. The average lifespan is around 4 to 5 years, exceptionally reaching 10 (Wolfe and Petteway, 1968) to 15 years (Hopkins et al., 1973). Despite its muddy taste, the Atlantic rangia is harvested and marketed for human consumption in eastern Mexico, where it has a relatively high economic value (Wakida-Kusunoki and MacKenzie, 2004).

Nowadays, when the inflow of metals to the lagoon is limited, the remobilization of Hg from the sediment is becoming more and more important. As a result of several biotic and abiotic processes, mercury has become remobilized into the marine environment. In this way, it can be bioaccumulated in benthic organisms and biomagnified in the subsequent links of the trophic chain, thereby representing the greatest threat to marine mammals, water birds and humans (Jackson, 1998). Despite numerous works on the biology and ecology of the mussel *Rangia cuneata*, there is a lack of scientific reports on the determination of mercury levels in the clams studied in this area. Only the level of phenolic contamination of Atlantic rangia in the Vistula Lagoon has been determined (Graca et al., 2021). Therefore, the aim of this study was to determine the concentration of total mercury in the clam *Rangia cuneata* from the Vistula Lagoon. The toxicity of mercury and its bioavailability is related to the form in which it occurs, so labile and stable forms of mercury were also determined in the samples. The study considered both the sex, size of the organisms and the physiological state of the clams (time of year), which can potentially determine the mercury concentration in the organism. To determine the role of the shell in body-shell transfer for xenobiotics has not been well recognized. Therefore, this work focused on the clam shell to verify the hypothesis that the clam body can detoxified Hg by depositing it in the shell, making it an effective site for Hg accumulation and deactivation, analogous to feathers and claws in birds and fur in mammals (Scheuhammer et al., 2007; Bustamante et al., 2016; Manceau et al., 2021). To understand the processes of Hg metabolism and detoxification of the clam, it is also important to know the pathways of entry of the xenobiotic into the clams, so total mercury and its thermal fractions were determined in the *Rangia cuneta* dietary: plankton and suspended particle matter, and in the habitat (sediment). Atlantic rangia is one of the largest representatives among Baltic invertebrates therefore it can serve as a model species to fill the research gap on the distribution and detoxification of mercury in organisms from lower trophic levels. As a result of *R.cuneata* becoming increasingly abundant in the southern Baltic and thus a common dietary component of higher trophic levels, it is important to determine its role in the trophodynamics of Hg in the study area.

2. Materials and methods

2.1. Sampling

Rangia cuneata organisms were sampled during a cruise by the research unit of the Sea Fisheries Institute in Gdynia - ŁM MIR 2, in the southern part of the Vistula Lagoon (N 54°17.114′ E 19°25.070) in April, August and October 2019 and April 2020 on station SUC-R2 (Fig. 1; Table 1), just off the mouth of the Elbląg channel. In addition, during the summer 2021 season, *R.cuneata* clams were collected from 7 measurement stations, in the western part of the Vistula Lagoon to determine the seasonal variability of the studied xenobiotic Hg (Fig. 1). Biometric parameters were measured, and tissues isolated in an analogous manner. Biological material was collected using a hand dredge (dimensions: 36 × 21 cm, trawl length 60 cm and mesh size 3 × 3 mm) from a depth of 2.5 m and then sieved through a 0.25 mm mesh sieve. Atlantic rangia individuals were hand-picked from the sieve and placed in aerated thermoses with floodwater. After transporting the samples to the laboratory, the organisms were placed in the aquarium in filtered water for 24 h for purification. After 24 h, biometric analyses of the organisms were undertaken.

In addition, the dietary components of *R.cuneata* were also collected at the research station. The seawater to filter out the suspended particulate matter (SPM) was collected with a bathometer from the near-bottom layer (Table 1). The water samples were collected into dark borosilicate glass bottles, previously cleaned with 4 M dilute nitric acid, rinsed with deionized water and heated (300 °C, 6 h). The samples, after being transported to the laboratory, were filtered through ignited (500 °C, 6 h) and pre-weighed Whatman GF/F glass microfiber filters (pore size: 0.7 μm, diameter: 47 mm). Plankton was collected from the water column using a 10 μm net. A surface layer of bottom sediments (top 10 cm) was also collected for analysis. The collected material was stored at −20 °C and lyophilized and homogenized immediately before analysis (Jędruch et al., 2019).

In situ measurements of the water were also taken during sampling. Salinity [PSU], water pH, temperature [°C] and visibility in the water column using Secchi disk were measured. Moisture content, organic matter content and the proportion of the finest fraction in the surface sediment were determined according to the method in papers: Saniewska et al., 2014.

2.2. Biological analysis

Rangia cuneata organisms ($n = 365$) were divided in the laboratory into nine size classes - based on shell length (first: 0–5 mm; second: 5.1–10 mm; third: 10.1–15 mm; fourth; 15.1–20 mm; fifth: 20.1–25 mm; sixth: 25.1–30 mm; seventh: 30.1–35 mm; eighth: 35.1–40 mm; ninth: 40.1–45 mm) using electronic calipers, and the wet weight of whole organisms was then determined. The sex of the organisms was determined based on the structure of the gonads under an Olympus IX71 optical inverted microscope. 142 females, 209 males and 14 juvenile specimens were determined. The soft body was then separated from the shell and samples of both soft body and shell were reweighed. Samples were stored at −20 °C until analysis.

Before proceeding with further chemical analyses, the *R.cuneata* specimens (both shell and soft tissue) were lyophilized and their dry mass was

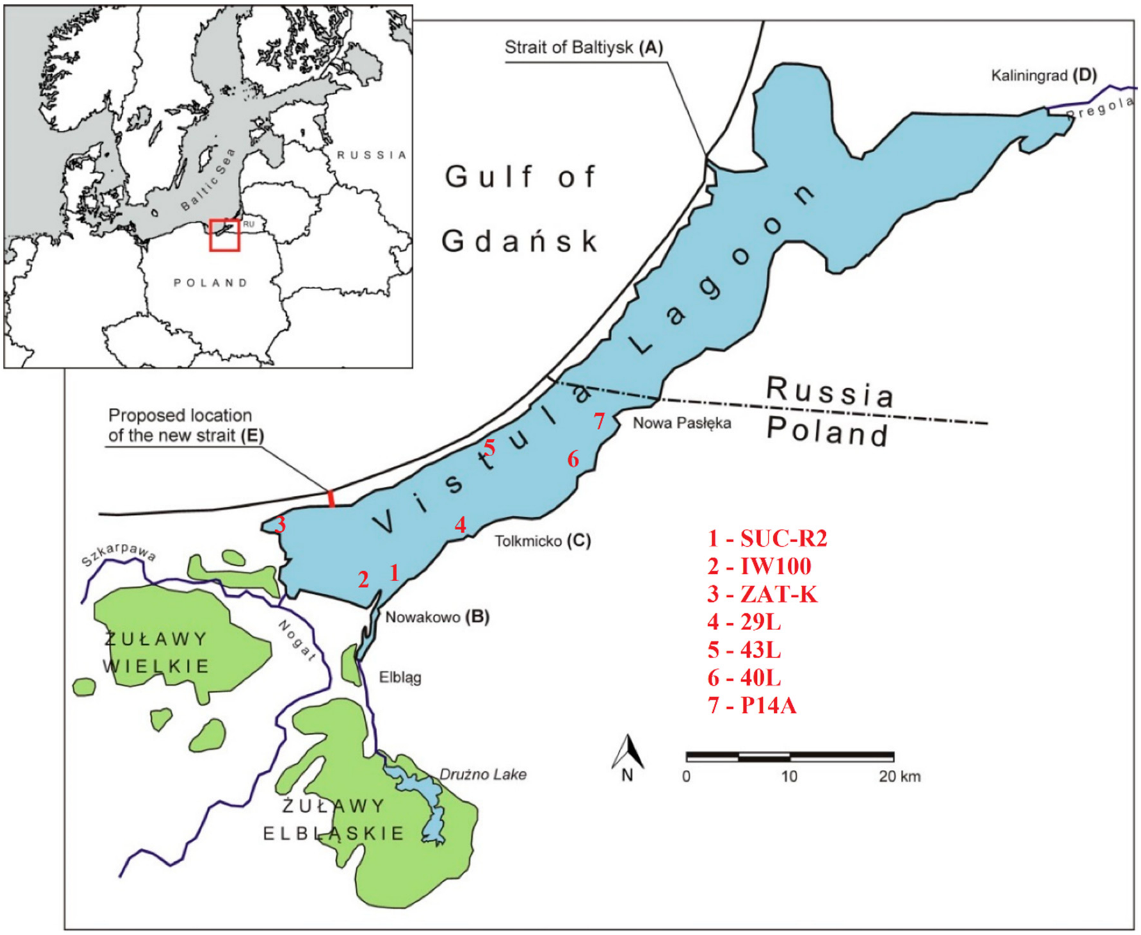

Fig. 1. Sampling area, Vistula Lagoon (southern Baltic Sea) based on (Szydłowski et al., 2021).

determined. The wetness of the organisms was determined based on the difference between wet and dry mass. The clams were then homogenized in an agate mortar. Owing to their low mass, the smallest size class individuals were homogenized jointly in order to obtain sufficient sample weight for mercury analysis. A total of 365 bivalve soft tissue and 365 shells were analyzed.

2.3. Total mercury analysis

Total mercury analysis was performed on a DMA 80 analyzer, by thermal desorption using an automatic DMA mercury analyzer (Milestone, Italy) according to the method published by Saniewska and Bełdowska (2017). This method does not require prior mineralization of the sample, which reduces the risk of contamination. About 0.1000 g of a sample (was placed in quartz vessels in triplicate. The concentration of total mercury was determined by incinerating the sample at 750 °C in pure oxygen. The correctness of the analysis was verified by the analysis of certified reference materials (tea leaves INCTTL-1 - Hg_{TOT}: 5 ng g^{-1}, soil NCS DC 87103-Hg_{TOT}: 17 ng g^{-1}, plankton BCR-414-Hg_{TOT}: 276 ng g^{-1}), with an average recovery of 98 % and a standard deviation not exceeding 5 %. Limit of detection (LOD) was calculated as the threshold of the standard deviation (SD) of Hg concentration in the ignited sample boat measured in 10 replicates. In the case of Hg_{TOT} analysis, the LOD was 1 pg of Hg.

Table 1
Comparison of total mercury concentrations in the soft tissue *Rangia cuneata* with other regions around the world.

Region	Hg concentration [ng g^{-1}]	References
Vistla Lagoon. southern Baltic	22.2 d.w.; 6.4 w.w.	this study
Texas. river	110.0 w.w.	Guthrie et al., 1979
Texas. river mouth	60.0 w.w.	Neff et al., 1978
Chef Menteur. New Orlean	4.5 d.w.	Byrne and DeLeon, 1986
Rigdets. Lousiana	< 1 d.w.	Byrne and DeLeon, 1986
Pungo river. North Carolina	32 w.w.	Di Giulio and Ryan, 1987
Mexican Bay	772.4 d.w.	Lewis and Chancy, 2008
Mobile Bay. Alabama	26 w.w.	Showalter, 2010
Caloosahatchee estuary. Florida	7.9 d.w.	Rumbold et al., 2018

2.4. Mercury fractionation

The use of a 5-step thermal desorption method allowed the separation of five groups of compounds with similar properties. This method involved simultaneous combustion of the same sample at five temperatures: Hg labile 1a (175 °C) (released mainly Hg bound to halides: HgI_2, $HgBr_2$, $HgCl_2$); Hg labile 1b (225 °C) (released mainly Hg bound to organic compounds like: $Hg(CN)_2$, $Hg(SCN)_2$, $Hg(CNO)_2$, $(CH3COO)_2$ Hg, methylmercury and mercury in complexes with organic matter); HgS (325 °C); Hg labile 2 (475 °C) (released: $HgSO_4$, HgO (red) and HgF_2); Hg residual (750 °C) (Bełdowska et al., 2018; Jędruch et al., 2018). The analysis of the samples was performed in three repeats. The analyses of the environmental samples were preceded by an examination of the Hg patterns specific to each fraction according to the method of Saniewska and Bełdowska (2017). Seal muscle was used as the pattern for the thermally labile fraction (1b), in which MeHg accounted for 98 % of the contribution to the total Hg_{TOT} (Jędruch et al., 2018) and the contribution of fraction 1b accounted for 98 % of the total Hg fraction in seal muscle, respectively. The use of Hg standards in this method is important, as there are no certified reference materials for individual forms of Hg in environmental samples.

The correctness of the analysis was verified by comparing the sum of Hg concentrations measured by the 5-stage fractionation method with the result of the Hg_{TOT} analysis. The accuracy of the analysis was on average 96 %. The quality of the method was also verified by the analysis of certified reference materials (in analogy to total mercury), carried out in triplicate, for which the recovery ranged from 96 to 105 %. Limit of detection (LOD) was calculated as the threshold of the standard deviation (SD) of Hg concentration in the ignited sample boat measured in 10 replicates. In the case of Hg_{TOT} analysis, the LOD was 1 pg. of Hg. The concentrations of Hg labile 2 and Hg residual fractions were below the limit of quantification (BLD) and therefore these mercury fractions were not included in the discussion.

2.5. Data analysis

Statistical analysis and graphic representation of the obtained results were carried out using STATISTICA 13 (StatSoft) and Microsoft Excel (2008). The analyzed data were not characterized by normal distribution (Shapiro-Wilk test $p < 0.05$). In order to determine the significance of differences between Hg and biometric parameters, the non-parametric U Mann-Whitney and ANOVA Kruskal-Wallis test was used. The relationships between the analyzed variables were determined on the basis of the Spearman's coefficient, with a confidence interval of at least 95 % and PCA analyses. The normalisation of Hg concentrations was performed in accordance with formulae presented in an earlier study by Jędruch et al. (2015).

The condition index CI (mg cm^{-3}), which is a good proxy of the physiological state of investigated mussels (Wołowicz et al., 2006; Pierścieniak et al., 2010; Jędruch et al., 2019), was calculated using the following formula (Beukema and de Bruin, 1979; Okumuş and Stirling, 1998):

$$CI = STDW\, L^{-3}, \tag{1}$$

where STDW is soft tissue dry weight after lyophilizing (mg) and L is shell length (cm). To evaluate the ability of benthic organisms to accumulate Hg from surface sediment, the biota-sediment accumulation factor (BSAF) was calculated following the formula suggested by Szefer et al. (1999):

$$BSAF = Hg_{mussel}\, {Hg_{sediment}}^{-1}, \tag{2}$$

where Hg mussel and Hg sediment are the concentrations of Hg_{TOT} (ng g^{-1} d.w.) in *D. polymorpha* surface sediments, respectively. The accumulation of Hg in *R.cuneata* with food was expressed by a bioaccumulation factor (BAF), according to the formula (Kwok et al., 2013):

$$BAF = Hg_{mussel}\, {Hg_{diet}}^{-1}, \tag{3}$$

where Hg mussel and Hg_{diet} are the concentrations of Hg_{TOT} (ng g^{-1} d.w.) in *R.cuneata* its diet. The BAF was calculated separately for different elements constituting the mussels' food in the study area: suspended particulate matter (SPM) and plankton.

3. Result and discussion

3.1. Mercury in soft tissue

No studies on Hg concentrations in *Rangia cuneata* have been carried out in the Vistula Lagoon as well as in the Baltic to date. Considering the average Hg_{TOT} concentrations in Atlantic rangia from spring 2019 - spring 2020 from the Vistula Lagoon (22.2 ng g^{-1} d.w., 6.4 ng g^{-1} w.w.), it was found to be four times lower than the average mercury concentrations in *R. cuneata* from the Pungo River in North Carolina in 1983–1984 (32.0 ng g^{-1} w.w.) (Di Giulio, 1987) and 4 times lower than average concentrations in *R.cuneata* from Mobile Bay, Alabama, during the period 2008–2009 (26 ng g^{-1} w.w.) (Showalter, 2010) (Table 1). In rangia from the Vistula Lagoon, mercury concentrations were 17 and 9 times lower, respectively, than in *R.cuneata* taken in the estuary at the Texas coast in 1975 (Neff et al., 1978; Guthrie et al., 1979). Hg concentrations were also lower (35 times) than in *R. cuneata* in the Gulf of Mexico between 1993 and 2002 (772.4 ng g^{-1} d.w.) (Lewis and Chancy, 2008) (Table 1).

The Atlantic rangia mercury concentration results obtained cannot be compared to other areas of Europe, as no such data have been published to date. Comparing data on total mercury concentrations in zoobenthic organisms from the 21st century to the 1990s, it can be concluded that in previous years, higher Hg concentrations were the result of more intensive mercury inputs to the environment (HELCOM, 2020; WHO, 2019). Nowadays, because of restrictions in the Baltic Sea on Hg emissions, a lower Hg load enters with riverine inflow, dry and wet deposition or with sewage (HELCOM, 2020). Consequently, although 12 rivers and water from four sewers enter the Vistula Lagoon, mercury concentrations in bivalve shellfish in this water body were relatively low compared to other regions of the world. The exceptions were mussels from lakes located in the United States, which were located in so-called pristine regions with little anthropological pressive influence. In contrast, higher concentrations of Hg in Atlantic rangia from the Vistula Lagoon were in comparison with the Rigolets Strait in Louisiana (<1 ng g^{-1} d.w.), with Chef Menteur Lake in New Orleans in 1980 (4.5 ng g^{-1} d.w.) (Byrne and DeLeon, 1986) and from the Caloosahatchee Estuary in Florida between 2011 and 2015 (7.9 ng g^{-1} d.w.) (Rumbold et al., 2018), 22, 7 and 3 times higher, respectively (Table 1).

Comparing the mean concentration of Hg_{TOT} in the soft tissue of Atlantic rangia clams from spring 2019 - spring 2020 from the Vistula Lagoon (22.2 ng g^{-1} d.w.; 6.4 ng g^{-1} w.w.) to other mussel species from the southern Baltic was found to be 7-fold lower compared to the mussel population from the Bay of Puck (143 ng g^{-1} d.w.) and almost 2-fold lower than the mean Hg concentration in the Zebra mussels population from the Vistula Lagoon (39.8 ng g^{-1} d.w.) (SI) (Jędruch et al., 2019; Wilman et al., 2022). Atlantic rangia is one of the largest bivalve species in the southern Baltic. This may indicate a rather rapid increase in muscle and shell mass of the clam and, consequently, the dilution of Hg in its tissues, more efficiently than in other clam species in the study area. Additionally, these clams in the Vistula Lagoon are characterized by their high lipid content, (Graca et al., 2021). Mercury has a strong affinity for peptides or proteins, while it binds weakly in lipids (Rossignol, 2019; Ajsuvakova et al., 2020). Hence, the low concentration of Hg in the soft tissue of this mussel species may also result. The storage of a high lipid content in tissues may also be an adaptation of the alien species to live in new bodies of water, where water temperature conditions are lower than in the native areas of occurrence - the Gulf of Mexico (average annual water temperature is 31 °C). The WHO limit value for total mercury in human food is 500 ng g^{-1} w.w. Considering the average concentration of total mercury in the Atlantic rangia wet weight rangeland: (6.4 ng g^{-1} w.w.), it was found to be 78 times lower than the WHO recommended limit. The permissible concentration of Hg in food is 500 ng g^{-1} w.w., and according to EFSA the permissible weekly intake (TWI) from food is 4 μg Hg per kilogram of body weight. The ingestion of 1948 g of *R.cuneata* mussel from the Vistula Lagoon would deliver an acceptable weekly dose of the xenobiotic to the human body.

Above this, rangeland is not an obligate filter-feeder like the mussel and the rockfish. In addition to filtering water from suspended particles, it feeds on organic matter and detritus from the sediment. Darnell (1958) noted that the digestive tract of this mussel contained 70 % detritus debris, 10 % sand and 17 % algae, as well as diatoms, borers and vascular plant remains. This species can take up organic matter directly from the sediment (Tenore et al., 1968). This difference in the diet of these clam species is also indicated by the percentage of labile and stable Hg fractions (SI; Fig. 2a). The proportion of the mercury fraction $Hg_{lab.1b}$ was 20 % and 30 % higher in *R.cuneata* compared to *M.trossulus* and *D.polymorpha*, respectively. Mercury in this fraction occurs mainly as organic mercury (including MeHg) and Hg in complexes with organic matter. As much as 95 % of this fraction may indicate that Atlantic rangia in the Vistula Lagoon feeds heavily on organic matter probably originating from the degradation of animal tissues. Such a high proportion of $Hg_{lab.1b}$ is observed in predatory fish (da Silva et al., 2020), so this may indicate biomagnification of mercury

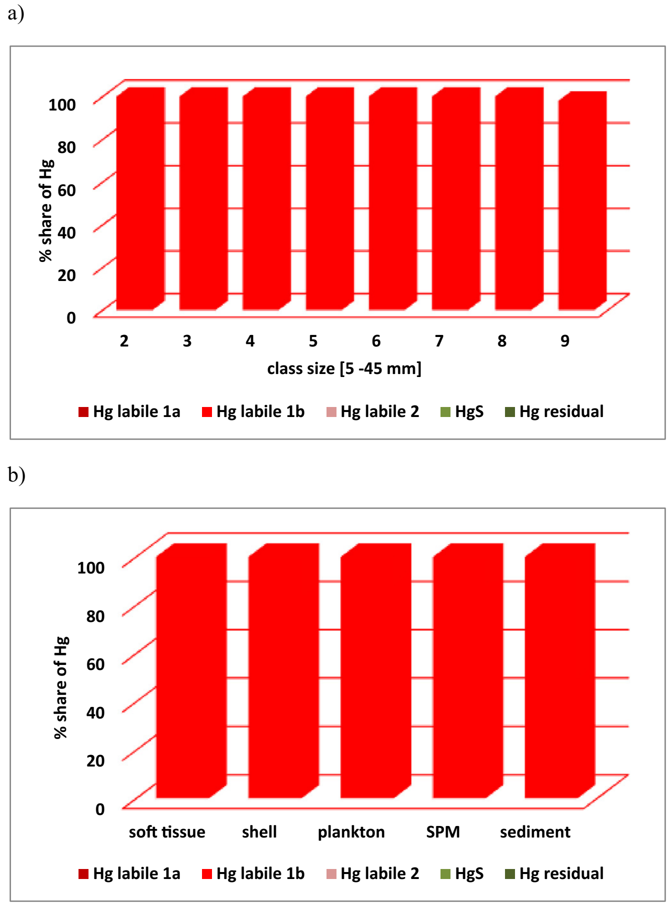

Fig. 2. Percentage of mercury fraction in the soft tissue of *Rangia cuneata* in successive size classes (a) and relating the fractional contribution in the soft tissue to the other matrices analyzed: shell, plankton, SPM, sediment (b).

in the organisms of the studied bivalve. Inorganic mercury in the sediment precipitates into insoluble mercuric sulphide (the stable form of mercury), which can be ethylated by bacteria, resulting in the most toxic form of Hg, methylmercury. Sulphate-reducing bacteria are found in anaerobic conditions, typical of muddy silty (fine-dispersed) sediments of rivers, lakes and oceans, where methylmercury concentrations are usually highest (Parks et al., 2013). Consequently, organic forms of mercury may be remobilized from the sediment to the headwaters, as a result of bioturbation and bio-irrigation of the sediment by bivalves, successively adsorbed by matter and suspended solids, and subsequently accumulated and biomagnified by clams (Jackson, 1998). Organic forms of mercury in the sediment in the study area accounted for an average of 48 % of the total Hg_{TOT} contribution, while in suspension it was 83 % (Fig. 2b). Additionally, an interaction between the species *R.cuneata* and *D.polymorpha* is observed in the Vistula Lagoon, where often individuals of Atlantic rangia are overgrown by smaller zebra mussels (Kornijów et al., 2017). As a result, the host (*R.cuneata*) suffers energy losses for carrying 'passengers', is hindered in its vertical migration in the bottom sediments and competes for food. Overgrowth by individuals of *D.polymorpha* can alter the availability and quality of suspended overlying water. By occurring higher above the sediment, they can more selectively filter water from plankton and microalgae, in which mercury halides - $Hg_{lab.1a}$ - constitute a large percentage (Bełdowska et al., 2018; Wilman et al., 2022; Fig. 2b). Consequently, individuals of *R.cuneata* feed on larger suspended particles, in which the proportion of inorganic forms of Hg was lower (Fig. 2a); hence, also the lower proportion of this mercury fraction in the Atlantic rangia body compared to obligate filter-feeding (SI) bivalves may result.

The calculated coefficient of BSAF and BAF_{SPM} and BAF phytoplankton was characterized by a value <1 (SII). Irrespective of the physiological state of the bivalve (condition index), mass or size of the organism, the bioaccumulation factor did not change statistically significantly ($p > 0.05$). This indicates a limited accumulation of Hg in the corpuscle of *R. cuneata* from food. In the region of the present study, papers by Jędruch et al. (2019) and Wilman et al. (2022) showed Hg accumulation from food (SPM and phytoplankton) in the bivalve *D. polymorpha* (Vistula Lagoon) and *M. trossulus* (Puck Bay). This difference is probably due to the food preferences of the mussels. In the diets of zebra mussel and blue mussel, SPM and phytoplankton make up 100 % of the proportion, which is typical of obligate filter-feeding bivalves. In contrast, Atlantic rangia feeds on a variety of plant-derived matrices and organic matter from the sediment, as described earlier. Hence, the lower soft tissue concentrations of *R.cuneta* relative to other clams species in the study area may also result.

3.2. Accumulation and elimination of R.cuneata from mercury

Principal component analysis (PCA) separated the determinants of the processes that mercury and its labile and stable forms underwent in the soft-bodied Atlantic rangia (Fig. 3). The first of these, explaining 60 % of the variability, were biometric parameters such as body size and weight, sex and condition of the bivalve (Fig. 3). These determined the concentration level of Hg_{TOT} especially the fractions $Hg_{lab.1a}$ and $Hg_{lab.1b}$.

Taking into account the concentration of total mercury as well as its fraction in adults, no statistically significant differences were found between males and females ($p > 0.05$; Fig. 4). Therefore, in further considerations, values were considered without separating the sexes - males and females. A similar trend has been reported in other native species: crab *Rhithropanopeus harrisii* and clam *Dreissena polymorpha* (Wilman et al., 2019, 2022). The median concentration of total mercury in Atlantic rangia in males and females was 29.2 ng g^{-1} d.w. This was statistically significantly lower than in juvenile individuals at 38.0 ng g^{-1} d.w. ($p < 0.05$; Fig. 4). Oxygen isotopic analysis of δ18 O in *R.cuneata* clam in the work of Lécuyer et al. (2012) showed that bivalves up to 20 mm (to V class size in this study), had the highest oxygen demand. Smaller (younger organisms), while filtering water for the respiration process (they respire with oxygen dissolved in the water), are more efficient at additionally storing calcium ions and carbonates for shell calcification and nutrients (energy, calories) necessary for the growth process (Marsh, 1986; Yin et al., 2017). Also they accumulated metals, including mercury more efficient. Therefore,

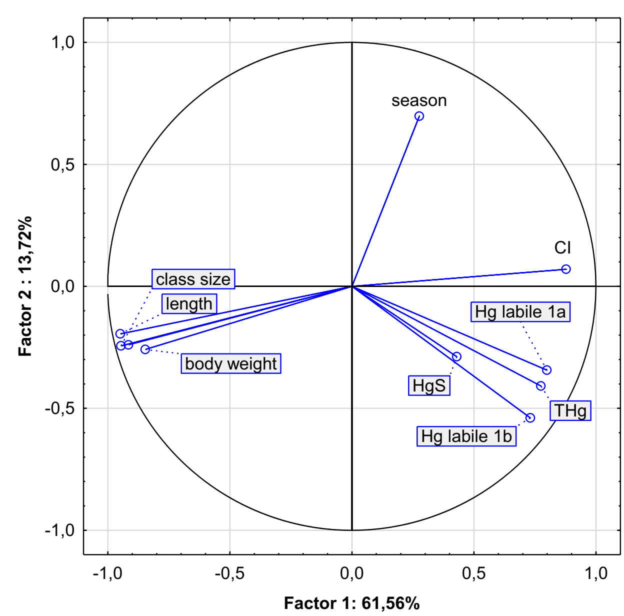

Fig. 3. Principal component analysis (PCA) of the concentration of total mercury and its fraction in the soft tissue with *Rangia cuneata* biometric parameters and their classification.

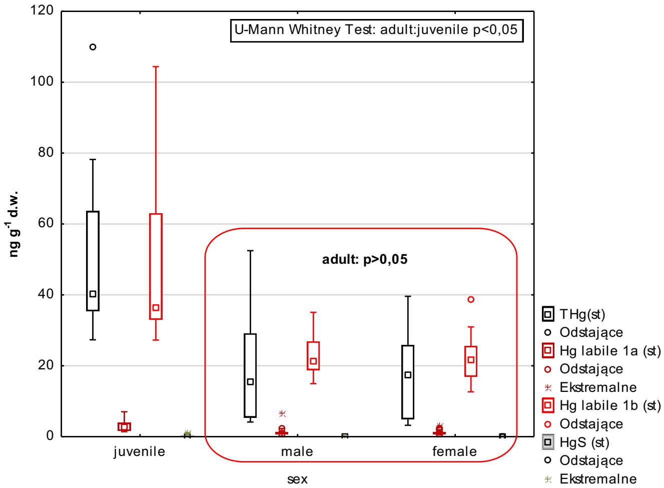

Fig. 4. Variability of concentration of total mercury and its fractions in the soft tissue of *Rangia cuneata* by sex of clams.

in juvenile and smaller (to V class size) specimens, concentrations of mercury and its labile and stable forms were could significantly higher ($p < 0.05$) than in adults.

A similar relationship was observed by Graca et al. (2021), for phenol compounds in Atlantic rangia in the Vistula Lagoon. The same relationship was observed across measurement seasons. The consequence was that the concentration of mercury and its labile and stable forms decreased with increasing mass and size of organisms (SIII), and these changes were statistically significant ($p < 0.05$). This may indicate a dilution process of the concentration of total mercury and its fractions during growth and weight gain, due to the formation of new tissues of *R. cuneata*. Metal concentrations increase with age and size when the growth of the organism is slow relative to the rate of metal accumulation (Roditi and Fisher, 1999), whereas in this case the rate of organism growth outweighed the bioaccumulation of Hg. An analogous process was observed in the bivalves *D.polymorpha* and *M. trossulus* for total mercury, $Hg_{lab.1b}$ and mercury sulphide (Wilman et al., 2022; Jędruch et al., 2019). The decrease in $Hg_{lab.1a}$ concentrations in subsequent size classes in rank is the opposite process to that observed in blue mussels and zebra mussels. This indicates a different source of mercury compounds, namely food. Variegated zebra mussels and blue mussels are obligate filter feeders, feeding only on plankton and microalgae, in which mercury halides - $Hg_{lab.1a}$ (Bełdowska et al., 2018) - constitute a large proportion. In contrast, Atlantic rangia in the Vistula Lagoon may prefer to detritus-eat and feed on larger suspended particles (what had been discussed in chapter 4.1.). The percentage as well as the concentration of the $Hg_{lab.1b}$ fraction in the body of *R.cuneata* was the highest of all fractions (Fig. 2a). $Hg_{lab.1b}$ occurs as mercury in complexes with organic matter in the sediment and suspension (Fig. 2b), being the main source of this fraction in the Atlantic rangia body (Fig. 2a).

The clam condition index (CI) seems to confirm the relationships discussed above. It varied linearly from one size class to another (SIV). Clams from the smallest size classes had the best condition, while those from the largest size classes (8, 9) were in the weakest condition. The condition of the clams had an inversely proportional effect on the concentration of Hg_{TOT} and its fraction. Clams in better condition had higher concentrations of total mercury and its fractions, while clams in poorer condition, had the lowest concentrations, as evidenced by negative, statistically significant ($r = -0{,}78$; $p < 0.05$) CI correlations, with Hg_{TOT} concentrations and its fractions (SIV). An analogous relationship was observed by Jędruch and Bełdowska (2020) in blue mussels from the Puck Bay and Wilman et al. (2022) in zebra mussels from the Vistula Lagoon. As a result of the high energy demand for growth and respiration, the mussels loosed on condition, but the growth process is so intense that the process of Hg dilution in the tissues prevails over accumulation. Additionally, organisms decrease their metabolism under stressful conditions and thus bivalves have valve-closure for preventing stressor exposure, which effected in lower Hg accumulation in *R.cuneata* body. A reduction in the Hg accumulation process due to dilution caused of clam growth was observed by Peltier et al. (2009) in the US region, while in the China region, work by Tran et al. (2020), the clam condition factor was directly proportionally correlated with metal concentration, indicating accumulation with growth and mass. Presumably, these differences may be related to the pollutant load entering the water body. The inverse correlation of CI with metal concentration may indicate limited emissions of Hg into the environment.

The second factor explaining the 14 % variation was the measurement season (Fig. 3). Abiotic factors, such as water temperature, stimulate clam development (reproduction and growth) and their metabolism. The concentration of total mercury and its labile and stable form - HgS, differed statistically significantly ($p < 0.05$) in all seasons except for the fraction $Hg_{lab.1a}$ and HgS in juvenile organisms ($p > 0.05$) (Fig. 5). The highest concentrations of Hg_{TOT} and labile and stable form of mercury in the soft body were determined in spring - in both 2019 and 2020 in both juvenile and adult specimens, but higher values were recorded in 2019 (Fig. 5). The climatic spring in 2020 was cooler than in 2019, additionally the month of March 2020 was a cooler month than March 2019 (https://klimat.imgw.pl). In March 2020, there were more days with frost ($T < 0$ °C). The water temperature was also on average 2 °C cooler in the months leading up to spring 2020, relative to spring 2019. As a result, in spring 2019, through higher air and consequently water temperatures (a phenomenon characteristic of the shallow Vistula Lagoon), organisms emerged more quickly from winter stagnation and began to take in food more intensively and prepare for reproduction, consequently accumulating Hg. Comparing to all seasons, in spring 2019, just before reproduction, the median value of total mercury concentration in adults was the highest. The increase in total mercury concentrations and fractions in spring 2020 was due to the high demand for food after the winter season. Because of the increased accumulation of Hg with food, as well as a decrease in body weight during winter, which contributes to the increase in Hg concentrations. In contrast, the lowest concentrations of Hg_{TOT} and labile and stable forms of mercury in the soft tissue were determined in summer 2019 (Fig. 5). The decrease in the concentration of total mercury and its labile forms during the summer was probably related to the reproduction of the organisms. Hg is transferred as MeHg (in this study could be a part of the Hg labile 1b fraction) from somatic tissues to reproductive tissues, which are released from *R.cuneata* during reproduction (Ram et al., 1978). Under favorable conditions, reproduction can continue throughout the year. Gametogenesis in *R. cuneata* begins when the water temperature reaches over 15 °C and the salinity is between 0 PSU and 15 PSU (Cain, 1975). In summer, when the water temperature was 22.2 °C, *R.cuneata* organisms had the most favorable conditions for reproduction during the entire measurement period. According to Kornijów et al. (2018), the growth of *R.cuneata* in the Vistula Lagoon takes place during the summer, followed by its inhibition in the autumn-winter-spring period. Therefore, no juvenile organisms were observed in summer in the present study. Consequently, in addition to reproduction, the process of clearing Hg from the bivalves in summer is intensified by a faster growth rate - the dilution of Hg in the tissues of Atlantic rangeland. In autumn, reproduction is inhibited, and the bivalves prepare for winter stagnation, thus consuming more food. At this time, the lagoon waters are rich in plankton and tryptone (Kornijów et al., 2017). As a result, the clams accumulated more mercury with their food, so an increase in the concentration of Hg and its fraction was observed (Fig. 5). In addition, in autumn, adult organisms had half the mercury concentrations of juvenile organisms (Fig. 5), indicating an important role of reproduction in the detoxification of adult rangia in summer. Juvenile organisms had higher Hg concentrations and Hg fractions in autumn than in spring 2020 (Fig. 5) because, unlike adults, they did not cleanse their organism by reproduction in summer, further indicating the important role of reproduction in organism detoxification. A similar relationship was reported in the common heartworm (*Cockle Cerastoderma glaucum*) from the French lagoon (Szefer et al., 1999) and in the variegated crayfish (Wilman et al., 2022),

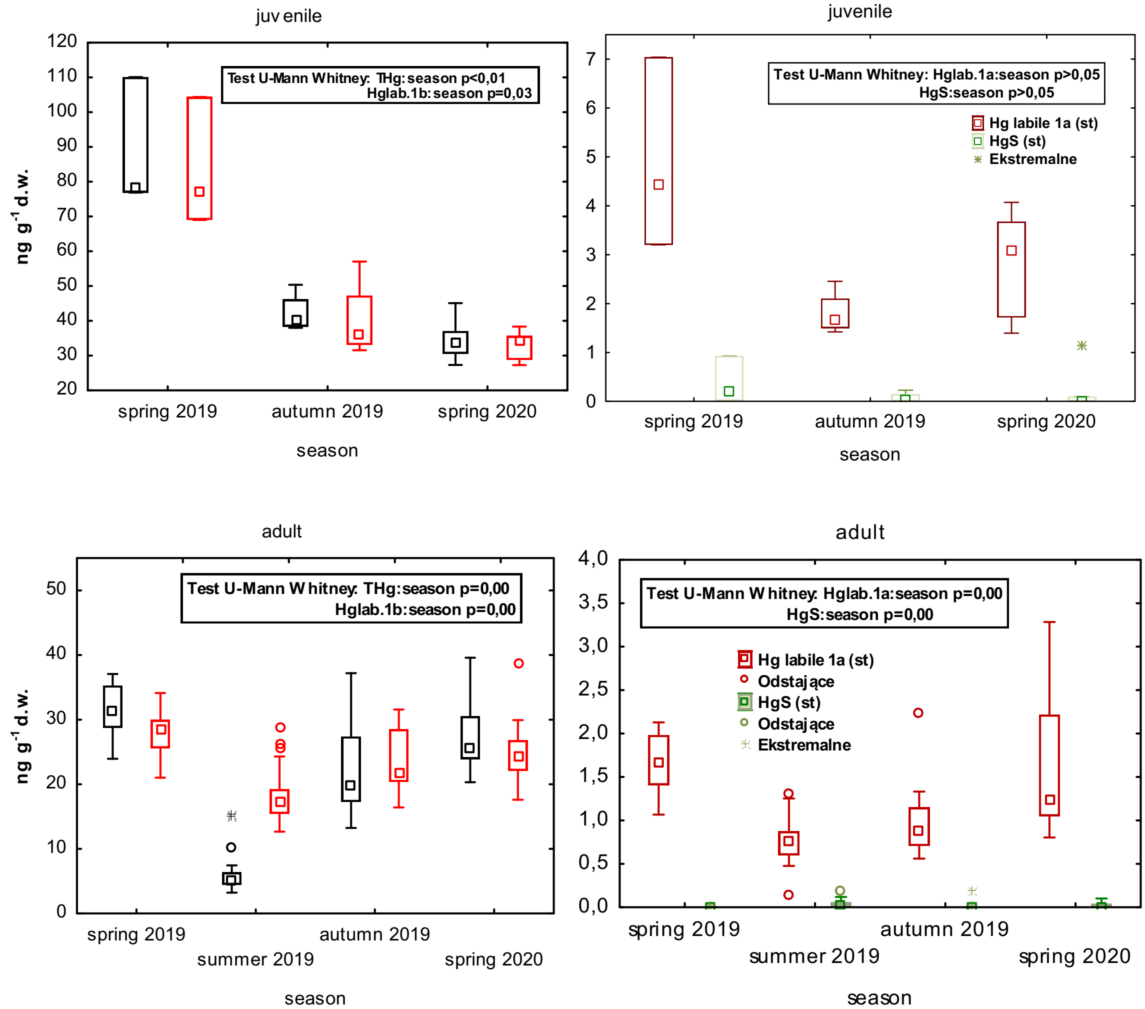

Fig. 5. Seasonal variation in the concentration of total mercury and its fractions: (a) in the soft tissue of juvenile specimens (b) in the soft tissue of adult *Rangia cuneata.*

where smaller organisms had higher concentrations of fractions and Hg_{TOT} compared to older, reproductively capable individuals. Additionally, higher concentration in Atlantic rangia body during spring-winter - rainfall season could cause by weather phenomena. Erosion of Hg associated with soil particles and intrusion of Hg bound to suspended particulate matter from the land (river discharge and flushing) of the estuary may increase bioavailability of Hg in the estuarine systems (Chakraborty et al., 2019; Gębka et al., 2019). Additional metal source is deposition of atmospheric mercury from coal combustion during heating season (Beldowska et al., 2014). Consequently, clams including Atlantic rangeland are exposed to the xenobiotic Hg, which feeds on organic matter. In this way, it can be bioaccumulated in benthic organisms and biomagnified in the subsequent links of the trophic chain (Jackson, 1998).

3.2.1. The role of shell in the detoxification of the body from Hg

Previous work on determining the concentration of contaminants including mercury in clams has focused on the soft tissue, as this is what is consumed by organisms, including humans. The results presented here on Hg and its fraction in the shell of *R.cuneata* are among the first in the literature where an attempt has been made to determine the role of the shell in the transfer of Hg in the bivalve organism. Mechanisms of the muscle-shell transport for the studied mercury forms derivatives are unknown. The mean mercury concentration in the clam shell was 1.2 ng g^{-1} d.w. This was 18 times lower than the mean Hg concentration in the body of *R.cuneata*. PCA analysis showed an indirect effect of biometric factors on the concentration of total mercury as well as its labile and stable forms in the *R. cuneata* shell (SV). Rather, Factor 1 and Factor 2 indicate a parallel increase in Hg_{TOT} and $Hg_{lab.1a}$, $Hg_{lab.1b}$ and HgS concentrations - influenced to some extent inversely by shell growth - dilution. Considering shell weight, and body weight in successive size classes, it can be observed that they increase in a linear manner, and the increase in shell weight was much more intense: an order of magnitude greater than body weight (SVI). This may indicate that the dilution of Hg in the shell is systematic in the process of shell biomineralization, regardless of the size and age of the bivalve.

In the Atlantic rangia shell, biometric parameters did not determine the concentration of Hg and its forms, the opposite of what was observed in the soft tissue. No differences in metal concentration were observed between adult and juvenile specimens ($p > 0.05$; SVII). Also, no statistically significant change was observed in the concentration of Hg and its forms, from one size class to the next. The variation in the concentration of Hg and its fractions in the shells was significantly more influenced by water temperature - season (factor 3; SV), which was closely related to the physiological state of the bivalves. The concentration of Hg_{TOT} and its fractions in the shell was positively correlated with the concentration of Hg_{TOT} and its fractions (except HgS) in the soft tissue in spring and autumn (Table 2). An increase in the concentration of the metal in the body during these seasons resulted in an increase in its concentration in the shells. Linear correlations indicate that $Hg_{lab.1a}$ was transferred from the body to the shell and incorporated there as $Hg_{lab.1a}$ but also as $Hg_{lab.1b}$ and HgS (Table 2). Similarly, $Hg_{lab.1b}$ - however, these fractions were not transferred to HgS. It is likely that HgS was derived from the transformation of $Hg_{lab.1a}$ to the stable form. In summer, Hg_{TOT} concentrations were lowest in the shell, analogous

Table 2
Correlation coefficients of total mercury and its forms in the soft tissue and shell of *Rangia cuneata*.

	THg(st)	Hg labile 1a (st)	Hg labile 1b (st)	HgS (st)
THg(sh)	0.33	0.2	0.22	-0.01
Hg labile 1a (sh)	0.48	0.35	0.39	-0.01
Hg labile 1b (sh)	0.38	0.25	0.26	-0.01
HgS (sh)	0.21	0.2	0.16	-0.07

*In red are marked values for which $p < 0.05$ sh – shell; st – soft tissue.

to the soft tissue. However, no statistically significant correlation ($p > 0.05$) was observed during this season. It is likely that in summer, when the water temperature is highest, the bivalves have the most energy for the reproductive process as well as for growth, and there is an efficient dilution of Hg and its fraction in the process of shell calcification than the transfer of Hg from the body to the shell itself.

Although, in the chemical composition of *R.cuneata* shells in the Vistula Lagoon, proteins accounted for the largest share, on average 40 %, carbohydrates 30 %, lipids and mineral matter a total of 30 % (Dąbrowska, 2017), the concentration of Hg in ng g^{-1} d.w. and its fractions was at low levels, most likely due to the saturation of the shell with calcium carbonate, which is used in the calcification process - shell growth. Concentrations of Hg_{TOT} and its fractions ranged from BLD in adults, to 3 ng g^{-1} d.w. in juvenile specimens (SVII). Therefore, the shell does not play an important role in the transfer and detoxification of the bivalve organism from toxic Hg. On the one hand, the concentration of Hg_{TOT} and its labile and stable forms is higher in the bivalve body relative to the shell. However, on the other hand, considering the mass of Hg accumulated in the whole clam body and in the whole clam shell, it was found that the mass of Hg accumulated in the body was similar to the mass of Hg accumulated in the shell during the spring and autumn seasons, while in summer the mass of accumulated Hg in the shell was higher than in the body (Table 3; SVIII). With growth (age of the bivalve), the mass of accumulated mercury and its fraction in the whole shell increased. Hg sulphide was almost 100 % (of the total accumulated in the whole body) accumulated in the whole shell of *R.cuneata* (Table 3). The lowest concentration of Hg in the corpuscle in summer, when it has the most energy for the detoxification process, in addition to dilution due to growth and reproduction, was intensified by the deposition of Hg in the shell where Hg_{TOT} was 65 % accumulated (Table 3; SVIII) by elimination of the metal from the corpuscle. In spring and autumn, the percentage of the $Hg_{lab.1a}$ fraction in the shell increased in subsequent size classes (SIX). As a result of preparation for reproduction in spring and preparation for winter stagnation in autumn, by more intensive filtering of water from the food, the bivalves were able to transfer loosely adsorbed inorganic halogenated forms of mercury together with calcium ions and carbonates into the shell, and the process was intensified with shell growth. Consequently, $Hg_{lab.1a}$ in spring and autumn was 67 % and 59 %, respectively, accumulated in the shell mass (Table 3). This process is also confirmed by factor 3 (12 %) of the shell PCA analysis, where the concentration of the Hg labile fraction 1a was partly seasonally dependent (SV). After the winter stagnation due to inhibited detoxification processes of *R.cuneata* from mercury, the transfer of mercury and its fractions into the shell in the warm spring was of least importance in the detoxification process of the organism from the xenobiotic, indicating that the process of Hg body-shell transfer is probably inhibited in winter.

Table 3
Hg and its forms storage (%) in juvenile and adult shells of *Rangia cuneata*.

		Storage of shell [%]			
		HgT	Hg labile 1a	Hg labile 1b	HgS
Spring 2019	Juvenile	14	25	8	94
	Adult	41	67	29	100
Summer 2019	Adult	65	43	14	95
Autumn 2019	Juvenile	26	29	13	96
	Adult	49	59	29	98
Spring 2020	Juvenile	17	14	12	82
	Adult	43	39	33	98

4. Spatial changes

The Vistula Lagoon is a small estuary with limited water exchange and the shallow nature of the reservoir bottom and strong water dynamics. This could potentially indicate a homogeneous mercury contamination of the reservoir. However, analysis of Hg concentration and its fraction in the *Rangia cuneata* showed spatial variability in the Vistula Lagoon ($p < 0.05$; Fig. 6). Bivalves at stations ZAT-K, 43 L and 40 L had the highest concentrations of Hg_{TOT} in the soft tissue (median: 143, 115, 137 ng g^{-1} d.w. respectively) (Fig. 6). These are the easternmost and westernmost regions of the Vistula Lagoon, characterized by sandy sediment (the least contains of FSF) (Fig. 1; SX). In the central part of the Vistula Lagoon at stations IW100, 29 L and P14A, the median concentration of total mercury was at a similar level - 80 ng g^{-1} d.w. (Figs. 1; 6). The lowest median concentration of Hg_{TOT} as well as in small (V - VII class size) and large organisms (VIII - X class size) was determined in the bivalve body just off the mouth of the Elbląg river at station SUC-R2: median 56 ng g^{-1} s.m. (Figs. 1; 6). In geographical variation, analogous to the above discussion, the highest Hg_{TOT} concentrations were determined in smaller organisms: from 20 to 35 mm (V - VII class size), while lower Hg_{TOT} concentrations were determined in larger organisms: from 35 to 46 mm (VIII - X class size) (Table 5). Considering small (V - VII class size) and large organisms (VIII - X class size), Hg concentrations were highest at stations in ZAT-K, 40 L and 43 L, located closest to land. The highest Hg concentrations in bivalves were measured where sandy sediment was present (Table 5). Analysis of the habitat (sediment) in terms of Hg concentration and organic matter (MO) content and the proportion of the finest fraction (FSF) showed the opposite trend for the stations: in areas where Hg concentrations in *R. cuneata*

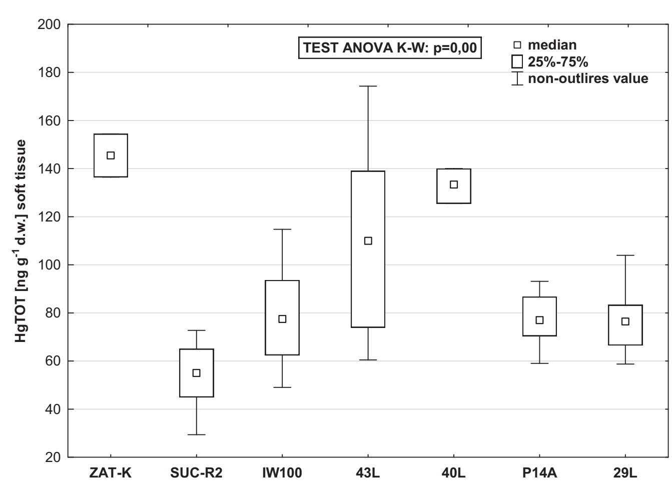

Fig. 6. Spacial changes of mercury concentration – Hg_{TOT} [ng g^{-1} d.w.] in *Rangia cuneata* body from Vistula Lagoon, August 2021.

Table 4
Total mercury concentration normalized to organic matter and fine fraction in sediments from the Vistula Lagoon (August 2021).[a]

Station	Hg[ng g^{-1}]	MO[%]	FSF[%]	Hg_{LOI}	Hg_{FSF}
ZAT-K	31	0.6	4	5167	775
SUC-R2	173	1.5	10	11,533	1730
IW100	177	1.6	11	11,063	1609
43 L	153.2	2.6	10	5892	1532
40 L	117	1.9	7	6158	1671
P14A	90.1	1.1	6	8191	1502
29 L	136	1.8	8	7556	1700

[a] MO – organic matter; FSF – fine sediment fraction.

were highest, the sediment was characterized by the lowest Hg_{TOT} values and when Hg concentrations were normalized to MO and FSF content (Table 4; SX). In contrast, in the areas where the lowest Hg concentrations were determined in the Atlantic rangeland body, the organic matter and sediment was most contaminated with mercury (Table 4; SX). This indicates a lack of bioaccumulation of Hg from the sediment and with organic matter from the sediment, as a result of the different food preference of *R. cuneata* (as discussed in section 4.1.).

This geographical variation in mercury concentrations in *R.cuneata* clams was also due to the form in which mercury was present in the sediment. Higher concentrations of Hg_{TOT} in the bivalve body were determined in the areas closest to the shore (ZAT-K, 43 L and 40 L), where higher concentrations of $HgSO_4$ and HgO ($Hg_{lab.2}$) were determined in sediments with a lower proportion of the fine fraction. Consequently, a directly proportional relationship was observed between the concentration of Hg_{TOT} in the clam and the concentration of $Hg_{lab.2}$ in the sediment ($r = 0.81$; $p < 0.05$), and this form of mercury in the sediment was inversely correlated with the fine fraction, FSF ($r = -0.78$; $p < 0.05$). At stations located in the mouth of the Elbląg river (SUC-R2), into which the largest river Elbląg flows, and in the central part of the Vistula Lagoon (IW100, 29 L and P14A), sediments with higher dispersion (FSF) were characterized by higher concentrations of $Hg_{lab.1b}$ ($r = 0.76$; $p < 0.05$) and HgS ($r = 0.87$; $p < 0.05$). Consequently, correlations were observed between Hg concentration/FSF and the concentration of $Hg_{lab.1b}$ fractions ($r = 0.8$; $p < 0.05$) and HgS ($r = 0.85$; $p < 0.05$) in the bivalve body. These were regions of accumulation of both fine fraction, organic matter and mercury. However, as a consequence of MO degradation processes, mercury underwent transformations that led to a situation in which the metal largely migrated to the stable form HgS and the poorly accessible form bound with humid compounds for organisms (which is part of Hg_{abs}). Consequently, regions with higher Hg concentrations in the sediment were characterized by lower Hg concentrations in the clams body compared to shallower stations (Fig. 6; SX). This is further confirmed by correlations between Hg concentration in the body and organic matter ($r = -0.85$; $p < 0.05$) and $Hg_{lab.1b}$ concentration in the body and organic matter ($r = -0.9$; $p < 0.05$). Consequently, at deeper stations, clams bioaccumulated and biomagnified mercury from the sediment less efficiently.

Table 5
Regional variability of total mercury concentration in the soft tissue of *Rangia cuneata* by size of organisms (August 2021).[a]

Station		Class size [mm]	
		Small V-VII [20–35 mm]	Large VIII-X [35–46 mm]
ZAT - K	HgTOT	145.4	–
	n	4	–
SUC-R2	HgTOT	55.9	51.8
	n	10	15
IW100	HgTOT	93.4	62.3
	n	11	16
43 L	HgTOT	116.2	89.8
	n	16	4
40 L	HgTOT	134.2	113.2
	n	4	2
P14A	HgTOT	85.4	73.8
	n	12	7
29 L	HgTOT	–	73.1
	n	–	20

[a] n - -number of clams.

The correlations obtained may indicate that the shallower areas had a more accessible (labile) form of mercury in the sediment. Hence, higher mercury concentrations in clams in areas where the sediment was least contaminated with the metal. Furthermore, in shallower regions with better oxygen conditions, oxidation of the stable form HgS to the labile $HgSO_4$ may occur (Saniewska et al., 2019). This may also be confirmed by the inverse relationship between HgS and $Hg_{lab.2}$ in the sediment ($r = -0.78$; $p < 0.05$).

Furthermore, Atlantic rangia in the Vistula Lagoon is an alien species of bivalve. The first reports of its occurrence date back to the 21st century. The variation in bivalve size at the measurement stations and in Hg concentration may indicate that its adaptation in the Vistula Lagoon is still in progress (Warzocha et al., 2016). The results obtained for stations IW100 and SUC-R2 in the area of the Elbląg river could indicate the classics of *R.cuneata* occurrence in the Vistula Lagoon, where the bivalve has established the most stable population. The organic matter-rich sediments in these areas, with a more muddy character (more finely dispersed), provide good living conditions for *R.cuneata* for the growth and reproduction process, compared e.g. to the most north-western (ZAT-K) and eastern (40 L and 43 L) stations. The percentage of labile and stable forms of Hg in the corpuscle had the same distribution for all measurement stations, where the $Hg_{lab.1b}$ fraction accounted for >90 % of the contribution to total Hg_{TOT} (Fig. 7). It is likely that, irrespective of the region and food availability and quality, clams feed on organic matter and SPM (Fig. 2b), where the $Hg_{lab.1b}$ fraction represents a high percentage and is the main source of these forms of mercury.

5. Conclusion

Mercury concentration in *Rangia cuneata* were low in the southern Baltic compared to other clams species from the area. The most intensive

Fig. 7. Percentage share [%] of mercury fractions in a) sediment b) *R. cuneata* soft tissue from Vistula Lagoon, August 2021.

detoxification processes of the soft tissue from Hg occurred in summer when the clams had the most energy (faster metabolism) and was stimulated by water temperature. Atlantic rangia grew faster than they accumulated mercury and its forms from food during the year, with a consequent dilution of mercury in the soft body (BSAF<1; BAF < 1). The lowest concentrations of Hg and its forms in the soft tissue of *R.cuneta* in the following size classes were observed during the summer season, which was further intensified by summer reproduction.

Geographical variation in mercury concentrations in Atlantic rangia body was related to the form of Hg in the sediment. In areas where were finer fraction and organic matter accumulated in the sediment, mercury was built in hardly decomposed organic matter and then was in less available form for living organisms like rangia. As a result of that, the clams had lower Hg concentrations in the body in this regions.

The transfer of mercury from the soft tissue to the shell contributed to the purification of the body from Hg, mainly from inorganic forms. This process was more efficient in adult specimens, relative to juvenile specimens. Mercury concentrations in *Rangia cuneata* were at low levels. Assuming that in the future this organism, due to its increasing prevalence, will become an increasingly common component of the fish diet, less toxic mercury will be introduced into the marine trophic chain, including humans. This is important because Hg has no positive function in living organisms and is toxic even at low doses. In addition, this result indicates that storage in the shell can be an important way of elimination of Hg and selected forms of Hg from clams soft tissue and the trophic chain. *Rangia cuneata* in estuaries or bays can be used to assess changes in the environment, considering the size of the organism or selecting organisms of the same size for spatial or temporal changes.

CRediT authorship contribution statement

1. Bartłomiej Wilman

- Sampling clams material
- preparation of material and chemical analyses
- statistical analysis; graphical presentation of results
- Writing original draft - preparation of each chapter of the article

2. Magdalena Bełdowska

- Supervision
- Conceptualization - Ideas
- Writing review and editing

3. Agata Rychter

- Sampling clams materials
- Biological analyses
- Conceptualization - Ideas
- Writing review and editing

4. Ryszard Kornijów

- Sampling clams material
- Writing review and editing

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160018.

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Publication 1

Supplementary material

SI Total mercury concentration [ng/g] and percentage of Hg fraction in clams from the southern Baltic region

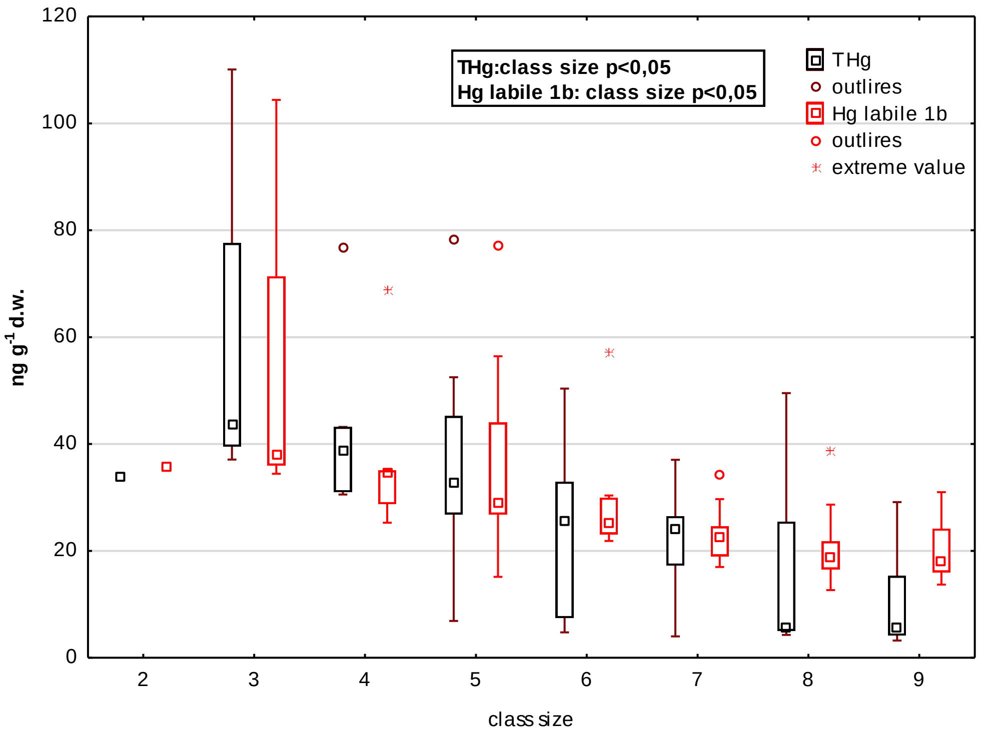
species	tissue	Hg_{TOT}	$Hg_{labile\ 1a}$	$Hg_{labile\ 1b}$	HgS	$Hg_{labile\ 2}$	$Hg_{residual}$	reference
D.polymorpha	soft tissue	40	32	64	2	1	1	Wilman et al.,
M.trossulus		148	28	71	1	0	0	Jędruch et al., 2019
R.cuneata		22	5	95	0	0	0	this study

SII Biota-sediment accumulation factor [BSAF] and biota accumulation factor [BAF] callculated for *Rangia cuneata* from Vistula Lagoon

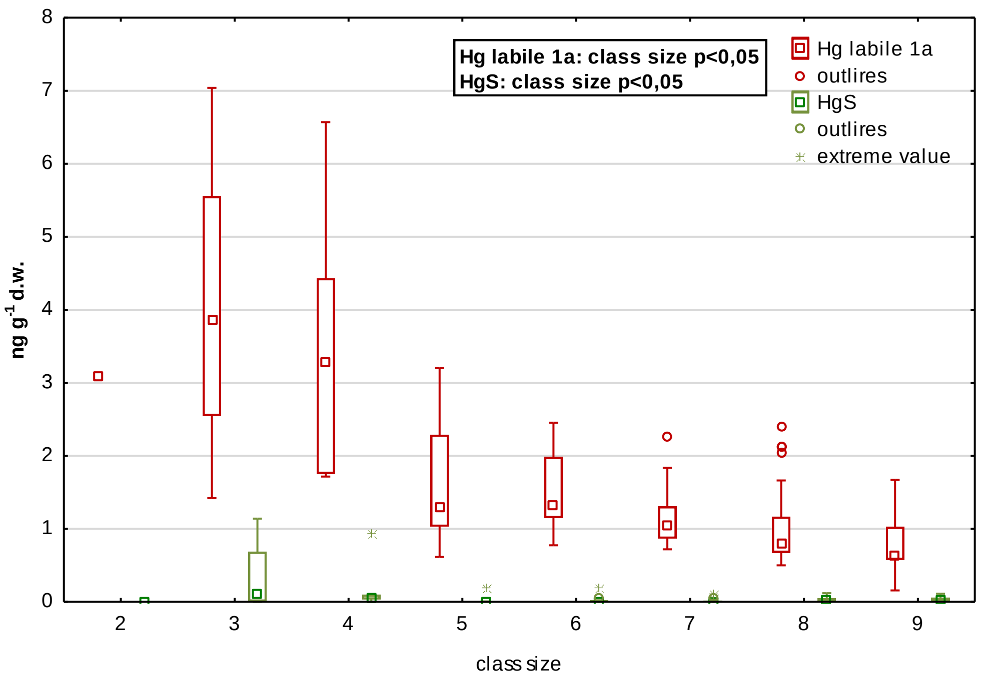
season	BSAF	BAF_{SPM}	$BAF_{plankton}$
spring 2019	<1	<1	<1
summer 2019	<1	<1	<1
autumn 2019	<1	<1	<1
spring 2020	<1	<1	<1

SIII Change in concentration of total mercury and Hg labile 1b (a) and Hg labile 1a and HgS (b) in the soft body of Rangia cuneata in successive size classes of clams

a)

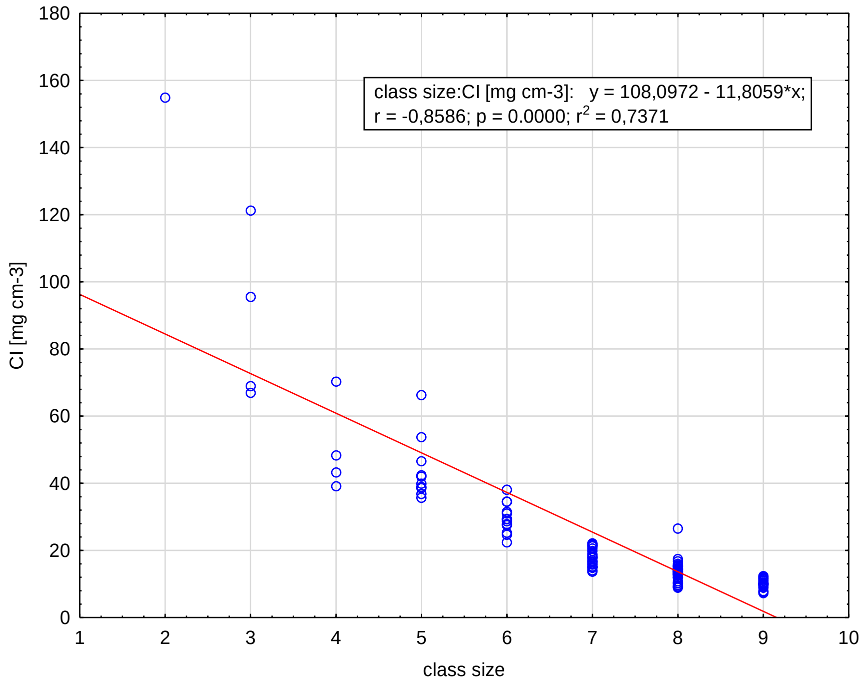


b)

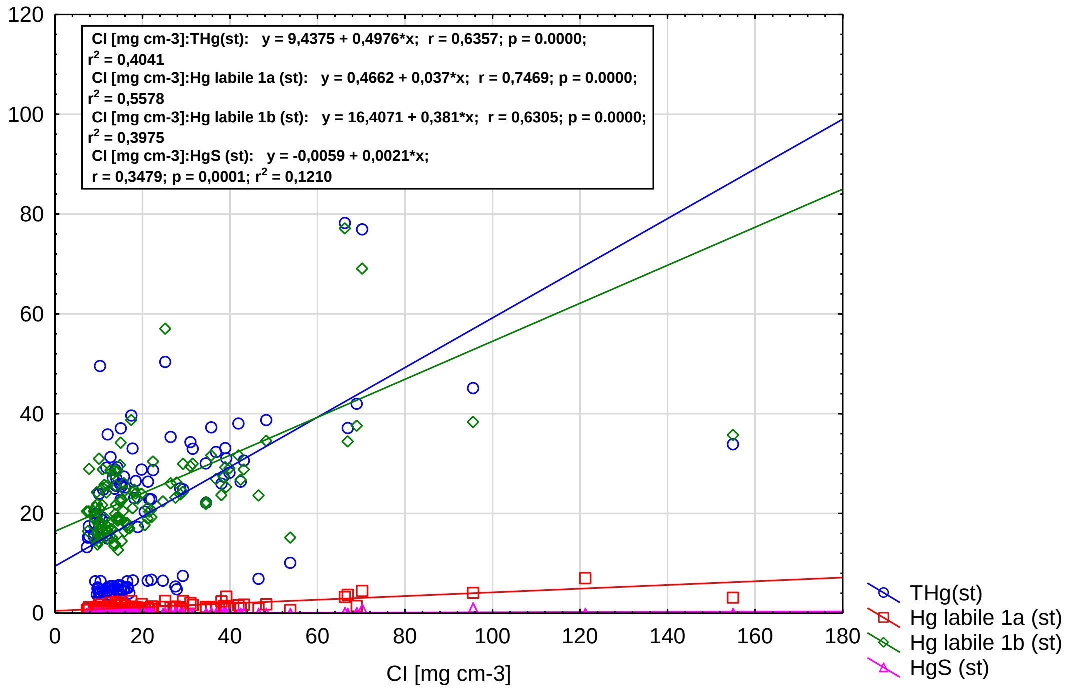


SIV Change in the condition index (CI) of clams in successive size classes (a) and the effect of clam condition on the concentration of mercury and its forms (b) in the soft tissue of *Rangia cuneata*

a)

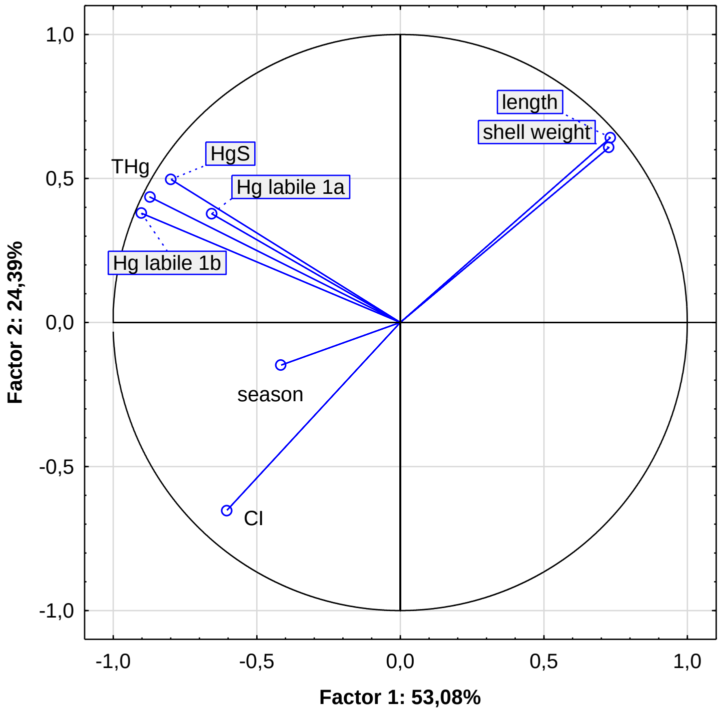


b)

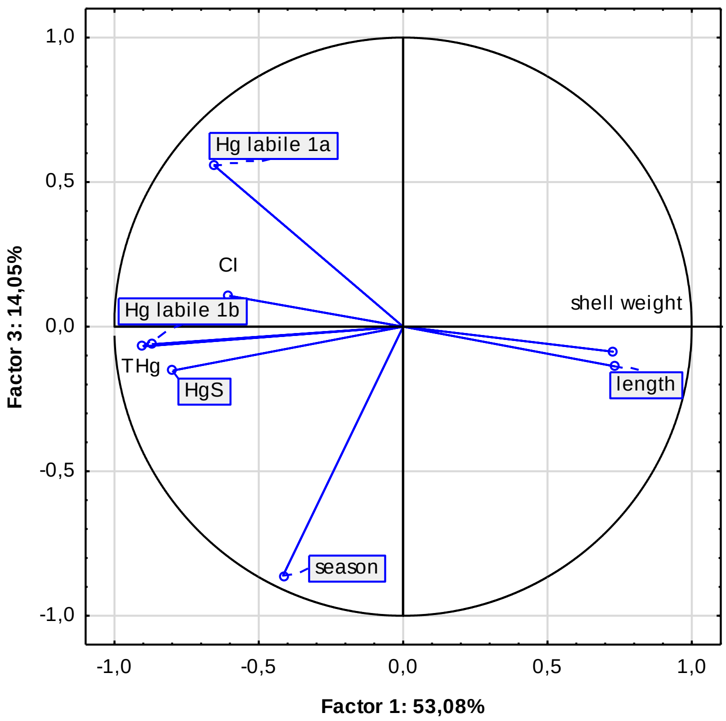


SV Principal component analysis (PCA) of total mercury concentration and its fraction with biometric parameters in *Rangia cuneata* shell: (a) factor 1:2 (b) factor 1:3 and their classification

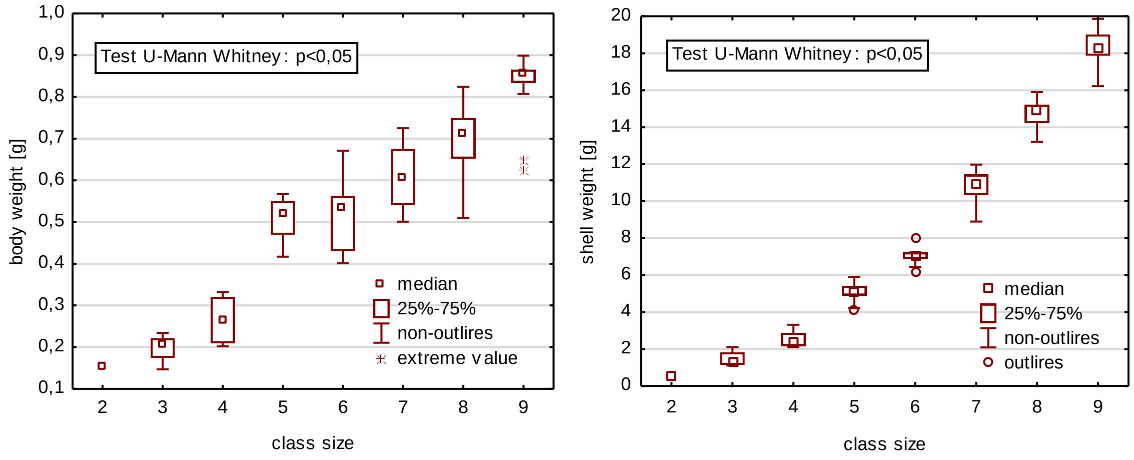
a)



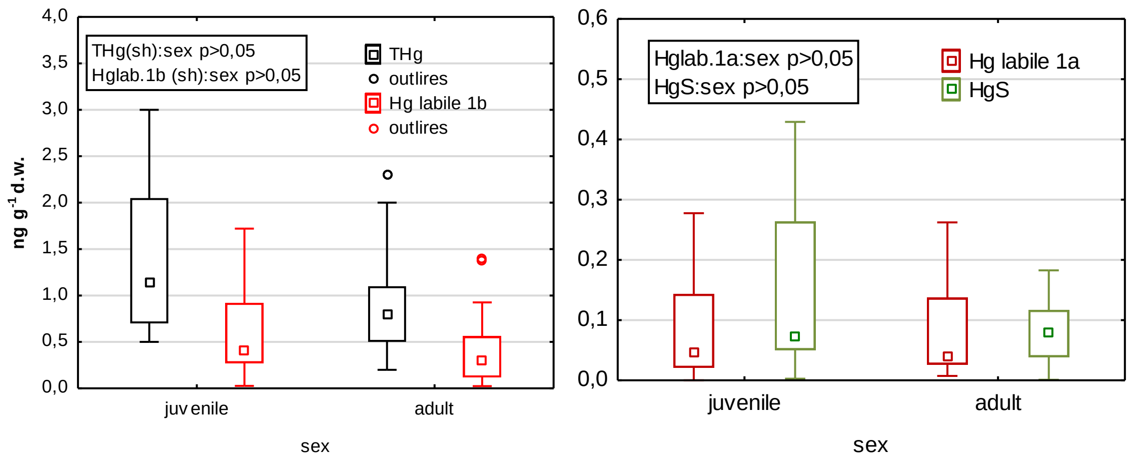
b)



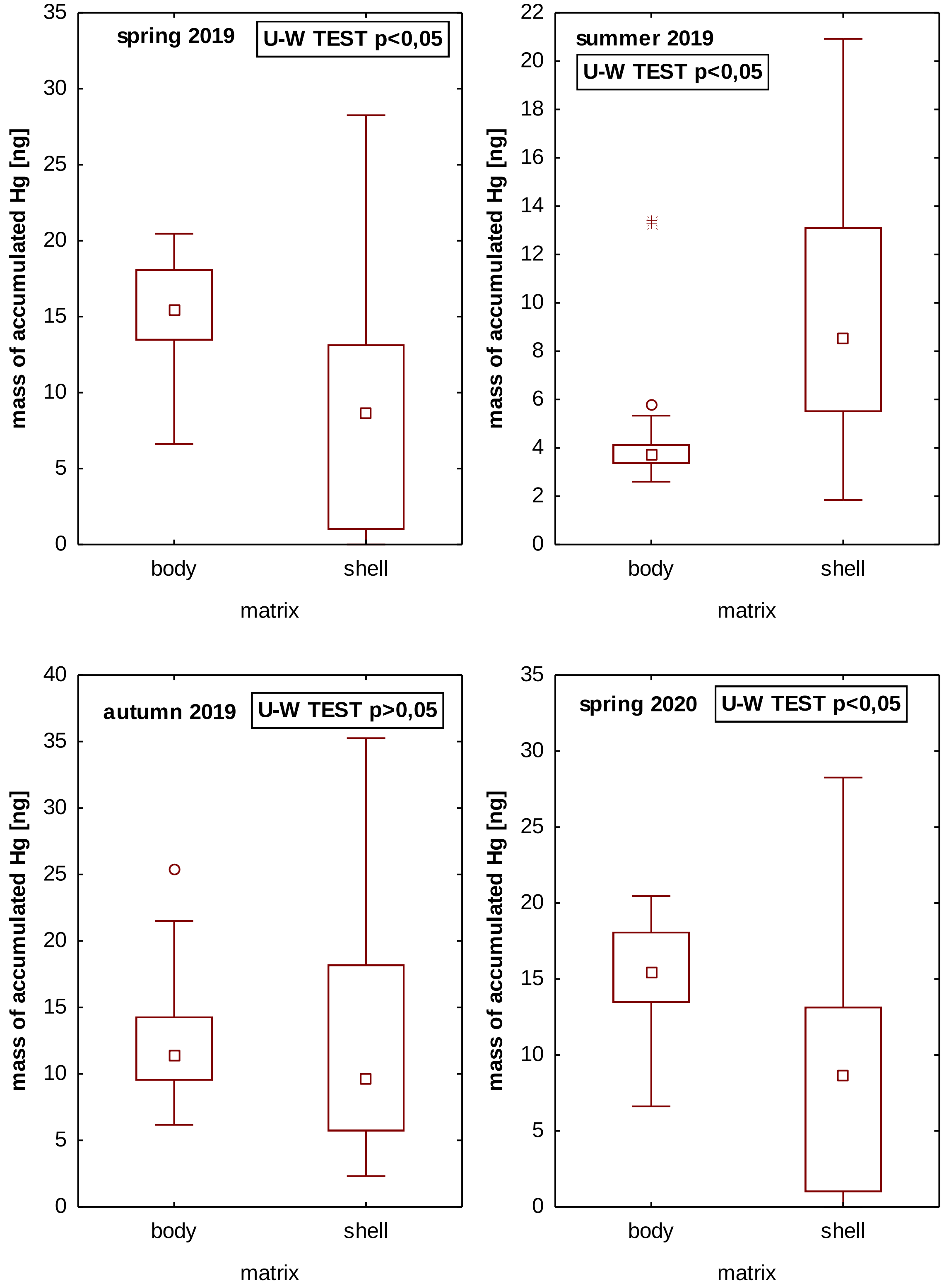
SVI Increase in tissue muscle mass (a) and shell weight (b) of *Rangia cuneata* clams in successive size classes



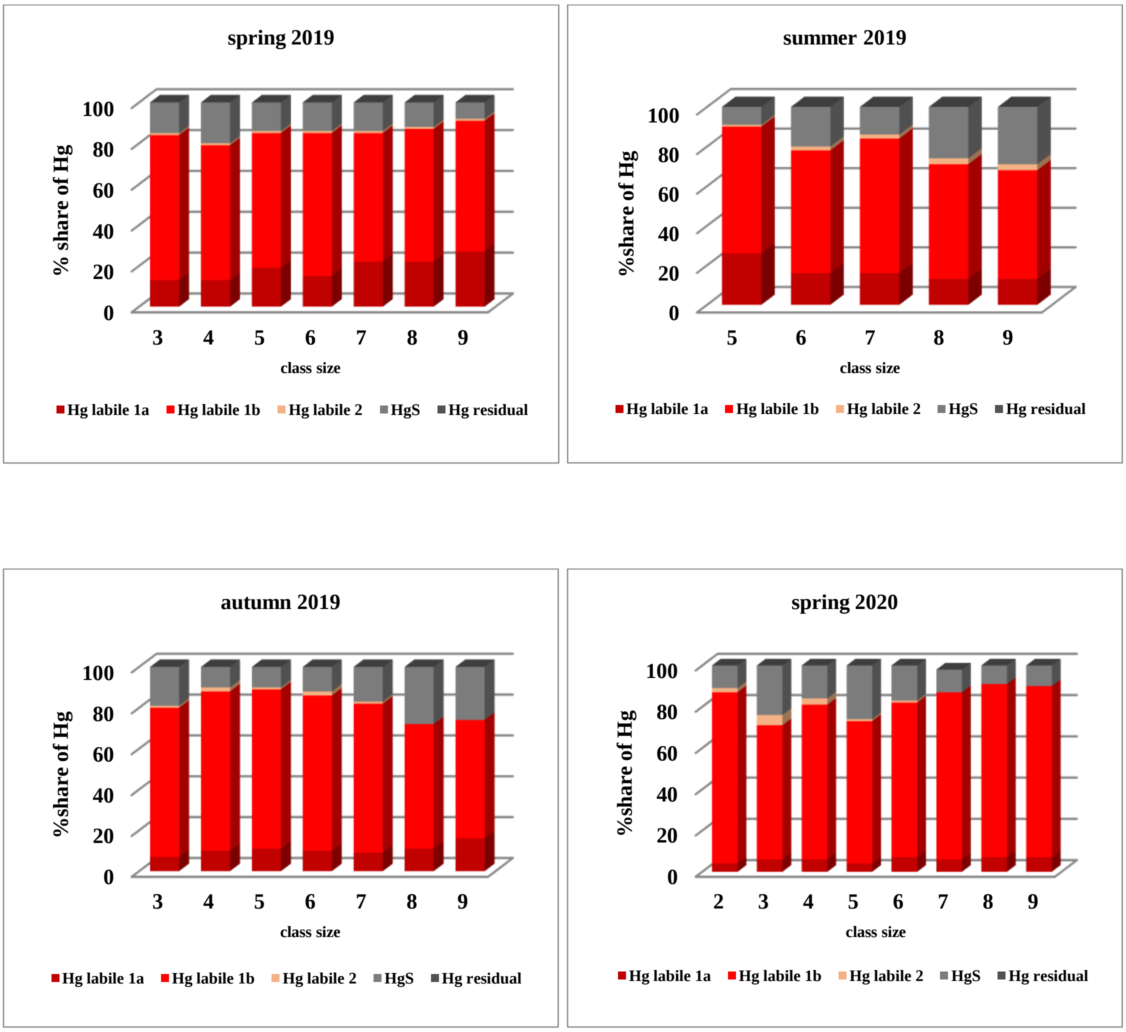
SVII Change in concentration of total mercury and Hg labile 1b (a) and Hg labile 1a and HgS (b) in the shell of *Rangia cuneata* by sex of clams



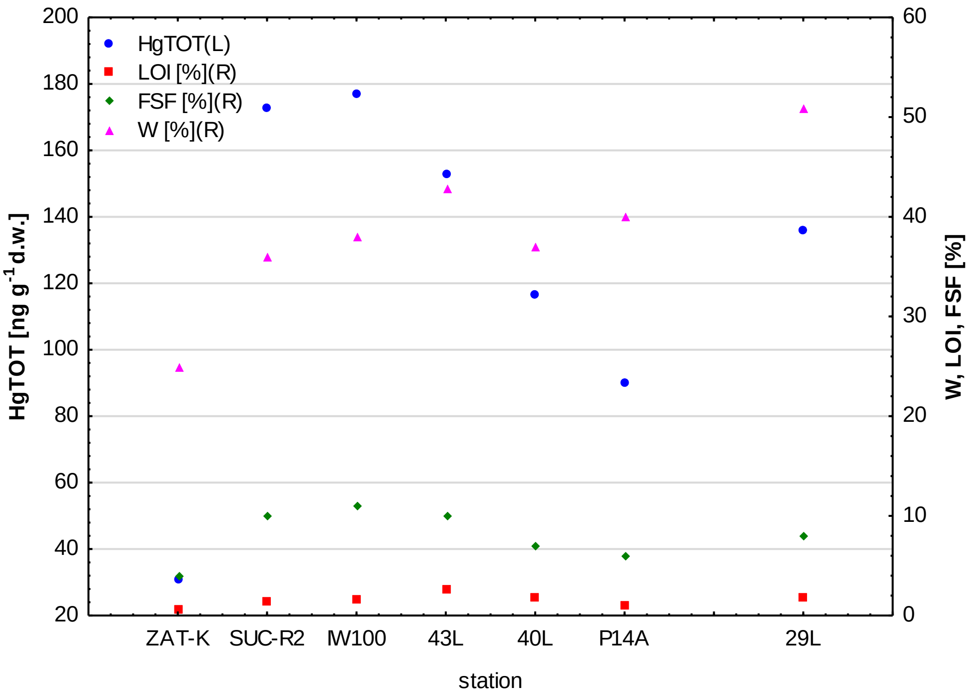
SVIII The mass of accumulated mercury in the body mass and in the shell mass in each measurement season



SIX Seasonal variability of the percentage of Hg forms in the shell of *Rangia cuneata* with respect to the size of the clams studied



SX Physico-chemical characteristic of habitat (sediment) *R.cuneata* from Vistula Lagoon, August 2021



Statement of co-authors

Individual authors contribution statement has been structured using the Contributors Roles Taxonomy (CRediT) recommended by Brand et al. (2015).

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I declare that my contrubution in publication :

Wilman, B., Bełdowska, M., Rychter, A., Kornijów, R. 2023. Different pathways of accumulation and elimination of neurotoxicant Hg and its forms in the clam Atlantic rangia (*Rangia cuneata*). *Science of The Total Environment*, 858, 160018. doi: 10.1016/j.scitotenv-.2022.160018.

Included in my doctoral dissertation entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- First author, corresponding author
- Collection and preparation of environmental samples for biological and chemical analyses and their implementation
- Processing and interpretation of results
- Preparation of original draft of the manuscript
- Responding to reviews, editing the manuscript

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Included in the doctoral dissertation by Bartłomiej Wilman entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- Supervision
- Conceptualization - Ideas
- Writing review and editing
- Resources and funding acquisition

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Included in the doctoral dissertation by Bartłomiej Wilman entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- Head of research cruises
- Analysis of biometric parameters of organisms, tissue preparation
- Support in the field of biology and ecology of mussels
- Writing review and editing

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Included as part of the doctoral dissertation by Bartłomiej Wilman, entitled: *Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels: A case study of clams and crabs from the Vistula Lagoon,*

comprised the following:

- Sampling clam material
- Writing, reviewing, and editing

Ryszard Kornijów

Publication 2

<u>Wilman, B</u>., Bełdowska, M., Rychter, A., Popławska, A. 2023. Factors determining bioaccumulation of neurotoxicant Hg in the zebra mussels (*Dreissena polymorpha*): influence of biometric parameters, sex and storage of shell. *Marine Pollution Bulletin*, *197*, 115718. doi: 10.1016/j.marpolbul.2023.115718.

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Factors determining bioaccumulation of neurotoxicant Hg in the zebra mussels (*Dreissena polymorpha*): Influence of biometric parameters, sex and storage of shell

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ABSTRACT

One of benthic organisms exposed to contact with mercury in the southern Baltic is alien species of clam: *Dreissena polymorpha*. As this organism is increasingly dynamic in various regions of the world including the southern Baltic region, it is reasonable to ask whether it tolerates elevated concentrations of xenobiotics? Does it effectively eliminate Hg? The study determined the effects of biometric parameters and water temperature on the rate of accumulation and efficiency of eliminating Hg from body. Investigations focused on the shell which represents poorly-recognized role in the process of Hg distribution in clams. The results showed that especially during warm season, clams effectively reduced the levels of Hg in their body by the biodilution of Hg and reproduction. Important factor influencing detoxification was Hg transfer from the soft tissue to the shell. This protects the soft tissue against the toxic effect of Hg.

1. Introduction

Mercury (Hg) is an element fulfilling no positive function in living organisms. It can have a toxic effect even in trace amounts (Castro-González and Méndez-Armenta, 2008; Hosseini et al., 2013). Extensively described cases of Hg poisoning in the municipality of Minamata on the Minamata Bay in Japan largely triggered global urgency for research on mercury and the threat related to the occurrence of the toxin in reference to the environment, water food chain, and human health (D'Itri, 1993; Malm et al., 1995). It was evidenced that Hg from natural and anthropogenic sources increasingly contributes to global problems related to human health and the health of other living organisms (Bosch et al., 2016; Jinadasa et al., 2013; Ye et al., 2016). Nonetheless, it is human activity that mainly contributes to an increase in the level of Hg in the atmosphere, on land, and primarily in water ecosystems (Asare-Donkor and Adimado, 2016; Driscoll et al., 2013; Jinadasa et al., 2013; Kojadinovic et al., 2006). Due to this, the presence of Hg in products of marine origin raises interest in the context of public health (Burger and Gochfeld, 2012; Gibb and O'Leary, 2014; Mozaffarian, 2009). The ability of Hg to be accumulated in and excreted from water and human organisms largely depends on its chemical form (Bosch et al., 2016). Organic mercury, mainly methylmercury (MeHg) is subject to biomagnification in subsequent links of the trophic chain. It is considered (next to dimethylmercury) the most toxic form of Hg (Branco et al., 2007; Kojadinovic et al., 2006).

In the past, a significant source of mercury supply to the sea (including the Gulf of Gdańsk and Vistula Lagoon) was uncontrolled discharge of untreated industrial sewage. Consequently, marine sediments have become important storage sites of Hg (Wrembel, 1999). The hydrological status of the Vistula Lagoon is primarily defined by the inflow of fresh water from 20 rivers and water exchange with the Baltic Sea, although to a much lesser extent. The main inflow is supplied by the Russian Pregolya River; the inflow from Polish rivers comes from the Bauda, Elbląg, Pasłęka, Nogat, and Szkarpawa Rivers (Ezhova et al., 2005; Uścinowicz and Zachowicz, 1996). The Vistula lagoon is currently polluted, mostly by residues of various plant protection products and fertilisers used in agriculture. Sewage from mechanical-biological wastewater treatment plants in Tolkmicko, Frombork, Braniewo, Stegna, and Krynica Morska continuously directly enter the lagoon. The Vistula Lagoon has therefore been included in the natural environment protection program Natura 2000, and entered into the list of Baltic Sea Protected Areas introduced by HELCOM (Szefer, 2002). Nowadays, the

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inflow of metals to the lagoon is limited, and the remobilisation of Hg from the sediment is becoming an increasingly pressing issue. As a result of a number of biotic and abiotic processes, mercury becomes remobilised into the marine environment. It can be bioaccumulated in benthic organisms, and biomagnified in subsequent links of the trophic chain, thereby representing the greatest threat to marine mammals, water birds, and humans (Jackson, 1998). The resuspension of Hg associated with fine sediment particles and the supply of Hg bound to suspended particulate matter from outside (river discharge and flushing) of the estuary may also increase the bioavailability of Hg in the estuarine systems (Chakraborty et al., 2019).

One of the benthic organisms exposed to mercury is zebra mussel (*Dreissena polymorpha*). These introduced (non-indigenous) species are non-native in the coastal waters of the Vistula Lagoon. The zebra mussel was introduced to Europe from the areas of the Caspian and Black Sea, probably in the ballast waters or attached to the hulls of carrier vessels. Its life expectancy is 3–5 years (Stańczykowska, 1977). *D. polymorpha* is a suspension feeder ingesting large quantities of detritus and phytoplankton. *D. polymorpha* is also an important food source for many animal species. The larval stages are eaten by fry and predatory planktonic crustaceans (Sprung, 1993; Knoll, 2004). Settled mussels are mainly eaten by crayfish, crabs, fish (roach *Rutilus rutilus*, eel *Anguilla anguilla*, bream *Abramis brama*, etc.), birds (coot *Fulica atra*, czernica *Aythya fuligula*, *Aythya ferina*, and many others), and mammals (e.g. muskrat *Ondatra zibethicus*) associated with the aquatic environment (Hamilton and Ankney, 1994; Molloy et al., 1994).

Despite numerous works on the biology and ecology of the mussel *Dreissena polymorpha*, no literature is available regarding the role of the species in the trophic web in the southern Baltic Sea region. There are also no scientific reports on the determination of mercury levels in the studied clams in this area. As a result, they appear and spread in the waters of the southern Baltic Sea and other regions more and more extensively (Karatayev et al., 2011). In this way, especially in the estuarine lagoons, they become an important element of the diet of trophic web organisms, particularly birds and benthic feeding fish (flounder, eel, bream, roach), commercially fished and consumed by humans. It is therefore important to determine the concentration level of mercury in the non-native species (Mingyang et al., 2008; Quinn et al., 2014). In addition, the important question arises whether a specie that spreads so dynamically, is it "resistant" to pollution? Therefore, do these clams effectively eliminate toxic Hg from their body so that they can expand the region? What processes are involved in this? The scientific aim was to study the processes of accumulation and detoxification of Hg by *Dreissena polymorpha* in different seasons taking into account sex and size class of the during the year, as a result of changes in physical and chemical conditions of water, the availability of food base, and individual development (growth and reproduction). These factors may significantly affect differences in Hg concentration and its labile and stable forms in the analysed species. For this purpose, the concentration of total mercury and its labile and stable forms were determined in the analysed organisms (soft tissue and shell). The majority of the literature has so far focused on the biomagnification and bioaccumulation of total mercury or its most toxic form, namely methylmercury and the problem of magnifying and accumulating mercury in organisms is very well recognized in the trophic web of marine ecosystems (Wren and MacCrimmon, 1986; Baeyens et al., 2003; Schmitt et al., 2011; Hong et al., 2013; Gentès et al., 2021). It is however poorly investigated what forms of mercury are supplied to organisms from lower trophic levels through the food web. Such a state of research is insufficient. It is important to study all forms of mercury, especially the labile ones – the most toxic and bioavailable to the soft tissue. Labile and stable forms of Hg were also determined in the sediment, plankton, and suspension particulate matter (SPM) constituting the food base of clams (main routes of exposure to Hg).

2. Materials and methods

2.1. Sampling

The organisms were collected during a cruise of a research vessel of the Marine Fishery Institute in Gdynia – ŁM MIR 2, in the western part of the Vistula Lagoon PW27 (N 54°18.791′, E 19°17.019′) in April, August, and October 2019, and in April 2020 (Fig. 1; Table 1). The biological material was collected by means of a manual drag (with dimensions: 36 × 21 cm, length of trawl of 60 cm, and net mesh size of 3 × 3 mm) from a depth of 2.5 m, and then sieved through a sieve with mesh of 0.25 mm. Individuals from species *Dreissena polymorpha* were collected manually from the sieve, and placed in aerated thermoses with lagoon water. After transporting the samples to the laboratory, the organisms were placed in an aquarium with filtered water for 24 h for purification. After 24 h, the organisms were subject to biometric analyses.

The dietary components of *D. polymorpha* were also collected at the research station (PW27). Seawater for filtering out suspended particulate matter (SPM) was collected by means of a bathometer from the near-bottom layer (Table 1). The water samples were collected into dark borosilicate glass bottles, previously cleaned with 4 M dilute nitric acid, rinsed with deionised water, and heated (300 °C, 6 h). After transporting the samples to the laboratory, they were filtered through ignited (500 °C, 6 h) and pre-weighed Whatman GF/F glass microfiber filters (pore size: 0.7 μm, diameter: 47 mm). Plankton was collected from the water column by means of a 10 μm net. The surface layer of bottom sediments (top 10 cm) was also collected for analysis. The collected material was stored at −20 °C, and lyophilised and homogenised immediately before analysis.

During sampling, water was also subject to in situ measurements. Salinity [PSU], water pH, temperature [°C], and Secchi depth were measured (Table 1). Wetness [W], organic matter content [LOI], and share of the finest fraction in surface sediment [FSF] was determined in accordance with the method described in papers: Saniewska et al. (2014).

2.2. Biological analysis

At the laboratory, the organisms were divided into four size classes in terms of length of shell (first: 5–10 mm; second: 10–15 mm; third: 15–20 mm; fourth; 20–25 mm) by means of an electronic calliper. Next, the wet mass of entire organisms was determined. The sex of the organisms was determined based on the gonad structure under an optical inverted microscope Mikroskop Olympus IX71. Only organisms from April 2019 had developed gonads, and sex was only identified in these samples. Next, the soft tissue was separated from the shell, and the samples were weighed again. The samples were stored at a temperature of −20 °C until the analysis.

Before proceeding with further chemical analyses, the *D. polymorpha* specimens (both shell and soft tissue) were lyophilized, and their dry mass was determined. The moisture of the organisms was determined based on the difference between wet and dry mass. The clams were then homogenised in an agate mortar. Owing to their low weight, the smallest size class individuals were homogenised jointly in order to obtain sample weight sufficient for mercury analysis. A total of 442 samples of mussel soft tissue and 100 shells were analysed (Supplement material I - SI).

2.3. Total mercury analysis

Total mercury analysis was performed on a DMA-80 analyser by means of the thermodesorption method with the application of an automatic mercury analyser DMA (Milestone, Italy) in accordance with the method published by Saniewska and Bełdowska (2017). This method requires no prior mineralisation of the sample, reducing the risk of contamination. Approximately 0.1000 g of a sample was placed in

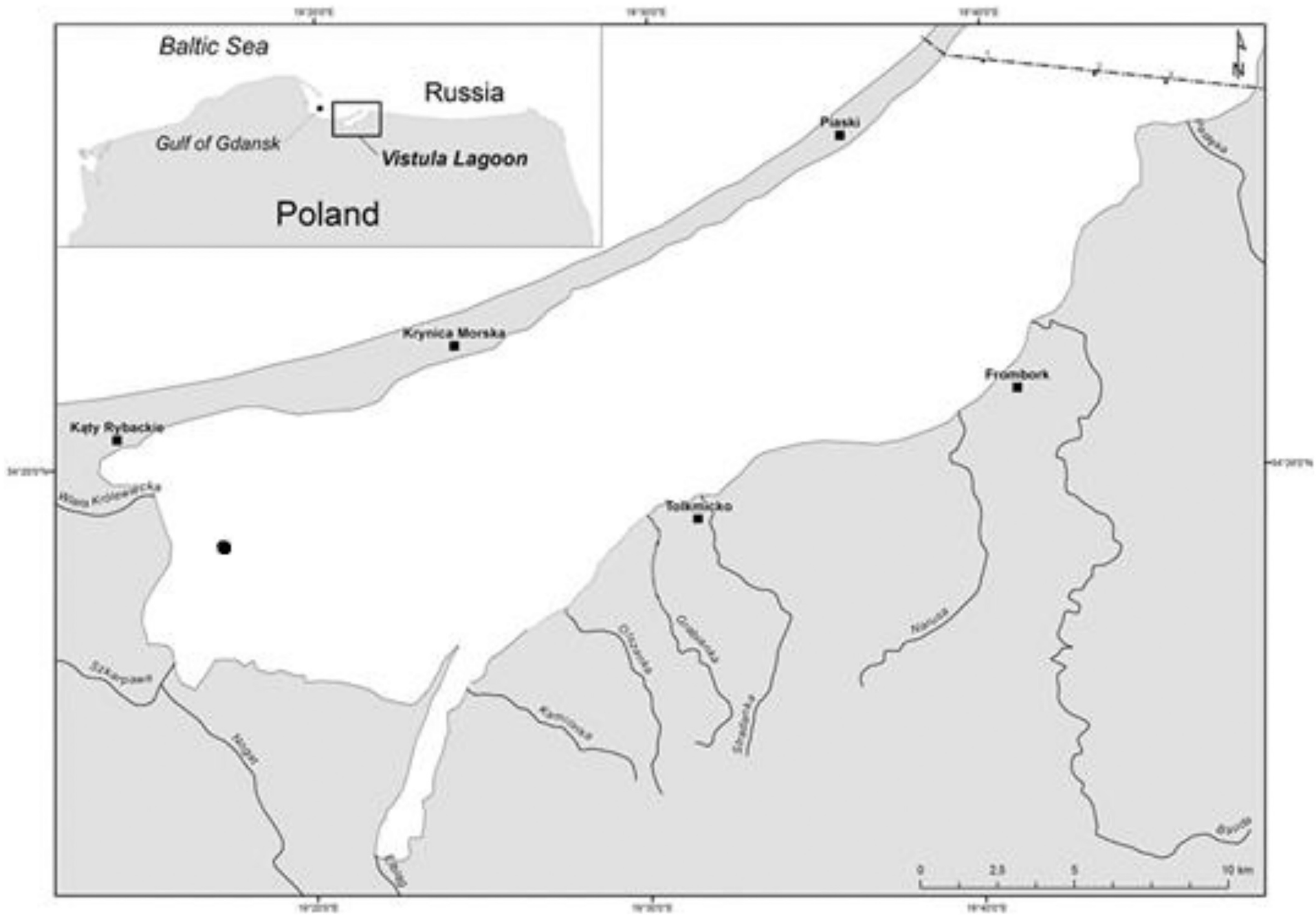

Fig. 1. Study area - Vistula Lagoon (estuary of the southern Baltic Sea).

Table 1
Habitat characteristics of *Dreissena polymorpha* from the Vistula Lagoon (southern Baltic Sea).

	Water									Sediment		
Location	Date	Depth [m]	Secchi disk [cm]	Temperature [°C]	O_2 [mg/L]	pH	Salinity [PSU]	SPM [mg dm^{-3}]	plankton [mg dm^{-3}]	LOI [%]	W [%]	FSF [%]
PW 27 N 54°18.791′ E 19°17.019′	04.2019	2.5	30	15	8.2	n.d	2.6	66	316	0,6	43	1.4
	08.2019	2.3	90	22.2	7.4	n. d.	2.9	34	260	0,8	55	2.1
	11.2019	2.3	70	6.3	11.1	6.5	3.5	n.d.	140	0,5	51	1.8
	04.2020	2.5	75	13.1	9.9	7.9	4.1	77	356	0,3	47	2.6

n.d. – no data; SPM – susppended particulate matter; LOI – lost in ignitron; W – wetnnes; FSF – fine sediment fraction (< 0,063 µm).

quartz vessels in triplicate. The concentration of total mercury was determined by incinerating the sample at 750 °C in pure oxygen. The correctness of the analysis was verified by the analysis of certified reference materials (tea leaves INCTTL-1 - Hg_{TOT}: 5 ng g^{-1}, soil NCS DC 87103–Hg_{TOT}: 17 ng g^{-1}, plankton BCR-414–Hg_{TOT}: 276 ng g^{-1}), with an average recovery of 98 % (89–107 %) and a standard deviation not exceeding 5 % (1.2–4.3 %). The limit of detection (LOD) was calculated as the threshold of the standard deviation (SD) of Hg concentration in the ignited sample boat measured in 10 replicates. In the case of Hg_{TOT} analysis, the LOD was 1 pg of Hg.

2.4. Mercury fractionation

The application of the 5-step thermodesorption method allowed the designation of five groups of compounds with similar properties (Wilman et al., 2019; Jędruch and Bełdowska, 2020; Korejwo et al., 2022; Wilman et al., 2023). The method involved simultaneous combustion of the same sample at five temperatures: 175 °C (released: mainly Hg bound to halides: HgI_2, $HgBr_2$, $HgCl_2$ ($Hg_{lab.1a}$)), 225 °C (released: mainly organic mercury such as: $Hg(CN)_2$, $Hg(SCN)_2$, $Hg(CNO)_2$, $(CH_3COO)_2Hg$, mercury in complexes with organic matter ($Hg_{lab.1b}$)); 325 °C (released: HgS ($Hg_{stab.\ 1}$)); 475 °C (released: $HgSO_4$, HgO (red), and HgF_2($Hg_{lab.2}$)); residual mercury 750 °C ($Hg_{stab.\ 2}$) (Bełdowska et al., 2018; Jędruch et al., 2018; Wilman et al., 2023). The analysis of the samples was performed in three repeats. The analyses of the environmental samples were preceded by an examination of the Hg patterns specific to each fraction according to the method of Saniewska and Bełdowska (2017). Seal muscle was used as the pattern for the thermally labile fraction (1b), in which MeHg accounted for 98 % of the contribution to the total Hg_{TOT} (Jędruch et al., 2018; Wilman et al., 2023) and the contribution of fraction 1b accounted for 98 % of the total Hg fraction in seal muscle, respectively. The use of Hg standards in this method is important, as there are no certified reference materials for individual forms of Hg in environmental samples. The analysis of the samples was performed in three repetitions. The correctness of the analysis was verified by comparing the sum of Hg concentrations measured by means of the 5-stage fractionation method with the result of the Hg_{TOT} analysis. The accuracy of the analysis averaged 96 %. The quality of the method was also verified by the analysis of certified reference materials (in analogy to total mercury), carried out in triplicate, with recovery ranging from 96 to 105 %. The limit of detection (LOD) was calculated as the threshold of the standard deviation (SD) of Hg concentration in the ignited sample boat measured in 10 replicates. In the case of Hg_{TOT} analysis, the LOD was 1 pg of Hg.

2.5. Processing results

The statistical analysis and graphic representation of the obtained results were carried out using STATISTICA 13 (StatSoft) and Microsoft Excel (2008). The analysed data were not characterised by normal distribution (Shapiro-Wilk test, $p < 0.05$). In order to determine the significance of differences between Hg and biometric parameters, the non-parametric U Mann-Whitney and ANOVA Kruskal-Wallis test was applied. The relationships between the analysed variables were determined based on the Spearman's coefficient, with a confidence interval of at least 95 % and PCA analyses.

The condition index CI (mg cm^{-3}) constituting a good proxy of the physiological state of the investigated mussels (Wołowicz et al., 2006; Pierścieniak et al., 2010; Jędruch et al., 2019) was calculated based on the following formula (Beukema and De Bruin, 1979; Okumuş and Stirling, 1998):

$$CI = STDW\ L^{-3} \tag{1}$$

where STDW is soft tissue dry weight after lyophilising (mg), and L is shell length (cm). To evaluate the ability of benthic organisms to accumulate Hg from surface sediment, the biota-sediment accumulation factor (BSAF) was calculated following the formula suggested by Szefer et al. (1999):

$$BSAF = Hg_{mussel}\ {Hg_{sediment}}^{-1} \tag{2}$$

where Hg_{mussel} and $Hg_{sediment}$ are the concentrations of Hg_{TOT} (ng g^{-1} d.w.) in *D.polymorpha* and surface sediments, respectively. The accumulation of Hg in *D.polymorpha* with food was expressed with the bioaccumulation factor (BAF), according to^{-1} the formula (Kwok et al., 2013):

$$BAF = Hg_{mussel}\ {Hg_{diet}}^{-1} \tag{3}$$

where Hg_{mussel} and Hg_{diet} are concentrations of Hg_{TOT} (ng g^{-1} d.w.) in *D. polymorpha*'s diet. BAF was calculated separately for different elements constituting the mussels food in the study area, namely suspended particulate matter (SPM) and plankton.

3. Results

3.1. Entire measurement period

The median of total mercury concentration in the soft tissue of *Dreissena polymorpha* from the Vistula Lagoon throughout the measurement period (04.2019–04.2020) ($n = 442$) reached 35.8 ng g^{-1} d.w. (mean: 36.3 ng g^{-1} d.w.) (SII). Minimum non-outliers Hg concentration: 15.9 ng g^{-1} d.w., maximum non-outliers Hg concentration: 75.3 ng g^{-1} d.w., as well as two outliers values were observed for organisms from the second size class in summer 2019. Labile forms of Hg (Hglab1a + Hglab.1b + Hglab2) in the soft tissue accounted for 97 % of total mercury (SII). The highest share was recorded for the mercury fraction $Hg_{labile1b}$ (primarily organic forms of Hg, including MeHg and Hg bound to organic matter), constituting 64 % of Hg_{TOT} (SIII). Labile forms of mercury primarily related to halogens ($Hg_{labile1a}$) constituted 32 % of Hg_{TOT} in the soft tissue of *D.polymorpha*. The remaining fractions showed a contribution from 1 to 3 % of Hg_{TOT} in the soft tissue of *D. polymorpha* (SII). The median of total mercury concentration in the shell of *Dreissena polymorpha* ($n = 100$) throughout the measurement period was 7 times lower than in the soft tissue, and reached 4.5 ng g^{-1} d.w. (SII). Considering the entire weight of the *D.polymorpha* organism, the soft tissue constituted an average of 8 %, and the shell 92 %. Labile forms of Hg in the shell constituted an 82 % share in total mercury (Fig. 2). The highest contribution was observed for mercury fraction Hg_{lab1b}, constituting (identically as in the soft tissue) 64 % of Hg_{TOT}. Labile $Hg_{labile1a}$ and HgS constituted 17 % of Hg_{TOT} in the shell of *D.polymorpha*, and the remaining mercury fractions reached only 1 % (Fig. 2). Labile forms of Hg in plankton constituted a total of 87 % share in total Hg_{TOT} (SIV). The highest contribution was recorded for the fraction $Hg_{labile1b}$ – 56 %. $Hg_{labile1a}$ accounted for 27 % of Hg_{TOT} (Fig. 2). In suspension, labile forms of Hg constituted 93 % of total Hg_{TOT}, and in sediment 60 % (Fig. 2). The highest share was determined for the fraction $Hg_{labile1b:}$ 55 % in SPM and 54 % in sediment. Sediment showed high content of Hg sulphate, stable fraction, reaching a share of 39 % in Hg_{TOT} (Fig. 2).

3.2. Seasonal changes

3.2.1. Spring 2019

Sex could only be identified in this season. Total mercury concentration as well as its labile and stable forms showed no statistical differences between males and females (SIII) ($p > 0.05$), either in the soft tissue or in the shell of *D.polymorpha*. Therefore, further discussion considered concentrations in both males and females. The median of total mercury concentration in the soft tissue of *Dreissena polymorpha* in spring 2019 was approximate to the median value from the entire study period, reaching 33.3 ng g^{-1} d.m., with maximum non-outliers concentration of 42.2 and minimum of 21.4 ng g^{-1} d.m (SII). The distribution of fractions was also approximate to the values calculated for the entire study period (SII). The median of total mercury concentration in the shell of *Dreissena polymorpha* in spring 2019 was 6.8 ng g^{-1} d.w., with non-outliers maximum concentration of 9.9 and minimum of 3.8 ng g^{-1} d.w. Labile forms of Hg in the shell constituted 86 % of total mercury. The highest contribution was primarily determined for Hg labile 1b constituting 60 % of Hg_{TOT} (Fig. 2).

3.2.2. Summer 2019

The median of total mercury concentration in the soft tissue of *Dreissena polymorpha* in summer 2019 was 28.1 ng g^{-1} d.w. The value is 20 % lower in comparison to that from the entire measurement period. The highest value was reached by mercury fraction $Hg_{labile1a}$ constituting the greatest (53 %) share of Hg_{TOT} in the entire measurement period (SII). Among all analysed periods, the labile form of mercury $Hg_{labile1b}$ showed the smallest percent share, constituting 40 % of Hg_{TOT} in the soft tissue of *D.polymorpha* – 40 % (SII). The median of total mercury concentration in the shell of *Dreissena polymorpha* in summer 2019 was approximate to that of the entire measurement period – 4.9 ng g^{-1} d.w. (SIII). The highest share was reached by mercury fraction $Hg_{labile1b}$ constituting 68 % of Hg_{TOT}.

3.2.3. Autumn 2019

The median of total mercury concentration in the soft tissue of *Dreissena polymorpha* in autumn 2019 was the highest among all analysed periods, reaching 41.1 ng g^{-1} d.w, with maximum non-deviating concentration of 52.1 and minimum of 23.9 ng g^{-1} d.w. (SII). The highest share in all measurement seasons was reached by mercury fraction $Hg_{labile1b}$. constituting 82 % of Hg_{TOT}. The median of total mercury concentration in the shell of *Dreissena polymorpha* in autumn 2019 was 4.4 ng g^{-1} d.w. (SII). The highest share was determined for mercury fraction $Hg_{labile1b}$ constituting 61 % of Hg_{TOT} (Fig. 2).

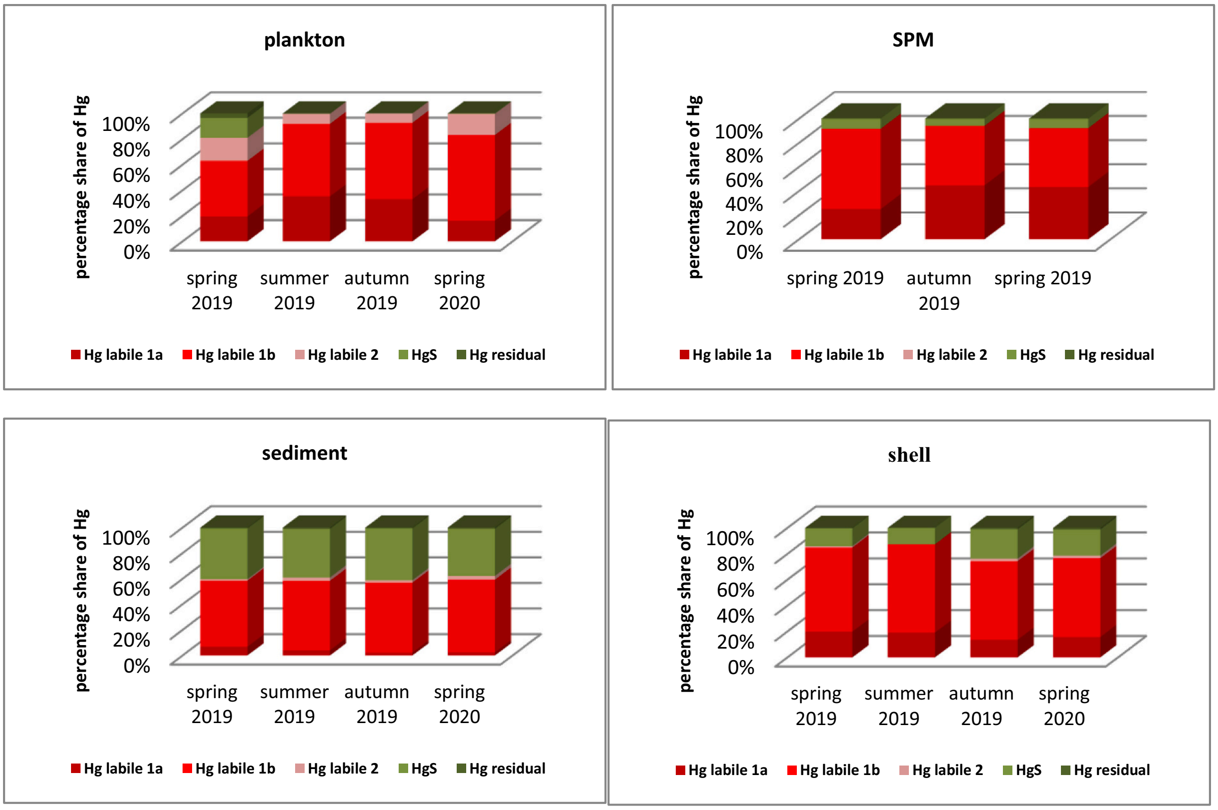

Fig. 2. Percentage of labile and stable forms of mercury in the plankton, suspended particulate matter (SPM), the sediment and shell of *D. polymorpha* over the entire measurement season.

3.2.4. Spring 2020

The median of total mercury concentration in the soft tissue of *Dreissena polymorpha* in spring 2020 was 39.2 ng g^{-1} d.w. (Fig. 2). The highest share was reached by mercury fraction $Hg_{labile1b}$, constituting 85 % of Hg_{TOT} (SII). The median of total mercury concentration in the shell of *Dreissena polymorpha* in spring 2020 was 3.7 ng g^{-1} d.w. (SII). The highest share was determined for mercury fraction $Hg_{labile1b}$ constituting 61 % of Hg_{TOT}. Considering spring 2019 and spring 2020, in both cases winter was mild, although March 2020 was a cooler month than March 2019. In March 2020, more days with frost occurred ($T <$ 0 °C). Water temperature was also lower by an average of 2 °C in months preceding spring 2020 in comparison to spring 2019. When water temperature is too low, organisms can enter the state of anabiosis, where life activity is reduced. This way, they save energy instead of using it for the processes of among others the detoxification of the organism. Warm spring occurred earlier in 2019 than 2020. Therefore, spring 2019 was more similar to the summer season 2019, and the spring season 2020 to the autumn season 2019.

In the zebra mussel from the Vistula Lagoon, mean concentration of Hg ($n = 442$) was 39.7 ng g^{-1} d.w. (11.4 ng g^{-1} w.w.). The European Food Safety Authority (EFSA) limit value for total mercury in human food is 500 ng g^{-1} w.w.. Considering the average concentration of total mercury in the zebra mussel: (11.4 ng g^{-1} w.w.), it was found to be 44 times lower than the EFSA recommended limit. According to EFSA the permissible weekly intake (TWI) from food is 4 μg Hg per kilogram of body weight. The ingestion of 5237 g of *D.polymorpha* mussel from the Vistula Lagoon would deliver an acceptable weekly dose of the xenobiotic to the human body.

In other regions of the world, the value differed depending on the type of water body. In the Ebro River, mean Hg concentration in *Dreissena polymorpha* was 12 times higher than in the Vistula Lagoon, reaching 1570 ng g^{-1}. Such a high value was related to the supply of pollutants containing Hg from a factory producing chloroorganic solvents (Carrasco et al., 2008). A study conducted in the north-east of the German part of the Szczecin Lagoon (southern Baltic Sea) in 1996 showed mean mercury concentration in the soft tissue of the organisms 18 % higher than in the case of those from the Vistula Lagoon. Considering mean values from one month (may 1996) with reference to the designated size classes, it was determined that in small mussels (with shell size of 1.5–1.9 cm, corresponding with class 1, 2, and 3), Hg concentration reached 49.3 ng g^{-1} of mercury – i.e. it was 35 % higher (Wiesner et al., 2001) than the presented data from the analogical period. The comparison of mercury concentration in medium-sized mussels (2.0–2.5 cm, corresponding with size class 4) in both water bodies also shows mercury concentration 30 % higher in the organisms collected from the Szczecin Lagoon (46.2 ng g^{-1}) (Wiesner et al., 2001) than those in this study (mean: 32.4 ng g^{-1}). The differences in results may be related to the differences between the regions of sampling (the Oder River flows into the Szczecin Lagoon, and the Vistula River to the Vistula Lagoon), and the passing of time: 24 years passed between the studies. During the time, many restrictions regarding the emission of heavy metals (including mercury) have been introduced (Hylander and Meili, 2003).

Considering the identified mercury fractions, the soft tissue of *D. polymorpha* showed the average share of the fraction Hg associated with halogens ($Hg_{labile1a}$) 10 % higher than that in the soft tissue of benthic fauna organisms from the Puck Bay (a region geographically close to the Vistula Lagoon). In the soft tissue of *D.polymorpha*, the share of the fraction $Hg_{labile1a}$ was higher (32 %) than in the soft tissue of *Mytillus trossulus* from the Puck Bay (28 %) (Jędruch et al., 2019), and higher than in the soft tissue of *Limecola baltica* (22 %) (Table 2). The share of the organic Hg and Hg complexes with organic matter ($Hg_{labile1b}$) was at an approximate level, reaching 64 % of total Hg_{TOT} (Table 2) (Jędruch et al., 2018). This was several percent lower than in *M.trossulus* and in *L.baltica*, and constituted 64 % of Hg_{TOT} (Table 2). In water bodies where the zebra mussel population is very large, the

Table 2

Comparison of total mercury concentration [Hg_{TOT} ng g^{-1} d.w.] and percentage of Hg fraction in the soft tissue and in the shell of *D.polymorpha* (Vistula Lagoon) with other zoobenthic species from the Puck Lagoon (southern Baltic Sea).

Species	Tissue	Hg_{TOT}	$Hg_{labile\ 1a}$	$Hg_{labile\ 1b}$	HgS	$Hg_{labile\ 2}$	$Hg_{residual}$	Reference
D.polymorpha	Soft tissue	36	32	64	2	1	1	this study
L.baltica		111	22	70	6	1	1	Jędruch et al., 2018
M.trossulus		148	28	71	1	0	0	Jędruch et al., 2019
D.polymorpha	Shell	4.5	17	64	17	1	1	this study
L.baltica	Shell	4	44	37	16	1	2	Jędruch et al., 2018
E.sinensis	Carapace	12	20	77	1	1	0	Jędruch et al., 2018

mussels theoretically have a possibility of filtering the entire water volume within a relatively short time. In some Masurian lakes, they can filter the entire water within several or a dozen days, and in the Szczecin Lagoon (with water volume higher than that of the Vistula Lagoon by 0.28 km^3) within a month (Wiktor, 1969; Stańczykowska, 1977). Both zebra mussels and blue mussels are obligatory filtrators. By filtering water, they obtain necessary nutrients with plankton. The study by Bełdowska et al. (2018) showed that the fraction $Hg_{labile1a}$ accounts for a high percent share of phytoplankton and microalgae. As a consequence, considering the water filtering capacity of *D.polymorpha*, more mercury compounds in combination with halogens are supplied to the organism and subject to bioaccumulation.

The median of total mercury concentration in the shell of *D.polymorpha* reaching 4.5 ng g^{-1} d.w. (SII) was at a level approximate to that in the mussel of *L.balthica* from the Puck Bay. Three times higher concentrations of Hg_{TOT} were observed in the carapace of crab *E.sinensis*

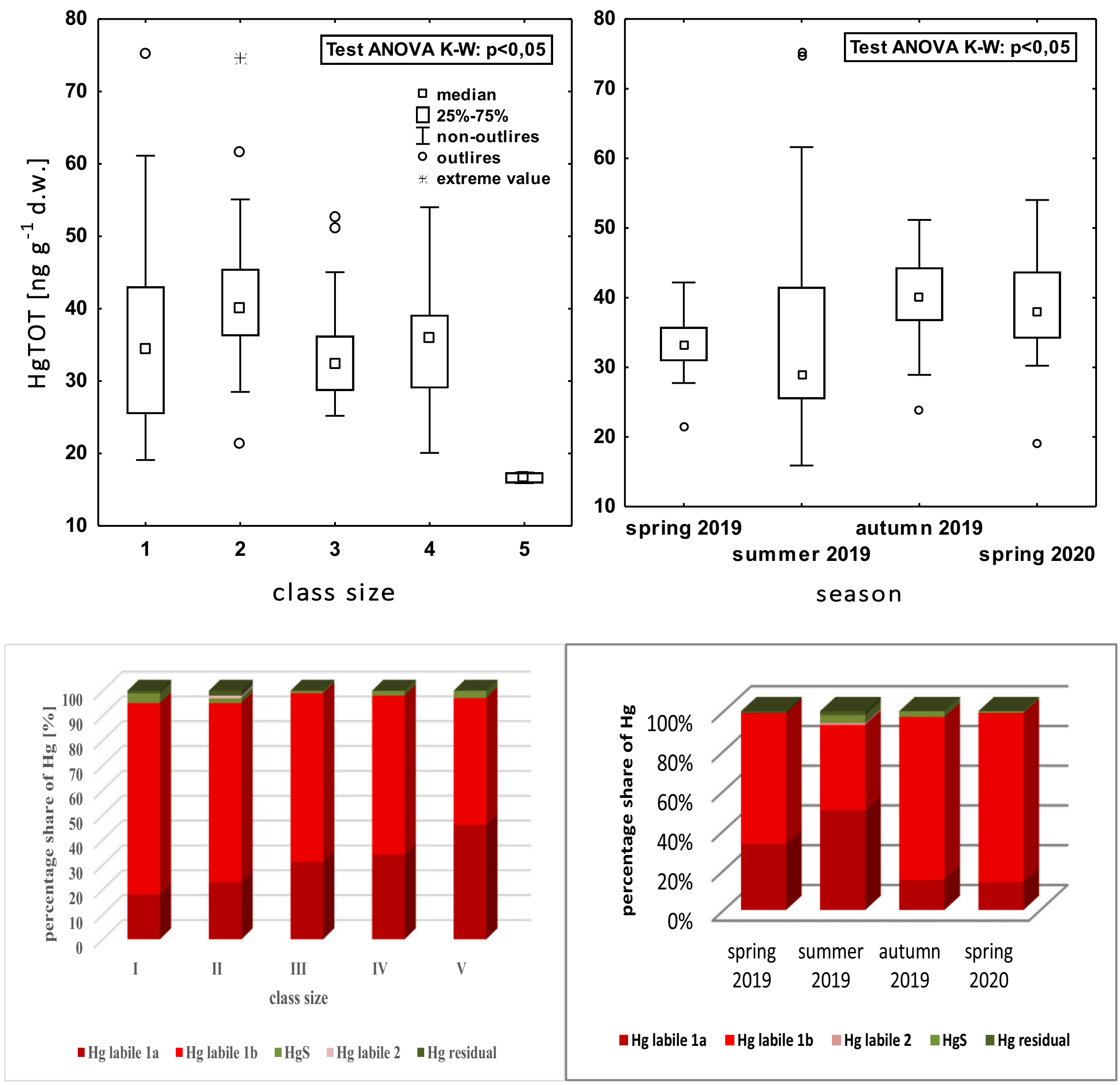

Fig. 3. Concentration of: (a) total mercury HgTOT changes in class size, (b) HgTOT seasonal changes, (c) and percentage share of Hg fraction in individual class size, (d) and individual season in soft tissue of *Dreissena polymorpha*.

from the Vistula Lagoon at a level of 12 ng g^{-1} d.w. (Jędruch et al., 2018). In the shell of *D.polymorpha*, the share of the fraction $Hg_{labile1a}$ (17 %) was more than twice lower in Hg_{TOT} in comparison to *L.baltica* (Jędruch et al., 2018). The share of the fraction $Hg_{labile1b}$ was higher, reaching 64 % of total mercury in comparison to *L.baltica* (37 %). *D. polymorpha* probably more efficiently detoxes its organism from the organic form of Hg in the soft tissue through its transfer to the shell. Hg sulphate reached an approximate share in the shells of both species, namely 16 % (Table 2). This may also be related to the different chemical composition of the shell (Ramnath et al., 2018).

4. Discussion

4.1. Accumulation and detoxification of zebra mussel from Hg

Consideration of all the results shows that the concentration of both Hg_{tot} and its fractions in the soft tissue of the zebra mussel varied from several to several tens ng g^{-1} (SII). One of the factors determining such a variability was the size of the organism expressed with size class (Fig. 3a). The median of mercury concentration and organic Hg and Hg complexes with organic matter (Hg_{lab1b}) in the soft tissue of *D.polymorpha* in size classes 1–5 was at an approximate level (Fig. 3a). A statistically significant difference ($p < 0.05$) in concentration in particular size classes resulted from the double decrease in Hg concentration in size class 5. Hg_{TOT} concentration was generally negatively correlated with particular size classes ($r = -0.21$; $p < 0.05$) and weight of the soft tissue ($r = -0.30$; $p < 0.05$). The concentration of fraction $Hg_{labile1b}$ was also negatively correlated with the weight of the soft tissue of *D. polymorpha* ($r = -0.37$; $p < 0.05$). This suggests a process of dilution of total mercury concentration and its most toxic organic forms during growth and increase in body weight, as a result of development of new tissues of *D. polymorpha*. Concentration of metals increases with age and size, when the growth of the organism becomes slow in comparison to the rate of accumulation of metals (Roditi and Fisher, 1999). An analogical dependency in *D. polymorpha* was observed in other papers (Wiesner et al., 2001; Carrasco et al., 2008). Moreover, the negative correlation between organic Hg content and the length of the shells may be related to the activation of defensive mechanisms in older organisms: the demethylation of MeHg and storage as inorganic forms of Hg (Harley et al., 2015).

In the presented study, the concentration of the fraction Hg associated with halogens ($Hg_{labile1a}$) as the only fraction increased with growth of the weight of the soft tissue of *D.polymorpha* ($r = 0.21$; $p < 0.05$), and was negatively correlated with the fraction Hg_{lab1b} ($r = -0.51$; $p < 0.05$). The concentration of the fraction $Hg_{lab.1a}$ was statistically significantly lower in size class 1 in comparison to size classes 2–5 (where the median was at an approximate level) (Fig. 3c). With growth of the mussels, their efficiency in filtering suspension and plankton from water increases. Moreover, larger mussels could also have larger gills which also affected obtaining food from water, including Hg_{lab1a} that constitutes a high share in suspension and microalgae (Bełdowska et al., 2018). Similar dependencies were observed in mussels *Mytilus trossulus*, where the concentration of the fraction Hg_{lab1a} in the soft tissue increased with the length and weight of the organisms, and in larger organisms Hg_{TOT} and its organic form in the muscle tissue was diluted (Jędruch et al., 2019).

Changes in Hg concentration in subsequent size classes as well as within a given class can be additionally related to the availability and quality of food and water temperature which stimulates development (metabolism and reproduction), i.e. to the season of the year (Fig. 3b). Total mercury concentration during warm spring (in 2019) was not statistically significantly different in particular size classes ($p > 0.05$). The concentrations of $Hg_{labile1a}$ and $Hg_{labile1b}$ were subject to opposite processes during the growth of the organisms and these trends were statistically significant ($p < 0.05$ $r = -0.60$) (Fig. 4a). Until size class 3, the concentration of $Hg_{labile1a}$ increased in the soft tissue, and the

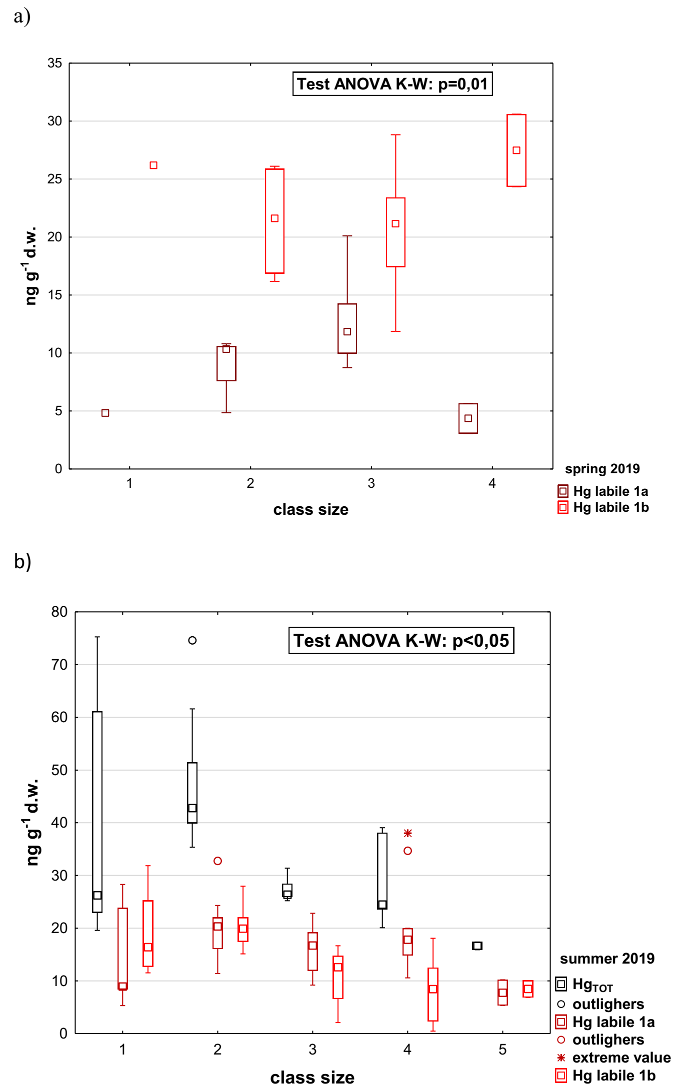

Fig. 4. Changes in concentration of labile mercury fractions 1a and 1b in successive size classes in the soft tissue of *Dreissena polymorpha* during the spring 2019 season (a) and change of total mercury HgTOT and labile fractions 1a and 1b by size class in the soft tissue of *Dreissena polymorpha* during the summer 2019 season (b).

concentration of Hg_{lab1b} decreased. In size class 4, the concentration of $Hg_{labile1b}$ increased, and concentration of fraction $Hg_{labile1a}$ decreased. Such a change in dependencies in size class 4 could be related to the difference in food accessibility. Adult zebra mussels settled at the bottom occur in colonies or in compact schools, rarely as single individuals (SIV). In Europe, their density usually varies from several hundred to several hundred thousand individuals per 1 m^2 of the bottom in zones of their occurrence. Maximum values for Poland were recorded by Wiktor (1969) in the Szczecin Lagoon. They reached 114 thousand individuals/m^2, with mean values of approximately 15 thousand individuals/m^2, and mean biomass of approximately 4.8 kg/m^2. This may lead to a situation where larger individuals are located closer to the sediment due to the fact of being overgrown by smaller organisms. Consequently, larger individuals could have limited access to suspension from the water column, and primarily filtered suspension from above the sediment, characterised by a higher share of Hg_{lab1b} than plankton (Fig. 2) (Kwasigroch et al., 2021).

The concentration of total mercury and its fractions $Hg_{labile1a}$ and $Hg_{labile1b}$ changed in summer in a way similar to that in spring 2019 in particular size classes in the soft tissue of *D.polymorpha*: it increased in classes 1–2, and then decreased in classes 3–5, whereas all the dependencies showed statistically significant differences ($p < 0.05$) (Fig. 4b). The rate of filtration by *D.polymorpha* largely depends on water temperature. Cooler water results in lower demand for metabolism, and even a moderate increase in temperature may increase the rate of metabolism and breathing in *D. polymorpha* (Rao and Khan, 2000). In summer, water temperature reached the highest value among all the measurement seasons, namely 22.2 °C (Table 1). As a result, they could filter water more efficiently, and obtain food in response to the demand for energy used for growth and respiration. In summer, plankton biomass was twice lower than in the remaining analysed seasons (Table 1). The bioaccumulation coefficient for $Hg_{labile1a}$ including plankton was the highest among the analysed seasons, and reached 191 (3 times higher than in spring) (SV). Low phytoplankton biomass and high bioaccumulation coefficient of this mercury fraction suggest that in summer, when water temperature was the highest and therefore the metabolism of the organisms intensified, the rate of water filtration from suspension was the most efficient. As a result, in summer, the concentrations and percent share of the $Hg_{labile1a}$ fraction and total mercury concentration was the highest among all measurement periods (Fig. 3d). In that season, the percent share of $Hg_{labile1a}$ in Hg_{TOT} in particular size classes doubled: from 34 % in size class 1 to 68 % in size class 4 (Fig. 3d).

A decrease in total mercury concentration and its labile forms in size classes 3–5 during warm spring and in summer could be additionally related to the reproduction of the organisms (Fig. 4). According to Mackie (1991), zebra mussels are sexually mature from the moment of reaching a length of shell of 10 mm (size class 2). Hg is transferred as MeHg (part of $Hg_{lab.1b}$) from the somatic to reproductive tissues that are released from zebra mussels during reproduction (Ram and McMahon, 1996). Spawning of *D.polymorpha* commences the most efficiently at water temperature from 17 to 18 °C, whereas it can begin already at a temperature of 12 °C (Ram and McMahon, 1996). In summer, when water temperature reached 22.2 °C, *D. polymorpha* organisms had the most suitable conditions for reproduction in the entire measurement period.

In cool seasons (autumn 2019, spring 2020), the highest share in Hg_{TOT} was determined for the mercury fraction $Hg_{labile1b}$ (averaging 82 %) (Fig. 3d). The highest bioaccumulation coefficient for Hg_{TOT} and its fraction $Hg_{lab.1b}$ (SV) was also determined in these seasons. As a result of weakened detoxification processes caused by a decrease in water temperature (reduced metabolism, lower rate of filtration, and lack of thermal optimum for spawning), the process of Hg accumulation prevailed over its excretion. As a consequence, the median of total mercury concentration was at an approximate level in subsequent size classes (I-IV) ($p > 0.05$), which was not observed in the remaining measurement seasons. Mercury fraction $Hg_{labile1a}$ increased in particular size classes ($p < 0.05$), and the concentration of the stable form of mercury HgS decreased ($p < 0.05$) (Fig. 5a; 5b). Larger organisms (larger gills – larger filtration surface) filter more suspension constituting the source of $Hg_{lab.1a}$ (Bełdowska et al., 2018). Due to this, the percent share of the fraction $Hg_{labile1a}$ increased on average from 9 % in size class 1 to 21 % in size class 4 (SVI). The share of HgS decreased from 10 % in size class 1 to 1 % in size class 4 (SVI), and fraction $Hg_{labile1a}$ was negatively correlated with HgS ($p < 0.05$ $r = -0.61$). A decrease in the share of HgS in subsequent size classes in cool seasons may be related to another process of organism detoxification, namely demethylation of Hg to inorganic forms, and then their transfer to the shell (Li et al., 2019; Li et al., 2022). The process probably occurs more efficiently with age (size) of the mussels (Fig. 5).

In all measurement seasons, the condition index (CI) was negatively correlated with the length of the shell (size classes), and the differences were statistically significant ($p < 0.05$). Smaller organisms (class 1–2) that were in a better condition were characterised by higher Hg concentration than mussels in size classes 3–5, i.e. than larger (older) organisms. An analogical dependency was observed by Jędruch and Bełdowska (2020) in blue mussels from the Puck Bay and in atlantic

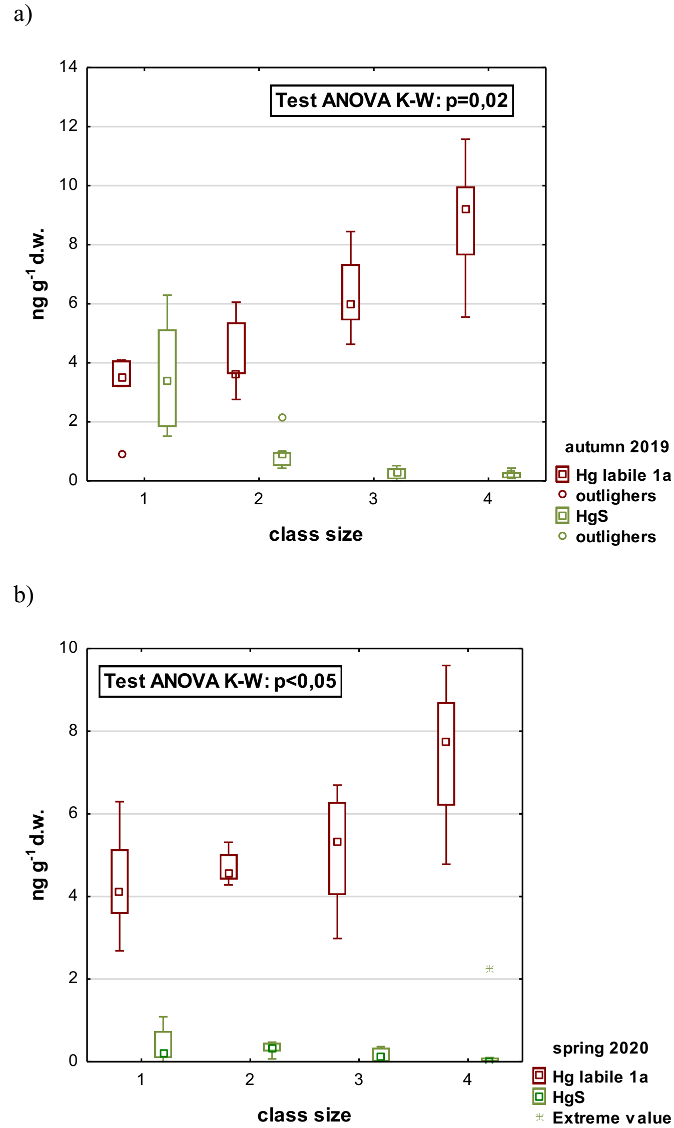

Fig. 5. Change in concentration of labile mercury fraction 1a and HgS by size class in the soft tissue of *Dreissena polymorpha* during (a) fall 2019 and (b) spring 2020.

rangia from Vistula Lagoon (Wilman et al., 2023). Mussels *D.polymorpha* were in a better condition in warm spring and summer than in autumn and during cool spring (SVIII). In warm spring and summer, the condition index was positively correlated with the mercury fraction $Hg_{labile1b}$ ($p < 0.05$). This suggests that organisms in good condition absorb more food as well as Hg, because this mercury fraction is primarily of trophic origin. In autumn and cool spring, the CI index was negatively correlated with mercury fraction $Hg_{labile1a}$ ($p < 0.05$), and positively correlated with mercury sulphate ($p < 0.05$). This confirms the previous statement that although in a warmer season larger organisms (size class 3–5) are in worse condition than smaller mussels (size class 1–3), they have more suitable conditions for detoxification from Hg and its organic form in the process of spawning or dilution of Hg in the soft tissue as a result of growth. In cooler seasons with lower activity and inhibited processes of detoxification from the organic form of Hg, larger mussels in worse condition are characterised by a share of Hg_{lab1a} greater than that in smaller ones in better condition. They probably use another system of detoxification – through demethylation of Hg to inorganic forms and removal of HgS from the soft tissue to the shell. A reduction in the Hg accumulation process due to dilution caused of clam growth was observed by Peltier et al. (2009) in the US region, while in the China region, work by Tran et al. (2020), the clam condition factor was directly proportionally correlated with metal concentration, indicating accumulation with growth and mass. Presumably, these differences may be related to the pollutant load entering the water body. The inverse correlation of CI with metal concentration may indicate limited emissions of Hg into the environment.

4.2. Hg accumulation in the shell of D. polymorpha

Previous work on determining the concentration of contaminants including mercury in bivalves has focused on the soft tissue, as this is what is consumed by organisms, including humans. The results presented here on Hg and its fraction in the shell of *D.polymorpha* are among one of the first in the literature where an attempt has been made to determine the role of the shell in the transfer of Hg in the clams. Mechanisms of the muscle-shell transport for the studied mercury forms derivatives are unknown. The concentration of total mercury and its fractions (except for HgS) in the shell of *D.polymorpha* showed no statistically significant differences between particular size classes throughout the measurement period ($p > 0.05$). Only the concentration of the stable mercury fraction HgS increased with the length of the shell of the mussel (Fig. 6) ($p < 0.05$ $r = 0.48$) and weight of the shell ($p < 0.05$ $r = 0.32$). Simkiss and Mason (1984) consider that Mt. complexes with cadmium and mercury may be metabolized more slowly than complexes with biologically essential metals, indicating one possible pathway for participation in detoxification. It may result of transfer of mercury sulphate from the soft tissue to the shell ($p < 0.05$, $r = -0.32$). It points to the process of detoxification of the organism through the integration of stable HgS in the shell, and increasingly intensive transfer in subsequent size classes (Chaudhary et al., 2022). A decrease in the concentration of $Hg_{labile1b}$ in the soft tissue with a simultaneous increase in the concentration of $Hg_{labile1b}$ in the shell ($p < 0.05$ $r = -0.31$) and Hg_{TOT} in the shell ($p < 0.05$ $r = -0.30$) also points to the process of detoxification of the organism from toxic Hg through its partial integration in the shell. This additionally contributes to a decrease in concentration of the most toxic organic forms of mercury in the soft tissue of the growing organisms in subsequent size classes. The processes occurred the most efficiently in the summer season, when HgS concentration showed statistically significant differences ($p < 0.05$ $r = 0.85$) and increased in subsequent size classes (SVII). It's percent share increased from 2 % in size class 1 to 22 % in size class 4 (SVII). In that

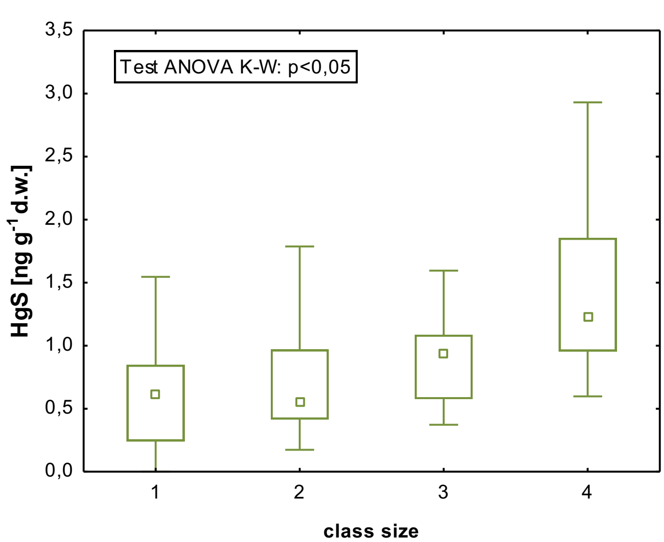

Fig. 6. Changes in the concentration of HgS in subsequent size classes in *Dreissena polymorpha* shell over the entire measurement period.

season, mercury sulphate in the shell was also negatively correlated with HgS concentration in the soft tissue ($p < 0.05$ $r = -0.58$), suggesting transfer of this mercury fraction from the soft tissue to the shell. In summer, when water temperature is the most favourable and therefore the metabolism is the most intensive, the mussel can have sufficient energy resources (calories) for more efficient processes of Hg detoxification from the soft tissue to the shell.

In autumn, water temperature was the lowest among all the analysed seasons. Due to this, the metabolism of the zebra mussel slowed down, and the detoxification processes became less efficient. The concentration of total mercury and $Hg_{labile1b}$ as well as stable HgS in the shell in autumn showed no statistically significant differences in particular size classes ($p > 0.05$). The concentration of the fraction $Hg_{labile1a}$ in the shell increased in particular size classes ($p < 0.05$ $r = 0.90$), and was positively correlated with the weight of the shell ($p < 0.05$ $r = 0.80$). A positive correlation was also determined between the concentration of $Hg_{labile1a}$ in the shell with $Hg_{labile1a}$ in the soft tissue ($p < 0.05$ $r = 0.83$), suggesting partial transfer of the fraction from the tissue to the shell. The concentration of the fraction $Hg_{labile1a}$ in the shell during cold spring (2020) was negatively correlated with the size class of the organisms ($p < 0.05$ $r = -0.78$) and with the weight of the shell ($p < 0.05$ $r = -0.71$) – opposite than in summer and autumn. No correlations were observed between transfer of Hg_{TOT} and labile and stable Hg fractions from the soft tissue to the shell. The processes of transformation and detoxification were probably inhibited by the winter period of stagnation.

The discussed processes of transfer of mercury and its forms from the soft tissue to the shell were confirmed by the multivariate principal component analysis, where 3 variables accounted for 83 % of the correlation (Table 3). The first component (accounting for 36 % of variance) points to transfer of Hg_{lab1b} from the soft tissue to the shell during the increase in the weight of the shell, with a simultaneous increase in the concentration of Hg_{TOT} and Hg_{lab1a} in the shell. This confirms that together with the growth (age) of the mussels, the processes of detoxification of the zebra mussel organism are more efficient. Moreover, an increase in the weight of the shell provides new places where Hg can be incorporated, as confirmed by factor 3 (accounting for 22 % of variance). In this case, the concentration of Hg was strongly dependent on the length and weight of the shell (Table 3). The second factor (explaining 25 % of variance) determining the processes of detoxification in the organism of *D.polymorpha* was water temperature (Table 3). Together with an increase in water temperature (warm season), the accumulation of Hg halogens in the soft tissue intensified. Increased metabolism led to increased water filtration, with a simultaneous decrease in the concentration of organic forms of Hg accumulated in the soft tissue: dilution in the muscle mass with growth and reproduction.

4.3. Storage of clams shell

The presented detoxification processes (dilution of Hg in the soft

Table 3
Principal component analysis (PCA) of total mercury (Hg_{TOT}). its labile and stable forms in the shell and in the soft tissue with biometric parameters *Dreissena polymorpha*. For bold value $p < 0.05$.

Variables	Factor 1	Factor 2	Factor 3
Temperature	0.19	**−0.80**	−0.02
Length	0.49	0.28	**−0.72**
Body mass	**0.80**	0.14	−0.10
Shell mass	0.40	−0.10	**−0.80**
$Hg_{labile1a}$(shell)	**0.74**	−0.24	0.32
$Hg_{labile1b}$(shell)	**0.76**	−0.22	0.27
HgS(shell)	0.50	0.46	−0.28
Hg_{TOT}(shell)	**0.83**	−0.01	0.14
$Hg_{labile1a}$(body)	0.19	**−0.89**	−0.35
$Hg_{labile1b}$(body)	**−0.60**	**0.42**	−0.27
HgS(body)	−0.48	−0.63	−0.03
Hg_{TOT}(body)	−0.52	−0.37	−0.45

tissue mass, transfer of Hg to the shell) appear to be very efficient. On the one hand, concentration of Hg_{TOT} and its labile and stable forms is higher in the soft tissue of the mussel than in the shell. On the other hand, considering the weight of Hg accumulated in the entire soft tissue and in the entire shell of the mussel, the opposite trend was recorded (Fig. 7). The mass of accumulated total mercury and all its labile and stable forms (except for $Hg_{residual}$) was higher in the shell than in the soft tissue of zebra mussels, and the differences were statistically significant A similar dependency was observed in particular measurement seasons (where $p < 0.05$). On average in the entire weight of *D.polymorpha*, the soft tissue constituted 8 %, and the shell 92 %. The mussel accumulates more mercury in the shell than in the soft tissue. This affects the transfer of toxic mercury in the trophic network, because primarily the soft tissue in *D.polymorpha* is eaten, e.g. by Harris mud crab or Chinese mitten crab. Birds or benthivorous fish that swallow the mussel whole partly excrete the shell during defecation or with pellets. Mercury in the shell of zebra mussels occurred at the highest concentrations in the organic form – Hg_{lab1b}. Higher content of mercury in the shell than in the soft tissue suggests that the shell constitutes the place of transfer of Hg and its deactivation, analogically to feathers and claws in birds and fur in seals (Scheuhammer et al., 2007; Bustamante et al., 2016; Falkowska et al., 2017; Grajewska et al., 2019; Manceau et al., 2021). The storage of shell shows that regardless of the season and the form of mercury in which Hg occurred, more metal was accumulated in the hard tissue (Table 4). Probably as a result of the growth and biomineralization of the shell, which constitutes over 90 % of the mass of the entire organism, mercury is more effectively accumulated in it as a result of the transfer of compounds necessary for the formation of the shell. Because mercury forms

Table 4
Hg and its forms storage (%) in shells of *Dreissena polymorpha*.

	Storage of shell [%]				
	HgTOT	Hg lab.1a	Hg lab.1b	Hg lab.2	HgS
Spring 2019	63	75	66	88	94
Summer 2019	72	79	61	81	95
Autumn 2019	66	74	59	80	98
Spring 2019	59	67	63	87	98

biological ligands with organic matter and complexes with Fe and Mn oxides, which are then redistributed to the shell, the mass of accumulated mercury in the entire soft body was lower than in the entire shell (Fig. 7). This protects the soft tissue of zebra mussels against the toxic effect of Hg. Moreover, a large portion of mercury in the shell was mercury sulphate (stable form – 17 %) (Fig. 2) which is the least toxic and stable form of Hg for the organism. Consequently, a smaller load of Hg in the mussel can be introduced to the circulation or biomagnified in the food web in the region where *Dreissena polymorpha* is an important link of the trophic chain.

5. Conclusions

Dreissena polymorpha is alien species which is dynamically settling in various areas of the world including the lagoons of the southern Baltic Sea. It is exposed to the presence of toxic mercury. The study of 442 organisms (soft tissue and shell) during four study periods, in five size classes show that the clams effectively protect your body from toxins by:

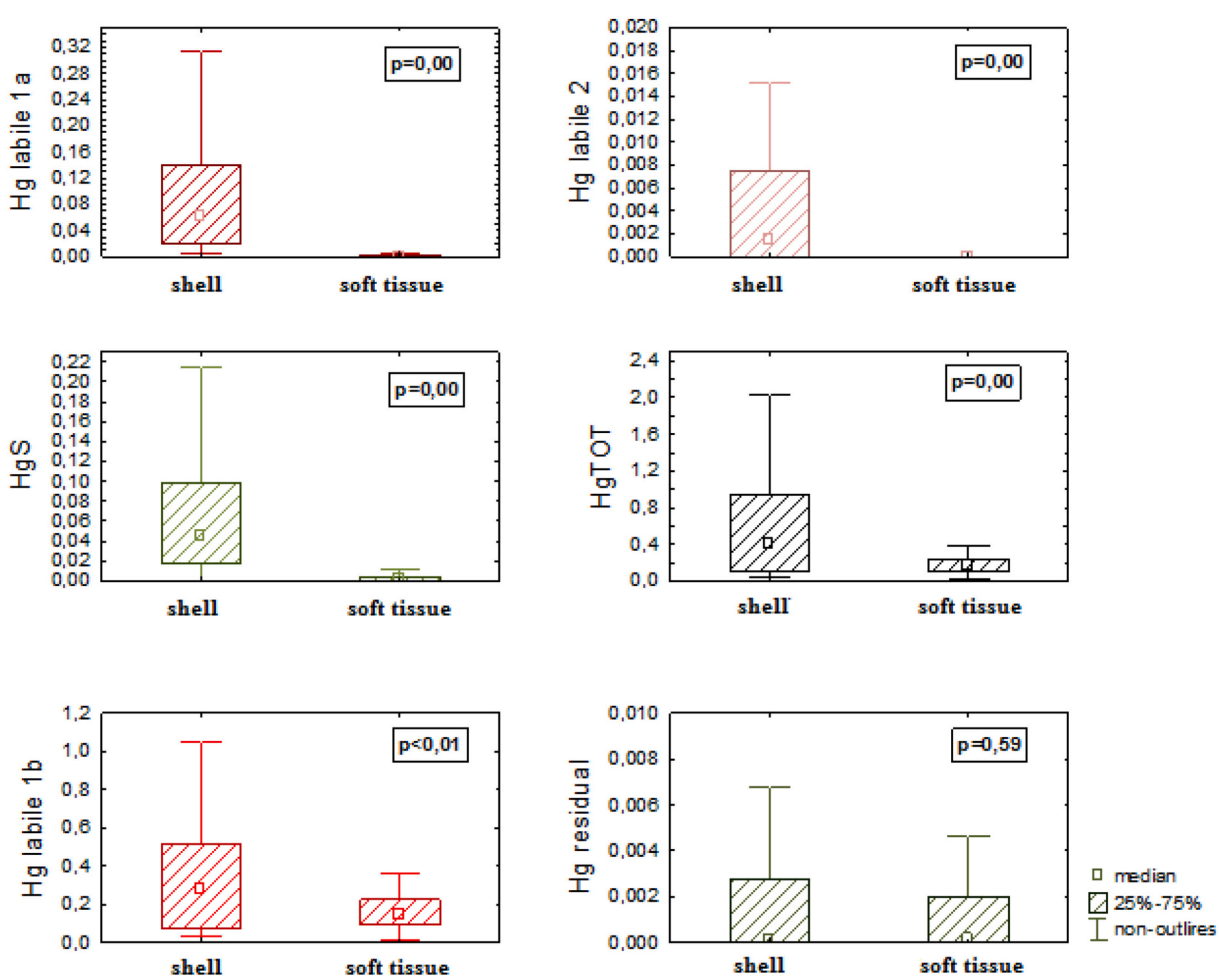

Fig. 7. Mass of accumulated total mercury and its labile and stable forms [ng Hg] in shell and soft tissue mass of *Dreissena polymorpha*.

biodilution Hg in the soft tissue, reproductive cycle and the most effective transfer of the Hg from the soft tissue to the hard tissue (the shell). The factor intensifying these processes was the water temperature. Due to all those processes, mercury concentration in zebra mussels was at a low level. With the assumption that in the future, due to its increasingly frequent occurrence, the organism will become a more and more common component of fish diet, a smaller load of toxic mercury will be introduced to the marine trophic chain. It is important, because Hg fulfils no positive function in living organisms, and is toxic even in low doses.

CRediT authorship contribution statement

Bartłomiej Wilman: corresponding author; collection of material; preparation of material and chemical analyses; statistical analysis; graphical presentation of results; Writing original draft - preparation of each chapter of the article.

Magdalena Bełdowska: Supervision; Conceptualization – Ideas; Writing review and editing.

Agata Rychter: manager of research cruises; analysis of biometric parameters of organisms, tissue preparation; support in the field of biology and ecology of mussels; Writing review and editing.

Angela Popławska: active participation in scientific research cruises; assistance with chemical analyses; preparation of background material: reference literature, compilation of meteorological data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115718.

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Publication 2

Supplementary material

SI Number of individuals [n] of *Dreissena polymorpha* in each size class in the measurement season.

		class size				
		I (5-10 mm)	II (10-15 mm)	III (15-20 mm)	IV (20-25 mm)	V (25-30 mm)
soft tissue	spring 2019	1	31	105	2	n.d.
	summer 2019	31	35	30	27	2
	autumn 2019	25	25	20	18	n.d.
	spring 2020	25	25	20	18	n.d.
shell	spring 2019	n.d.	6	6	n.d.	n.d.
	summer 2019	9	9	6	3	n.d.
	autumn 2019	9	9	9	6	n.d.
	spring 2020	9	9	6	6	n.d.

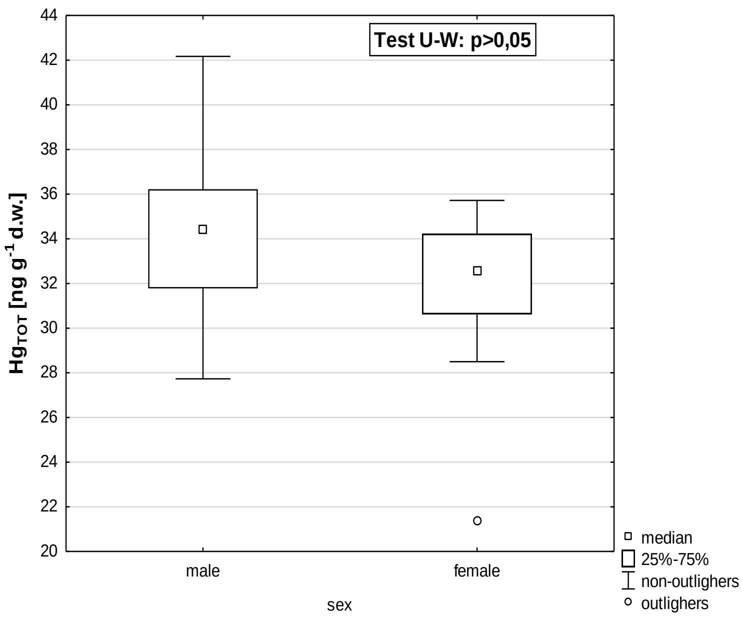
*** n.d. – no data**

SII Principal estimators of the concentration of total mercury (Hg_{TOT}) and its labile stable forms [ng g^{-1} d.w.] in the soft tissue and shell of *Dreissena polymorpha* for the entire measurement season.

		soft tissue					shell				
		Mean	**Median**	**Minimum**	**Maximum**	**SD**	**Mean**	**Median**	**Minimum**	**Maximum**	**SD**
HgTOT	**all sesons**	36.3	35.8	15.9	75.3	10.8	4.9	4.5	2.3	9.8	1.8
Hglabile1a		11.3	9.5	0.9	38.0	7.3	0.8	0.8	0.2	2.8	0.4
Hglabile1b		23.4	23.6	0.5	47.0	10.5	3.1	2.7	1.5	5.8	1.1
Hglabile2		0.3	<LD	<LD	4.1	0.7	0.1	<LD	<LD	0.5	0.1
HgS		0.9	0.3	<LD	6.6	1.5	0.9	0.8	<LD	2.9	0.6
Hgresidual		0.5	0.1	<LD	6.5	1.2	<LD	<LD	<LD	0.3	0.1
HgTOT	**spring**	33.0	33.3	21.4	42.2	4.1	6.4	6.8	4.0	9.8	1.9
Hglabile1a		11.0	10.8	3.1	20.1	4.2	1.3	1.1	0.7	2.8	0.6
Hglabile1b		21.7	22.7	11.9	30.6	4.9	4.1	4.1	2.6	5.8	1.1
Hglabile2		<LD	<LD	<LD	<LD	<LD	0.1	<LD	<LD	0.5	0.1
HgS		<LD	<LD	<LD	<LD	<LD	0.9	0.7	0.5	2.2	0.5
Hgresidual		0.3	0.3	<LD	0.9	0.2	<LD	<LD	<LD	0.1	<LD
HgTOT	**summer**	35.3	28.1	15.9	75.3	14.7	4.7	4.9	3.3	7.0	1.1
Hglabile1a		17.2	17.1	5.3	38.0	7.5	0.9	0.9	0.6	1.2	0.2
Hglabile1b		14.5	15.1	0.5	31.9	7.3	3.2	3.2	2.2	4.4	0.7
Hglabile2		0.7	<LD	<LD	4.1	1.1	<LD	<LD	<LD	0.1	<LD
HgS		1.6	0.8	0.1	6.6	1.8	0.6	0.5	<LD	1.6	0.5
Hgresidual		1.1	0.1	<LD	6.5	1.7	<LD	<LD	<LD	<LD	<LD
HgTOT	**autumn**	39.7	41.1	23.9	52.1	7.0	5.3	4.6	3.1	8.5	1.9
Hglabile1a		5.8	5.4	0.9	11.6	2.7	0.7	0.8	0.2	1.1	0.3
Hglabile1b		32.8	33.5	18.9	43.5	5.7	3.1	2.7	2.0	5.6	1.1
Hglabile2		<LD	<LD	<LD	<LD	<LD	0.1	0.1	<LD	0.3	0.1
HgS		1.2	0.4	<LD	6.3	1.7	1.3	1.0	0.6	2.9	0.7
Hgresidual		<LD	<LD	<LD	0.7	0.2	<LD	<LD	<LD	0.1	<LD
HgTOT	**spring**	38.7	39.2	19.1	55.0	8.8	3.5	3.8	2.3	4.7	0.8
Hglabile1a		5.5	5.1	2.7	9.6	1.8	0.5	0.5	0.2	0.7	0.2
Hglabile1b		33.0	32.6	16.3	47.0	7.9	2.1	2.1	1.5	3.0	0.5
Hglabile2		<LD	<LD	<LD	0.4	0.1	0.1	<LD	<LD	0.3	0.1
HgS		0.3	0.1	<LD	2.3	0.5	0.8	0.7	0.2	1.9	0.4
Hgresidual		<LD	<LD	<LD	0.2	0.1	0.1	<LD	<LD	0.3	0.1

*** LD – limit of detection; SD – standard deviation**

SIII Characteristic of total mercury concentrations [Hg_{TOT} ng g^{-1} d.w.] in male and female *Dreissena polymorpha* during the spring 2019 season.

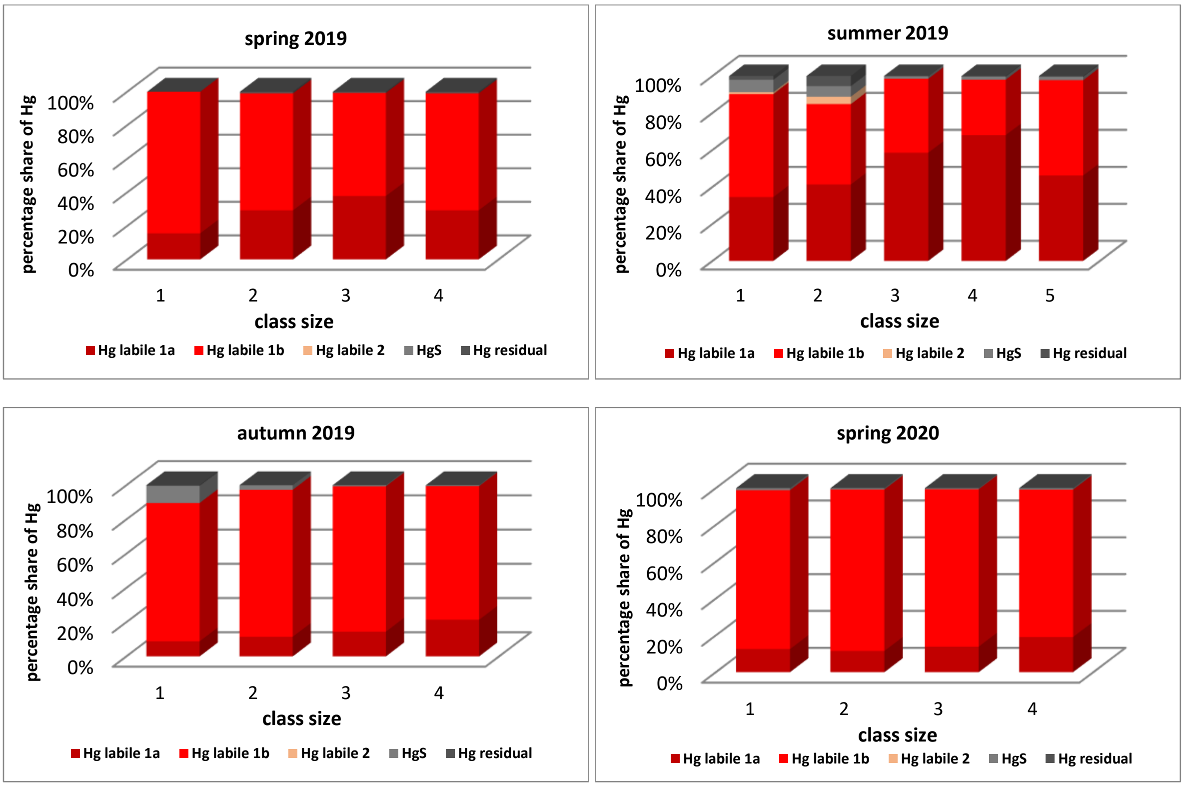


SIV Colony of *Dreissena polymorpha* in environment (photo: Andrzej Martin Kasiński).

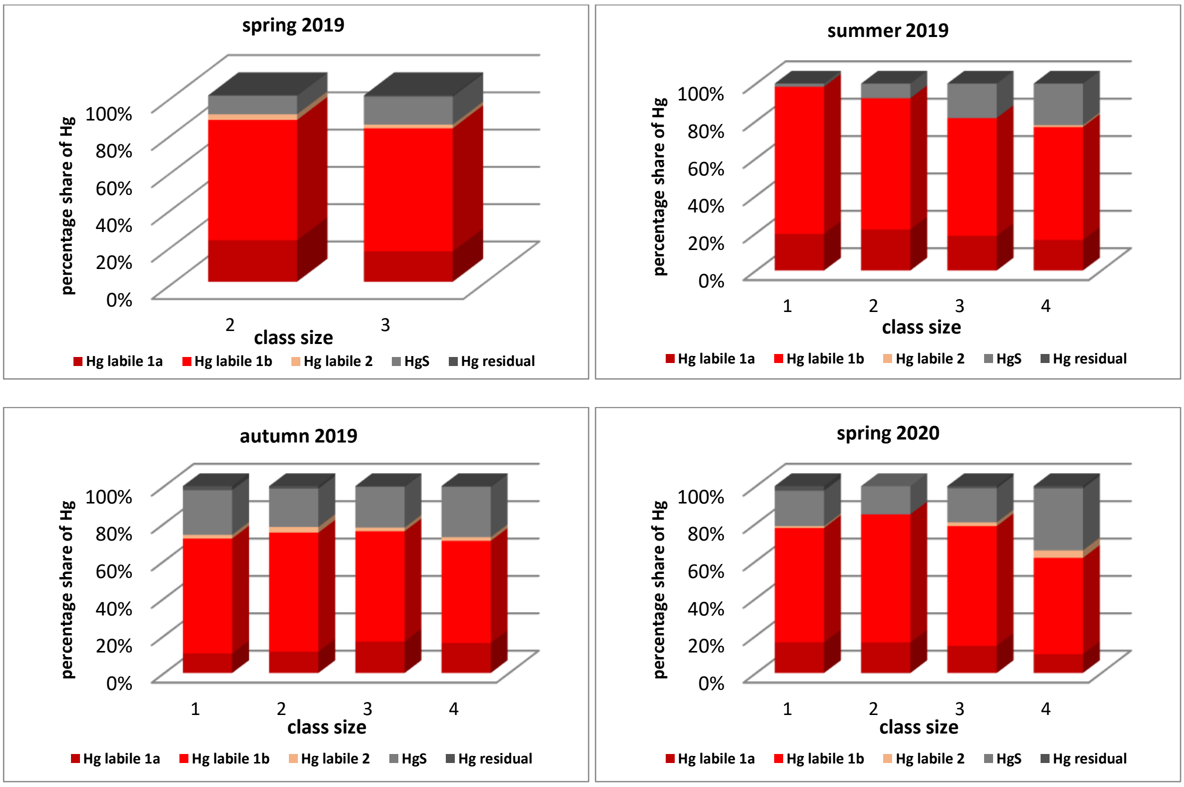
SV Biota-sediment accumulation factor (BSAF) of total mercury (Hg_{TOT}) and labile and stable forms Hg in the investigated *D.polymorpha* with the bioaccumulation factor (BAF) relative to the individual components of the mussel diet: suspended particulate matter (SPM) and plankton.

soft tissue/plankton (BAF)						
	HgTOT	**Hglabile1a**	**Hglabile1b**	**HgS**	**Hglabile2**	**Hgresidual**
spring 2019	**35**	**61**	**53**	**0**	**0**	**0**
summer 2019	**140**	**191**	**104**	**78**	**0**	**1**
autumn 2019	**285**	**116**	**410**	**123**	**0**	**0**
spring 2020	**353**	**275**	**471**	**17**	**1**	**2**
soft tissue/SPM (BAF)						
spring 2019	**29**	**39**	**29**	**0**	**0**	**0**
autumn 2019	**199**	**65**	**328**	**123**	**0**	**0**
spring 2020	**139**	**45**	**100**	**17**	**2**	**2**
soft tissue/sedyment (BSAF)						
spring 2019	**<1**	**<1**	**<1**	**<1**	**<1**	**<1**
summer 2019	**<1**	**<1**	**<1**	**<1**	**<1**	**<1**
autumn 2019	**<1**	**<1**	**<1**	**<1**	**<1**	**<1**
spring 2020	**<1**	**<1**	**<1**	**<1**	**<1**	**<1**

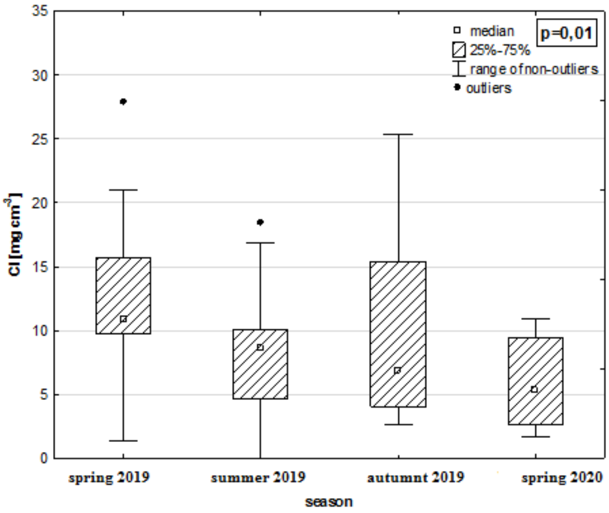
SVI Change in percentage of labile and stable forms of mercury in successive size classes in *Dreissena polymorpha* soft tissue during spring. summer fall 2019 and spring 2020 seasons.



SVII Change in percentage of labile and stable forms of mercury in successive size classes in *Dreissena polymorpha* shells during spring. summer fall 2019 and spring 2020 seasons.



SVIII Condition index - CI [mg cm^{-3}] of the clam *Dreissena polymorpha* in individual measuring seasons.



Statement of co-authors

Individual authors contribution statement has been structured using the Contributors Roles Taxonomy (CRediT) recommended by Brand et al. (2015).

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I declare that my contrubution in publication :

Wilman, B., Bełdowska, M., Rychter, A., Popławska, A. 2023. Factors determining bioaccumulation of neurotoxicant Hg in the zebra mussels (*Dreissena polymorpha*): influence of biometric parameters, sex and storage of shell. *Marine Pollution Bulletin*, *197*, 115718. doi: 10.1016/j.marpolbul.2023.115718.

Included in my doctoral dissertation entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- First author, corresponding author
- Collection and preparation of environmental samples for biological and chemical analyses and their implementation
- Processing and interpretation of results
- Preparation of original draft of the manuscript
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- Support in the field of biology and ecology of mussels
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Included in the doctoral dissertation by Bartłomiej Wilman entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- Active participation in scientific research cruises
- Assistance with chemical analyses
- Preparation of background material: reference literature, compilation of meteorological data

Publication 3

Wilman, B., Normant-Saremba, M., Rychter, A., Bełdowska, M. 2024. Total body burden of neurotoxicant Hg in Chinese mitten crab (*Eriocheir sinensis*) Considerations of distribution and human risk assessment. *Marine Pollution Bulletin*, *199*, 116028. doi: 10.1016/j.marpolbul.2024.116028.

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Total body burden of neurotoxicant Hg in Chinese mitten crab (*Eriocheir sinensis*) - Considerations of distribution and human risk assessment

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A R T I C L E I N F O

Keywords:
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Crustaceans

A B S T R A C T

The Chinese mitten crab (*Eriocheir sinensis*) is considered one of the 100 most invasive alien species in the world. Despite this, its role in ecosystems, among others, in the trophodynamics of pollutants including mercury, is still not fully understood. Becoming an increasingly important and widespread element of the trophic chain in new areas arouses interest from humans as consumers. Hence it is important to determine the level of contaminants (including Hg) in alien species. In the present study, great attention was paid separately to the soft tissues and hard tissues of the exoskeleton, which may play an important role in the detoxification of the crab's body from toxic Hg. The study was conducted on crabs collected in 2011–2021 in the Vistula Lagoon. Concentrations of total mercury and its forms were carried out using a Direct Mercury Analyzer, DMA-80 (Milestone, Italy). The present study showed that mercury accumulation of the crab's body largely occurred through the gills, followed by the oral route. The distribution of Hg in the crab's organs was related to the trophic origin of the mercury, while halide-bound mercury and semilabile forms from the respiration (filtration) process were redistributed into the crab's exoskeleton. Male crabs, compared to females, had a higher Hg burden on internal organs such as their hepatopancreas and gonads. Hg concentration in hard tissues was closely related to the type of mineralization of the carapace. The elimination of Hg from the muscles and from the hepatopancreas into the carapace was one of the important detoxification processes of the crab's body. Thus, moulting crabs effectively remove Hg protecting its body from the neurotoxin. As a result, a smaller Hg load is biomagnified, making the crab's muscle tissue fit for human consumption. The observed decrease in Hg concentrations from 2011 to 2021, as well as the spatial variability of Hg in the crab's muscles, testify that the crab can serve as a biomonitor for ecosystem changes.

1. Introduction

Because mercury (Hg) is a potent neurotoxin, negatively affecting the ecosystem, including human health, contributing to many neurological diseases (Alzhaimer, Parkinson, Autism), it is the subject of research by scientists worldwide (Risher and Amler, 2005; Ekino et al., 2007; Dikme et al., 2013). The distribution of Hg in organs and the elimination pathways of mercury from organisms belonging to higher trophic levels, i.e. fish, birds and mammals, is quite well understood and described in the world literature (Scheuhammer et al., 2007; Bustamante et al., 2016; Falkowska et al., 2017; Grajewska et al., 2019; Manceau et al., 2021). Research into organisms such as invertebrates has mainly focused on determining the concentration of Hg in soft tissue and the effect of seasonality on its variability (Amiard et al., 2006; Chiarelli and Roccheri, 2014; Jędruch et al., 2019). This is probably related to the size of the organisms and the small size of the individual organs, and thus the difficulty in obtaining a representative amount of material for chemical analyses. The Chinese mitten crab is the largest representative among Baltic invertebrates (Normant et al., 2000; Czerniejewski and Bełdowska, 2003). Therefore, it may serve as a model species to fill the research gap on the distribution and detoxification of mercury in species from lower trophic levels. Chinese mitten crab has a large carapace, which accounts for a large percentage of the crab's total mass (Rudnick et al., 2000). So the determination of hazardous substances (including mercury) in the whole individual (external carapace plus soft tissues) may be inappropriate for assessing the accumulation potential of contaminants or their metabolic pathways. Therefore, in the present study, great attention was paid separately to the soft tissues, and the hard

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tissues of the exoskeleton – which may play an important role in the detoxification of the crab's body from toxic Hg. Consequently, it was also hypothesized that elements of the exoskeleton may represent a site of accumulation and temporary inactivation of Hg in this species, so that it can also live in contaminated areas with its muscle tissue fit for human consumption. The study was therefore extended to analyse Hg in the exoskeleton, the so-called hard tissues, which are the attachment site for muscles and internal organs (soft tissues). Studies on another group of invertebrates have shown that the clam's shell plays an important role in the transfer of mercury along the soft tissue-shell (hard tissue) pathway (Wilman et al., 2023a). The production of Chinese mitten crab (CMC) is based mainly on aquaculture, which has increased from 3305 t in 1989–756,877 t in 2018, representing about 2/3 of the entire crab aquaculture production in China. The largest quantity of CMC in Europe is caught in the Elbe river basin. According to Fladung (2000), the catch size of CMC in the 1990s in the Elbe river basin was 100–140 t. Similar to other crustaceans, the inclusion of CMC in the human diet may be problematic due to the potential accumulation of dangerous organic and inorganic substances (e.g. toxic elements) (Barreto et al., 2009; Hoogenboom et al., 2015).

Fish and seafood are the main source of mercury getting into the human body (Mania et al., 2012). One such seafood is the Chinese mitten crab, a species that is non-native to the southern Baltic, first recorded in 1927 (Peters and Panning, 1933). Most individuals have been recorded in the southern Baltic Sea, although individual crabs have been observed every part of the Baltic Sea (Normant et al., 2000; Ojaveer et al., 2007; Ewers et al., 2023). In the southern Baltic, this crab did not form a stable population, where too low salinity limits reproduction of this species. Molecular studies indicate that crabs in Polish waters originate from a population established in Germany (Czerniejewski et al., 2012). Due to the lack of natural enemies in the Baltic Sea and its high adaptability (Kulmatycki, 1934; Czerniejewski and Bełdowska, 2003), the Chinese mitten crab migrates, however, in search of resources to areas in the south-eastern Baltic Sea. Consequently, it is becoming increasingly common in the estuaries, lagoons and bays of northern Poland (Ojaveer et al., 2007; Ewers et al., 2023). *E. sinensis* feeds mainly on vegetation, invertebrates, fish and detritus (Gollasch, 2006; Wójcik-Fudalewska et al., 2019). Feeding on food from different trophic levels, it is itself exposed to accumulation of the body with the xenobiotic Hg. This leads to a situation in which this crab contributes an unknown (so far) load of the xenobiotic Hg to the trophic chain of the southern Baltic, at the end of which is humans. Previous studies on mercury in crabs mainly focus on muscle analysis, which is insufficient, as all the soft tissues are eaten: both white (muscle) and brown (viscera) crab meat, for a specific taste (Hoogenboom et al., 2015; Nędzarek and Czerniejewski, 2021; Wang et al., 2021). Taking this information into account, the aim of the present study was to identify the concentration of total mercury and its labile and stable forms in the internal organs of the Chinese mitten crab from the Vistula Lagoon (southern Baltic Sea), in order to assess whether this species is safe for potential consumption compared to studies on crabs from other regions of the world for Hg. Studies on this crab species show a variable accumulation potential of the internal organs (Boszke et al., 2003; Hui et al., 2005; Wang et al., 2021). Mercury in any form is toxic; however, its toxicity varies depending on the concentration and form, which determines its physical and chemical properties (Kim et al., 2004; Sedlar et al., 2015; Wilman et al., 2023c). The present research also took into account if biometric parameters of the crabs such as the mass, size of the organisms and sex, can may determine the concentration of Hg and its labile and stable forms in *E. sinensis,* are also considered in this study. An additional aim of this study was the temporal and spatial variability of Hg concentration in Chinese mitten crab tested.

2. Materials and methods

2.1. Study area and sampling

The crabs were collected in October 2019 in the Vistula Lagoon in the area of Święty Kamień (area A), which is located between Tolkmicko and Frombork (54° .341743′ N, 19° 0.577823′ E) (area A; Fig. 1). The crabs were collected from traps set by local fishermen and immediately after collection were frozen at -20 °C for further analysis. In addition, crabs were collected in October 2019 from four fisheries in the south-eastern part of the Vistula Lagoon, in the area of Święty Kamień (area A), in the area of Nowa Pasłęka (area B), and in the area of Różaniec and the Russian border (area C; Fig. 1). Additionally, material from *E.sinensis* specimens collected in October 2011, 2017, and 2020, caught in the area of the Święty Kamień fishery (area A; Fig. 1), was analysed. The month of October is the time when crabs prepare to migrate in the southern Baltic to breed, as the salinity of the water in the Vistula Lagoon is too low. Their abundance during the study period was greatest in October, hence the sample was the most representative for chemical analysis. In addition, the choice of a single measurement season reduced the number of different variables affecting xenobiotic Hg concentrations in *E. sinensis*.

2.2. Biological analysis

In the thawed crabs, their sex was determined based on the structure of their abdomen (Schäferna, 1935) and then, after draining excess water on a fiber-free paper towel, they were weighed on a RADWAG WPS 1100/C/10 analytical balance (±0.01 g), followed by measuring their width (± 0.01 mm) in triplicate with a caliper at the widest point of the carapace. The collected crabs were then divided into groups:

I – to determine the concentration of total mercury and its fractions in whole individuals from the month of October 2019 caught in area A. For this purpose, 7 females and 7 males of *E. sinensis* were selected in the carapace width range: 57.10–80, 00 mm (Fig. 1; Table 1).

II – to determine the distribution of mercury and its forms in individual soft tissues: (fleshes walking legs – FWL, claws fleshes – FC) and internal organs: (gonads – GO, hepatopancreas – HP, gills – GI) and parts of the hard tissue, i.e. the exoskeleton, covering the soft tissues (carapace, dactylus, merus, claws, mittens – cuticular appendages). *E. sinensis* specimens from October 2019 were selected for dissection, (area A) 12 females and 27 males with a carapace width range of 60.00–76.80 mm (Fig. 1; Table 1).

III – in order to determine the multi-year variability of Hg concentration and its forms in Chinese mitten crab, five individuals each were selected from the month of October in 2011, 2017, 2019 and 2020 caught in area A between 60.01 and 70.00 mm carapace width (Fig. 1; Table 1). In these specimens, only the muscle tissue of the fleshes walking legs (FWL) was analysed.

IV – to determine the spatial variability of mercury concentration and fractions in crabs from the Vistula Lagoon, specimens from October 2019, caught in areas A,B and C in the carapace width range 60.01–70.00 mm were selected (Fig. 1; Table 1). In these specimens, only the muscle tissue of the fleshes walking legs (FWL) was analysed.

In all samples, their wet weight was determined on an analytical balance and placed in a freezer. Prior to the chemical analyses, samples were lyophilized by ice sublimation at -54 °C and homogenized in a ball mill (hard tissues) while the soft tissues were homogenized manually using an agate mortar.

2.3. Mercury analysis

Analysis of total mercury (HgTOT) was performed by thermal

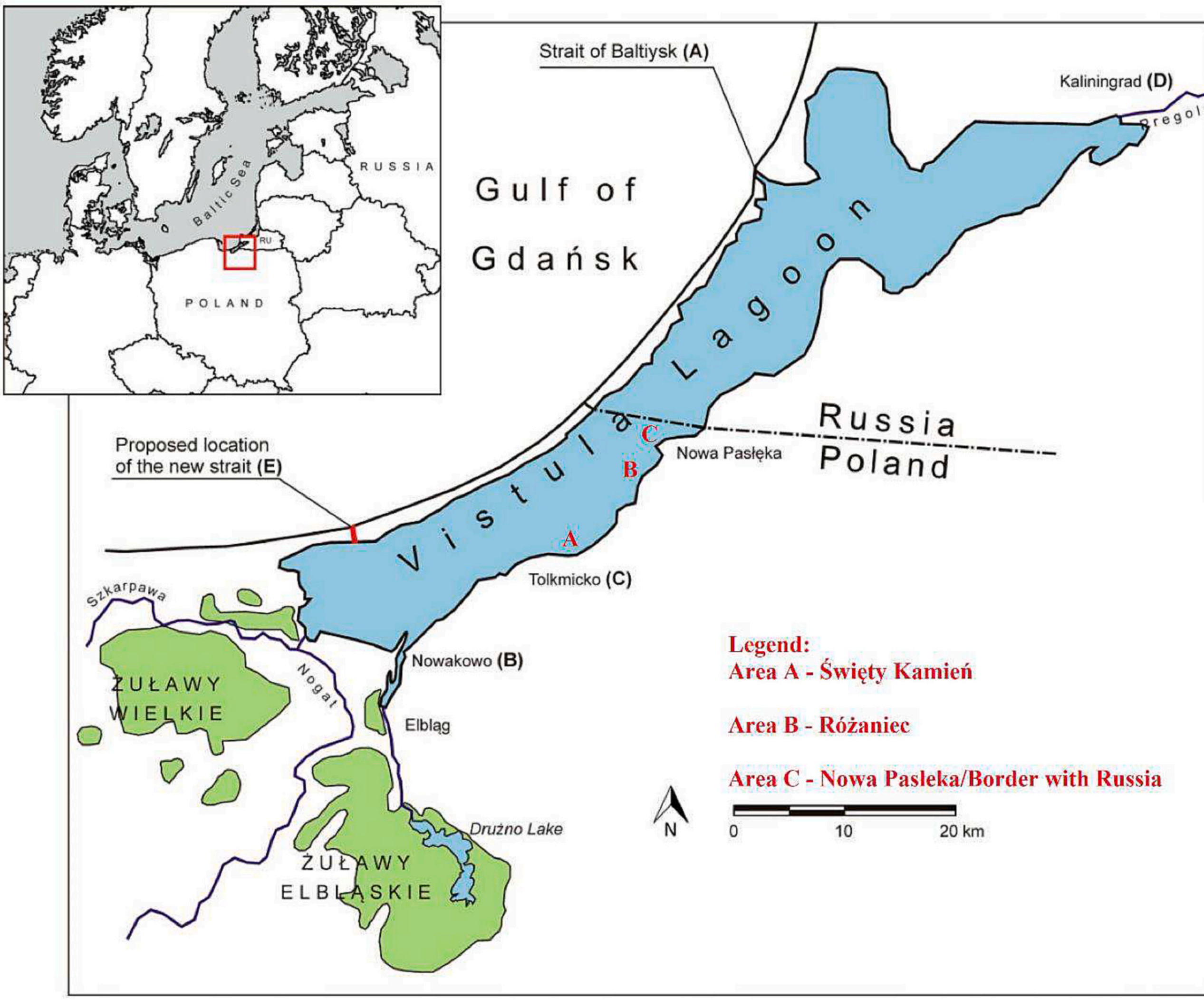

Fig. 1. Study area: Vistula Lagoon (southern Baltic Sea, Poland) based on (Szydłowski et al., 2021).

Table 1
Biometric parameters of *Eriocheir sinensis* from Vistula Lagoon (southern Baltic).

Group	Date/location	Sex	n	Carapace width [mm]	Mass [g]	GSI[%]	HSI[%]	K
I	Św.Kamień X 2019	female	7	69.2 ± 5.5	131.0 ± 30.4	n.d	n.d	0.20 ± 0.04
		male	7	68.6 ± 11.5	129.3 ± 74.0	n.d	n.d	0.21 ± 0.07
		all	14	68.9 ± 8.7	125.2 ± 54.7	n.d	n.d	0.20 ± 0.05
II	Św.Kamień X 2019	female	12	67.4 ± 8.1	133.4 ± 31.3	4.2 ± 1.3	4.9 ± 1.5	0.20 ± 0.03
		male	27	68.0 ± 8.3	156.7 ± 55.5	2.9 ± 1.2	4.7 ± 1.6	0.23 ± 0.08
		all	39	67.8 ± 8.1	149.6 ± 50.1	3.3 ± 1.4	4.7 ± 1.5	0.22 ± 0.05
III	Św.Kamień X 2011	all	5	62.9 ± 6.0	113.0 ± 8.5	n.d	n.d	0.18 ± 0.03
	Św.Kamień X 2017	all	5	69.4 ± 4.1	177.4 ± 23.7	n.d	n.d	0.25 ± 0.02
	Św.Kamień X 2019	all	5	69.0 ± 2.0	173.2 ± 13.8	n.d	n.d	0.26 ± 0.02
	Św.Kamień X 2020	all	5	67.9 ± 3.3	170.5 ± 13.0	n.d	n.d	0.25 ± 0.02
IV	Area A	all	5	69.0 ± 2.0	173.2 ± 13.8	n.d	n.d	0.26 ± 0.02
	Area B	all	5	64.6 ± 4.3	157.9 ± 42.0	n.d	n.d	0.24 ± 0.05
	Area C	all	10	65.9 ± 3.5	160.2 ± 18.2	n.d	n.d	0.25 ± 0.02

n.d. – no data.

desorption using a DMA-80 analyzer (Milestone, Italy), according to the method published by Saniewska and Bełdowska (2017). This method does not require prior mineralization of the sample, which reduces the risk of contamination. Approximately 0.1 to 0.2 g of sample was weighed on quartz boats in triplicate. Determination of the Hg concentration, and the share of individual forms of Hg, was carried out according to the thermal desorption method of Hg compounds, published by Wilman et al. (2023c) for biological material. Samples were weighed in triplicate on quartz boats and the sample mass (0.05–0.1 g), depending on the expected HgTOT concentration. The method was verified by analysing certified reference materials (mussel tissue SRM-2976 – HgTOT: 61 ng/ g d.w., scallop IAEA-452 – HgTOT: 160 ng/ g d.w., fish protein DORM-4 – HgTOT: 410 ng/ g d.w., dogfish liver DOLT-5 – HgTOT: 440 ng/ g d.w.) (sample weight: 0.1–0.2 g), with a mean recovery of 98 % and standard deviation not exceeding 7 %. The limit of detection (LOD) was calculated as the threshold of the standard deviation (SD) of the Hg concentration in the ignited sample boat measured in 10 replicates. In the case of Hg_{TOT} analysis, the LOD was 1 pg of Hg.

The application of the 5-step thermo-desorption method allowed the separation of five groups of compounds with similar properties (Jędruch et al., 2018; Wilman et al., 2023c). The method involved the simultaneous combustion of the same sample at five temperatures: In 175 °C (release of labile forms – HgF1 bound to halides: HgCl; $HgCl_2$, $HgBr_2$); 225 °C (release of labile forms – HgF2: Hg compounds combined with humic substances, MeHg, as well as other Hg compounds formed with ligands having strong complexing properties ($(CH_3COO)_2Hg$, Hg $(NO_3)_2{\cdot}H_2O$, $Hg(SCN)_2$); 325 °C (release of stable form – HgF3: HgS); 475 °C (release of semi-labile forms – HgF4: $HgSO_4$, HgO (red) and HgF_2 (2)); 750 °C (release of residual mercury (HgF5) (Bełdowska et al., 2018; Jędruch et al., 2018; Wilman et al., 2023c). The analyses of the environmental samples were preceded by an examination of the Hg patterns specific to each fraction according to the method of Saniewska and Bełdowska (2017). Seal muscle was used as the pattern for the thermally labile fraction (HgF2), in which MeHg accounted for 98 % of the contribution to the total HgTOT (Jędruch et al., 2018) and the contribution of fraction HgF2 accounted for 98 % of the total Hg fraction in the seal muscle, respectively. The use of Hg standards in this method is important, as there are no certified reference materials for individual forms of Hg in environmental samples. The analysis of the samples was performed in triplicate. The analysis was verified by comparing the sum of Hg concentrations, measured via the 5-step fractionation method with the result of the Hg_{TOT} concentration analysis. The mean recovery of the Hg form analysis was 111 %, with standard deviation not exceeding 5 %. The LOD for each fractionation method was calculated from ten replicates of the substrate analysis. The calculated LOD values were at the level of 1.2 pg of Hg, as in the case of HgTOT.

2.4. Processing results

Statistical analysis and graphic representation of the obtained results were carried out using STATISTICA 13 (StatSoft) and Microsoft Excel (2008). The analysed data were characterized by non-normal distribution (Shapiro-Wilk test $P < 0.05$). In order to determine the significance of differences between the Hg concentration and biometric parameters, the non-parametric U MannWhitney and ANOVA Kruskal-Wallis tests were used. The relationships between the analysed variables were determined on the basis of Spearman's coefficient, with a confidence interval of at least 95 %. $P < 0.05$ was regarded as a statistically significant difference. The crab tissues were grouped based on their Hg concentration using a multidimensional statistical technique – an aggregation analysis. The gonadosomatic index – GSI, and hepatosomatic index – HSI, of the crabs were calculated using the following formulas (Shao et al., 2013):

$$GSI\ [\%] = \frac{100 \text{ x Gonad wet weight}}{\text{Crab's body wet weight}} \quad (1)$$

$$HSI\ [\%] = \frac{100 \text{ x Hepatopancreas wet weight}}{\text{Crab's body wet weight}} \quad (2)$$

The condition of the crabs was calculated only for complete individuals (not missing appendages) using Fulton's condition index (K), with the formula: $K = W/L^3$, where W = wet weight (g), and L = carapace width (mm) (Ricker, 1975). For a more complete interpretation of the data, the biochemical composition determined for the organs of *E. sinensis* in the paper was used, based on results Shao et al. (2013) (Table 2). The dry weight concentrations of Hg in crabs were converted to the wet weight basis using the water content (average 80 %) in the soft tissues (Hamilton and Clifton, 1980).

Table 2
Biochemical compounds of *E.sinensis* organs based on Shao et al. (2013).

Hepatopancreas	Female	Male
Protein	5.30 %	6.30 %
Total lipid	47.10 %	41.20 %
Carbohydrate	1.10 %	1.20 %
Gonads	Female	Male
Protein	23.50 %	13.20 %
Total lipid	12.60 %	0.70 %
Carbohydrate	1.40 %	0.40 %
Muscles	Female	Male
Protein	17.70 %	17.0 %
Total lipid	1.0 %	1.0 %
Carbohydrate	0.90 %	0.50 %

3. Results and discussion

3.1. Hg total body burden in the organism of E. sinensis

The median total mercury concentration (HgTOT) in whole individuals of Chinese mitten crab from the Vistula Lagoon ($n = 14$) was 62.5 ng/g d.w., with the median HgTOT being higher ($P > 0.05$; Fig. 2a) in females (70.0 ng/g d.w.) compared to males (55.0 ng/g d.w.). A similar lack of any differences in mercury concentration was also found in other invertebrate species from the Vistula Lagoon, i.e. the American mud crab (*Rhithropanopus harrisii*) and the clams: *Rangia cuneata* and *Dreissena polymorpha* (Wilman et al., 2019; Wilman et al., 2023a, 2023b). Biometric parameters such as individual size (carapace width), crab weight and the K index also did not determine a different mercury concentration in whole individuals ($P > 0.05$). The lack of difference in metal concentration may have been due to the same dietary relationships of the males and females. A study by Wójcik-Fudalewska et al. (2019) showed that the sex of an individual has no effect on the stomach fullness in *E. sinensis*, despite the fact that the prey choice is often a function of sexual dimorphism (morphology and size of claws) and differences in foraging behavior (Spooner et al., 2007). In addition, the sex of an individual did not affect the type of food consumed in the case of *E. sinensis* (Wójcik-Fudalewska et al., 2019). Similar characteristics in the whole crab's mercury burden may have been due to a lack of detoxification of the body from Hg through reproduction, as this species reproduces just before death. Similarly, the food preference of crabs from the Vistula Lagoon is reflected in the percentage of individual forms of Hg in whole individuals of *E.sinensis*, which was uniform in males and females (Fig. 2b). In both cases, 97.2 % in HgTOT was accounted for by the HgF2 fraction (Hg in complexes with organic matter), which is mainly of trophic origin and represents a large proportion in animal tissues as well as detritus (Jędruch et al., 2018; Wilman et al., 2023c). These Hg compounds are easily transported inside the organism's cells – they are absorbed more completely than inorganic salts because they are more lipid-soluble and because they bind to sulfhydryl groups (Broussard et al., 2002). They also undergo bioaccumulation and magnification in the trophic chain (Cavoura et al., 2019). In contrast, 1.8 % was the HgF1 fraction (mainly halogenated forms of mercury), which is an important form of Hg in micro- and macroalgae and suspension (Bełdowska et al., 2018; Wilman et al., 2023c,c). However, because it is the most labile form of Hg and has a lower affinity for mercury toxicity target groups, its contribution to HgTOT was negligible in *E.sinensis* (Aksentov and Sattarova, 2020; Wilman et al., 2023a). They mostly form loose surface compounds with the substrate, and can be partially absorbed from the GI tract, which is connected with the water solubility of these compounds (Broussard et al., 2002; Aksentov and Sattarova, 2020). The other forms of Hg accounted for a total of 1 % of the contribution to HgTOT, and their concentrations were negligible (Fig. 2b).

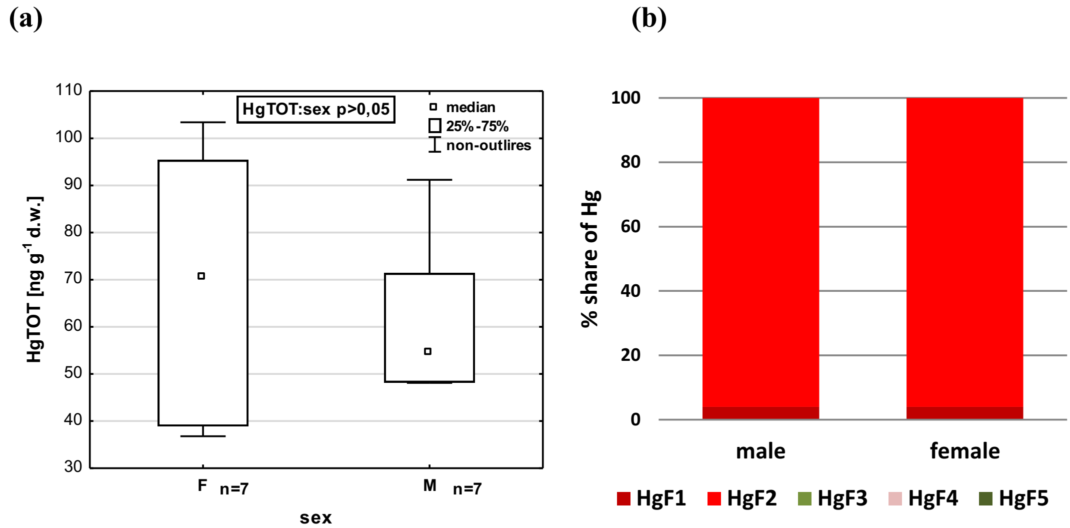

Fig. 2. Effect of sex on the concentration of total mercury (HgTOT) (a) and its forms (b) measured in whole specimens of *E.sinensis* (F-female; M-male).

3.2. Accumulation of Hg in crab tissues

Discussing Hg accumulation from the crab's body through the consumption of whole individuals may be inaccurate, as it is mainly the internal organs are the site of the bioaccumulation and biomagnification of mercury which are mostly consumed by humans (Branco et al., 2007; Kojadinovic et al., 2006). Cluster analysis categorized the results of HgTOT concentration and its fraction in *E. sinensis* soft tissues into two groups (Fig. 3). The tissues with the highest value of total mercury concentration, 'white crab meat': i.e. the fleshes walking leg (FWL) and fleshes claws (FC), were assigned to the first group (Fig. 3a; Fig. 4a). The second group was assigned the internal organs of the cephalothorax also known as "brown crab meat", i.e. the gills, hepatopancreas and gonads, which had Hg concentrations an order of magnitude lower compared to the *E.sinensis muscles* (Fig. 3a; Fig. 4a). The difference in the concentration of the test metal between white crab meat and brown crab meat was statistically significant (Fig. 4a). The reason influencing this variation is the biochemical composition of the individual tissues of Chinese mitten crab (Table 2). A study by Shao et al. (2013) showed that the muscles of *E.sinensis* crab are high in protein, with a low fat content compared to the hepatopancreas or gonads. Sulphydryl groups, included in many amino acids and peptides, are crucial for toxic mercury to form biological ligands with it by a direct interaction of Hg cations with SH groups, e.g. cysteine (Ajsuvakova et al., 2020). Hence, the bioaccumulation of Hg sourced from food base and water (filtration during respiration) in muscle is higher compared to other internal organs. The presence of proteins in muscle and internal organs influenced the fact that mercury was 100 % present there in labile forms, which are potential more bioavailable and toxic to the body (Fig. 4b). There are relatively few scientific reports in the world literature on the distribution of Hg in *E. sinensis* tissues. In the present study, organ loading in the Chinese mitten crab was analogous to that in the same species from the Bay of Puck, i.e. muscle > gills > hepatopancreas > gonads (Boszke et al., 2003). Also, Chinese mitten crab muscles from Chinese waters had higher Hg concentrations than hepatopancreas (Zhou et al., 2018). In contrast, the present study obtained the opposite relationship to other trace metals in *E. sinensis* from the Elbe River, in which higher concentrations of Zn, Cu, Mn and Fe occurred in the hepatopancreas and gonads, but not in the muscles (Nędzarek and Czerniejewski, 2021). Also, in another species of crab – *Portunus pelagicus* (blue crab) from the Persian Gulf – there was a higher bioaccumulation of Se, as Pb was observed in the hepatopancreas and gills rather than in muscle (Abdolhay et al., 2020). The other elements was not study in this study. The present study may indicate that, in Brachyura, mercury compared to other metals may undergo different metabolic pathways in internal organs. However, this issue requires more research on the distribution of Hg in crab tissues.

For the hard tissues of the exoskeleton, cluster analysis separated three groups in terms of their xenobiotic Hg concentration, which differed statistically significantly (Fig. 3b; Fig. 4c). The tissues with the lowest Hg concentrations were categorized in the first group: dactylus and carapace; in the second: merus – the largest segment of the legs, containing the greatest muscle mass – and claws; the species-specific mittens, in which the highest Hg concentration was determined, were categorized in the third group (Fig. 4c). The three outer layers of the cuticle are hardened by calcite or amorphous calcium carbonate deposited within the chitin-protein matrix (Roer and Dillaman, 1984; Lowenstam and Weiner, 1989; Giraud-Guille and Bouligand, 1994). In a

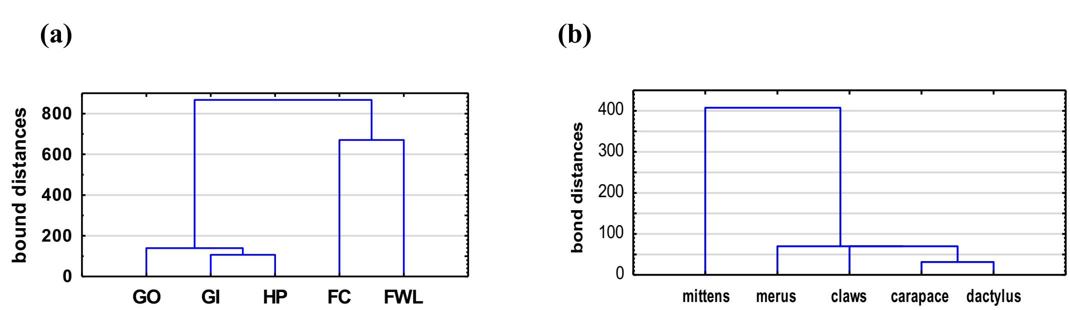

Fig. 3. Cluster analysis of the soft tissues (a) and hard tissues (b) of the Chinese mitten crab. Binding strength was determined by the concentration of total mercury (HgTOT) in each crab's body parts (fleshes walking legs – FWL, claws fleshes – FC; gonads – GO, hepatopancreas – HP, gills – GI).

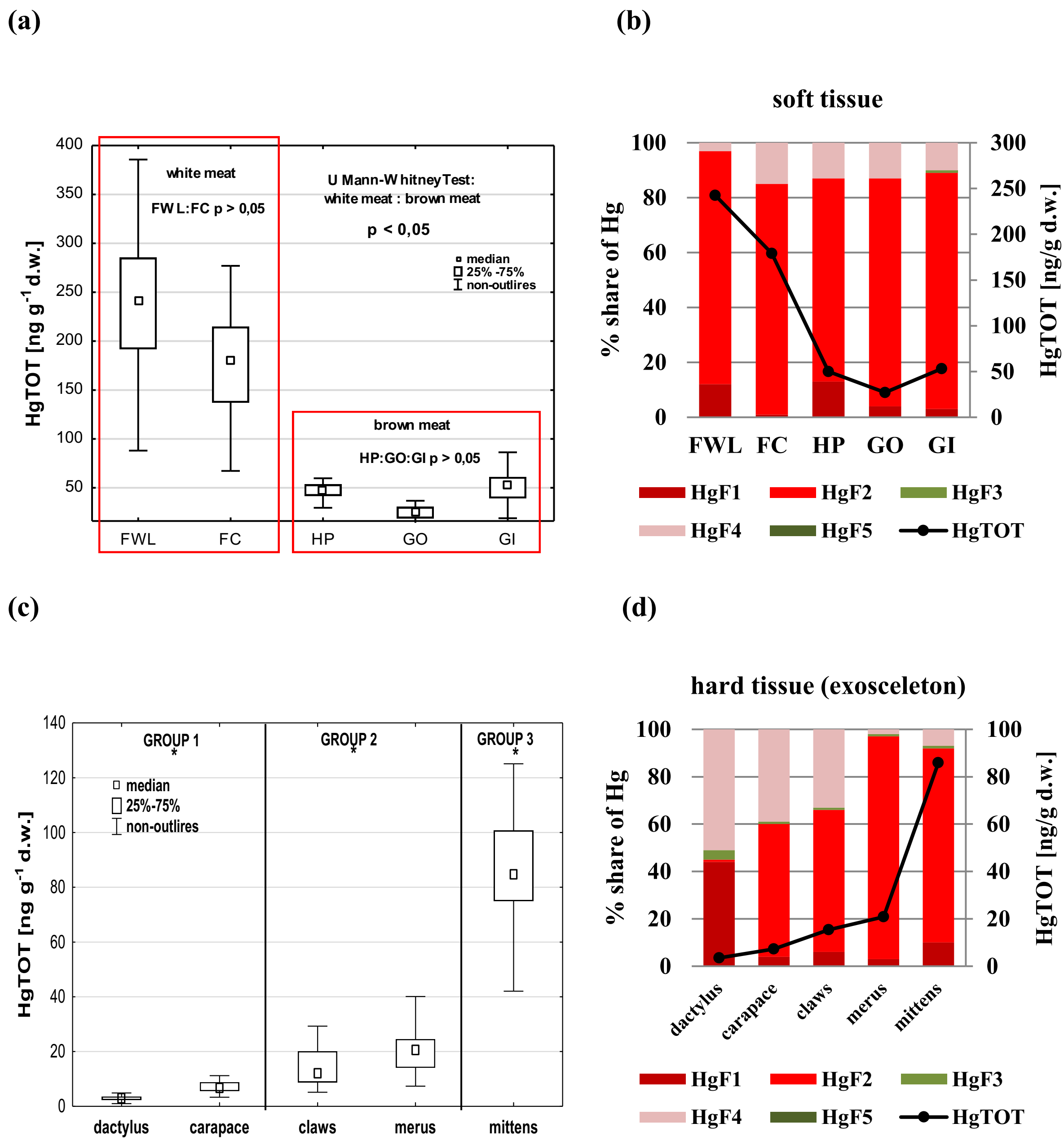

Fig. 4. Variability of total mercury stearance (HgTOT) in the soft tissues of *E.sinensis* (a) and the proportion of individual forms of Hg (b). Similarly, the variability of HgTOT in hard tissues (c) and the percentage of Hg forms in them (d) were presented. The tissues were divided into groups according to the result of cluster analysis. Asterisks indicate groups for which $P < 0.05$ (fleshes walking legs – FWL, claws fleshes – FC; gonads – GO, hepatopancreas – HP, gills – GI).

study by Wilman et al. (2023a), it was shown that calcification of the shells of the clams *Dreissena polymorpha* or *Rangia cuneata* results in a dilution of Hg in its mass, which resulted in a low concentration of the metal. However, higher concentrations of the xenobiotic were determined in the hard tissue of the exoskeleton of the Chinese mitten crab than in the clam shells. Because the cuticle is periodically moulted, calcium is conserved by being put into solution and transferred through the integumentary epithelium to the blood. It is mostly transported to cells, tissues, and other organs for temporary storage, and remobilized for deposition into the new exoskeleton immediately postmoult. As a result of these processes, Hg can also be bound in the carapace by substitution, due to the similar ionic flux of Hg^{2+} to Ca^{2+} (Rae et al., 2009). However, adult specimens moult less frequently, once a year, with the consequence that the rate of the Hg accumulation in the carapace may be greater than its elimination during the moulting process. In addition, in the exoskeleton are well-developed, high-density pores containing long, flexible tubules (each about 1 μm wide) that play a crucial role in the transport of ions (including Hg^{2+}) and nutritive material during the establishment and strengthening of the new exoskeleton after moulting (Cameron, 1989; Chen et al., 2008). The elevated Hg concentration in the crab carapace may also be due to the presence of an additional structure in the exoskeleton, namely the procuticle. The procuticle is divided into two parts, an exocuticle and an endocuticle, each with a similar composition and structure (Raabe et al., 2005). The procuticle is built from the long-chained polysaccharide chitin, in which mercury can be bolding, and makes bioligand (Chen et al., 2008). The lowest Hg concentration determined in the dactylus Chinese mitten crab among the hard tissue parts examined may indicate different neurotoxin binding processes than those discussed above. The tips of the claws and dactylus walking leg, while being darker in colour than the adjacent cuticle, tend to be somewhat translucent and flexible, potentially indicating a lack of typical mineralization. Schofield et al. (2003) showed that as calcium levels decline, chlorine (Cl^-) takes its place as the dominant inorganic element. Cribb et al. (2009) addressed the structure and composition of the non-calcareous exoskeleton at the ends of the dactyls of the crab *Metopograpsus frontalis* in their study. They found significant concentrations of Cl^- (chloride) and Br^- (bromide) ions. It is likely that the same pattern of mineralization occurs in the dactylus of *E. sinensis*, which can be confirmed by the mercury fractionation results, as the proportion of halogenated forms of Hg (HgBr, $HgCl_2$, HgCl) was precisely the highest in the dactylus of all the tissues analysed, both hard and soft (Fig. 4d). Because it is the most labile form of Hg and has a lower affinity for the target groups of mercury toxicity, it bioaccumulates more poorly, resulting in the lowest Hg concentrations in the crab dactylus (Fig. 4c). The different pattern of dactylus mineralization in crabs may be due to its role in overcoming obstacles, e.g. when climbing vertical surfaces on the migration pathway. Interpretation of the highest Hg concentration in the mitten of *E.sinensis* is difficult due to the lack of studies on the chemistry of mittens or their specific role in the crab body. The density and mass of the mittens is one of the features of sexual dimorphism that distinguishes females from males. In the males, the area covered by the pincer mittens is larger than in the females (Gollasch, 2006). However, there was no statistically significant difference ($P > 0.05$) in Hg concentration in the mittens between males and females of *E. sinensis*. Hypothetically, mittens in the crab may represent sites of Hg accumulation and deactivation, analogous to hair in mammals or feathers in birds, as cuticular outgrowths are shed along with the carapace during moulting. By way of moulting feathers in birds, or hair replacement in mammals, it is known that a hot-spot of Hg accumulation is formed at the site of the formation of new cuticular outgrowths, which is then incorporated into these structures (Scheuhammer et al., 2007; Bustamante et al., 2016; Falkowska et al., 2017; Grajewska et al., 2019; Manceau et al., 2021). Hence, the incorporation of Hg into appendages by the crab may be important in its elimination from the organism. However, mittens may be exposed to the direct adsorption of Hg from water and provide a habitat for small invertebrates, which may further influence higher Hg concentrations in these body parts (Normant et al., 2007; Normant et al., 2013).

3.3. Accumulation and detoxification pathways of Hg in E. sinensis

The presented levels of Hg bioaccumulation in individual soft tissues and the exoskeleton of the Chinese mitten crab are related, among other things, to the body's pathways of Hg accumulation, invertebrate adaptation mechanisms to high metal concentrations, and direct detoxification of the body from the xenobiotic (Migula, 1991).

The main routes of metal ingress in invertebrates are the gills and the digestive system. During exposure to toxic metals in water, the gills of crustaceans, which are the main entry site, act mainly as a transient tissue store for accumulated metals (Soegianto et al., 2022). Branched gills are characterized by large lamellar surface areas, high membrane permeability and the countercurrent flow of water and hemolymph, which increase the exchange of respiratory gases and inorganic osmolytes and also enhance the uptake of toxic metals (Foster and Howse, 1978). Hence, Hg concentrations in the gills of *E.sinensis* were higher compared to the hepatopancreas or gonads (Fig. 4a). Toxic water-borne metals affect crustaceans by adsorption on the cuticular surface of the gills, then are transported by epithelial cells into the hemolymph, where they are eventually transported to the internal organs. In the Chinese mitten crab, the results show Hg transfer from the gills to the hepatopancreas and gonads at the same level ($r = 0.5$ and $P < 0.05$) for both cases (Table 3). The enrichment factor was also at the same level (Table 3). To a lower extent, mercury accumulated in the gills was transported to the leg muscles, where EF amounted to 1.7 ($r = 0.7$ and $P < 0.05$; Table 3). Highly lipid-soluble complexes of MeHg and Hg^{2+} in the form $HgCl_2$ rapidly cross the cell membranes. MeHg is more homogeneously and rapidly distributed than inorganic mercury. In the medial portion of the gill stem, mercury is randomly distributed within lamellae in the muscle (Laporte et al., 2002) – hence also the highest proportion of the HgF2 fraction in both gills and muscle in *E.sinensis* (Fig. 4b).

Table 3
Statistical dependencies of total mercury concentrations in tissues of *Eriocheir sinensis*.*

	Gills (GI)		
	r	Regression equation	Enrichment factor [EF]
Gonads (GO)	0.5	y = 3,44x + 8899	3.4
Hepatopancreas (HP)	0.5	y = 3,53x + 9002	3.5
Fleshes walking leg (FWL)	0.7	y = 1,76x + 10,334	1.7

* For each r-value, $P < 0.05$.

Crabs have an open circulatory system. The hemolymph is pumped by the heart and forced directly into the body cavity, which washes over the organs thus supplying nutrients and oxygen as well as hazardous substances (Barreto et al., 2009; Hoogenboom et al., 2015). Some tissues, such as the hepatopancreas, are considered target organs for metal accumulation (Yılmaz and Yılmaz, 2007). The very high levels of metals in the hepatopancreas compared to other tissues may be related to the metallothionein (Mt) protein content in this organ. In general, metal accumulation in the hepatopancreas may be due to the abundance of metallothionein proteins in these tissues compared to gills and muscle tissue (Sen and Semiz, 2007). Mt. proteins sequester metals into stable complexes, in this case into HgS, less toxic to the body. However, mercury concentrations in the hepatopancreas were at lower levels compared to the muscle or gills in *E.sinensis* (Fig. 4a.). This may indicate a different detoxification process, namely Hg demethylation in the hepatopancreas in the Chinese mitten crab. In this organ, an inverse relationship was observed between HgF2 (Hg bounding with organic complexes) and the stable form of mercury, HgS ($r = -0.80$ and $P < 0.05$). The Mt. protein contains a high percentage of the amine group, nitrogen and sulphur, which sequester the metals into stable complexes. Hence, the further stable form of mercury, HgS, was redistributed to other organs: gonads ($r = -0.98$ and $P < 0.05$) or muscle ($r = -0.50$ and $P < 0.05$). Delakorda et al. (2009) consider that Mt. complexes with cadmium and mercury may be metabolized more slowly than complexes with biologically essential metals, indicating one possible pathway for participation in detoxification – hence, the lower Hg concentrations in the hepatopancreas than in the muscles or gonads of *E.sinensis.*. No correlation was observed between hepatopancreas metal concentration and HSI ($P > 0.05$), possibly indicating that this crab effectively copes with the body burden of the xenobiotic Hg. An additional determinant of lower Hg concentrations in the hepatopancreas may be the ability to compartmentalize metals in granules and vesicles (Migula, 1991). Marine invertebrates have storage vesicles in the hepatopancreas, where metals remain unchanged and metabolically inactive in these structures. Hg concentrations in the hepatopancreas of *E.sinensis* females were statistically significantly lower than those of males (Fig. 5a). This may be due to the organ loading with feed from different trophic levels. The male crab feeds more on fish and bivalves, whereas the female crab feeds more on shrimps, plants and benthic organisms (Hosseini et al., 2013). This relationship may also be confirmed by statistically significantly higher concentrations of halogenated forms of Hg (HgF1) in the liverpancreas and gonads of females (Fig. 6a,b). These forms of Hg may be a major component of the micro and macroalgae and suspension (Bełdowska et al., 2018; Wilman et al., 2023c), which are an important part of the diet of females. The statistically significant higher concentrations of HgS in the hepatopancreas and gonads of males than females may indicate a more efficient mechanism of demethylation to stable, less toxic forms as a result of a higher organ load of neurotoxin, (Fig. 6c,d). Hui et al. (2005) in their study observed a decrease in methylmercury concentrations in the hepatopancreas of *E.sinensis with the* size of the

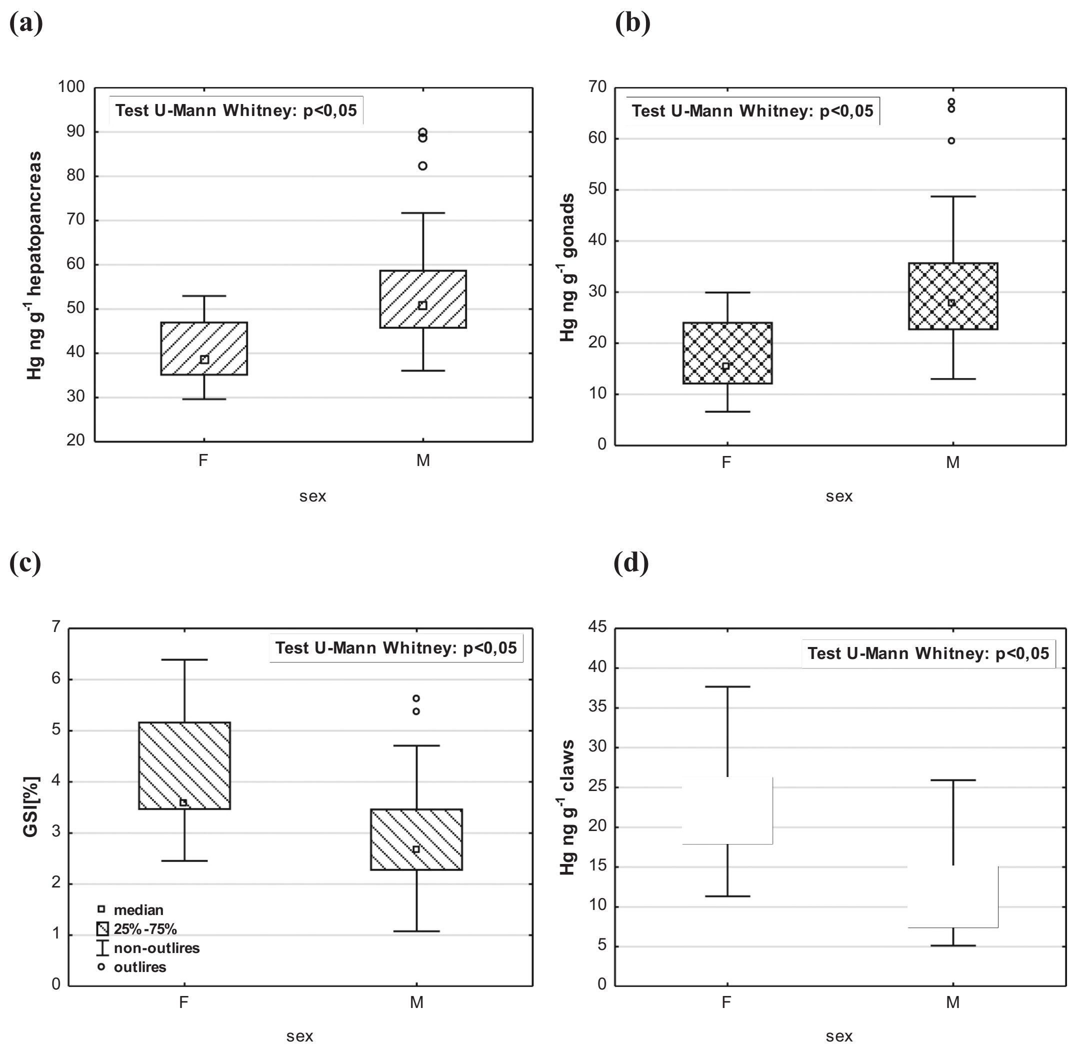

Fig. 5. The effect of gender on total mercury (HgTOT) concentrations in the hepatopancreas (a) and gonads (b), and in claws hard tissue (d). Figure (c) shows the GSI index in female and male *E.sinensis* (F-female: M-male).

individual, indicating more efficient demethylation of Hg with age in larger male crabs. A similar relationship was also observed by Hui et al. (2005) in the hepatopancreas of the San Francisco Bay Chinese mitten crab.

The median mercury concentration in the muscle of *E.sinensis* (220 ng/g d.w., 154 ng/g w.w.) from the Vistula Lagoon (southern Baltic) in 2019 was higher than in the muscle of fish from the southern Baltic in 2016, which ranged in planktivorous fish (sprat and herring) from 10.0 to 39.3 ng/g w.w, whereas it ranged from 64.1 to 148.1 ng/g w.w. in the muscle of predatory fish (cod and eel) in a length range similar to *E. sinensis* (67 ± 8.1 mm) (Polak-Juszczak, 2018). The lower concentration of the metal in muscle, especially in predatory fish, may be due to the dilution of mercury in fish muscle, which grows larger compared to crab muscle. The biodilution effect of Hg was also observed in bivalves in the study area (Jędruch et al., 2019; Wilman et al., 2023c). In the present study, no decrease in muscle Hg concentration was observed with the size or weight of Chinese mitten crab. This is probably due to the fact that mainly adult specimens are found in the study area. The protein metallothionein, which plays a significant role in the regulation and detoxification of metals in hepatopancreatic tissue, the synthesis of which in invertebrates is induced under the influence of metal ions such as Cd, Zn, and Hg in such complexes is probably bound in crab muscles (Sen and Semiz, 2007). In such a form, mercury could be redistributed from the hepatopancreas to the muscles of *E.sinensis*, as confirmed by the directly proportional relationship of HgTOT concentrations in these parts of the organism ($r = 0.7$ $p < 0.05$).

Hg is transferred from the somatic to reproductive tissues that are released from crustaceans and fish during reproduction (Ram and McMahon, 1996). Mercury concentrations determined in the gonads of *E.sinensis* had the lowest values of the soft tissues analysed (Fig. 4a). Statistically significantly lower HgTOT concentrations were also observed in female oocytes compared to male spermatophores (Fig. 5b). Female *E.sinensis* produce between 250,000 and one million eggs, which was characterized in our study by a statistically significantly higher gonadosomatic index (GSI) compared to males (Fig. 5c). Consequently, there may have been Hg dilution in the gonads of females due to new tissue formation. As shown by Shao et al. (2013), the small spermatophore of *E.sinensis* males is characterized by protein structures (mature spermatids) with a low lipid content compared to the gonads of females (Table 2). Consequently, male gonads may have more Hg bioligand formation sites, and thus higher HgTOT concentrations were observed in them (Fig. 5b). *E. sinensis* live approximately five years and breed only once in their lifetime (Veilleux and Lafontaine, 2007). Both males and females die after reproduction and the young reach sexual maturity after three to five years of life (Dittel and Epifanio, 2009). Hence, the elimination of Hg together with the gametes in this crab species may be the least important for detoxification, while at the same time they do not pass on the excess xenobiotic to their offspring, which is perhaps one of

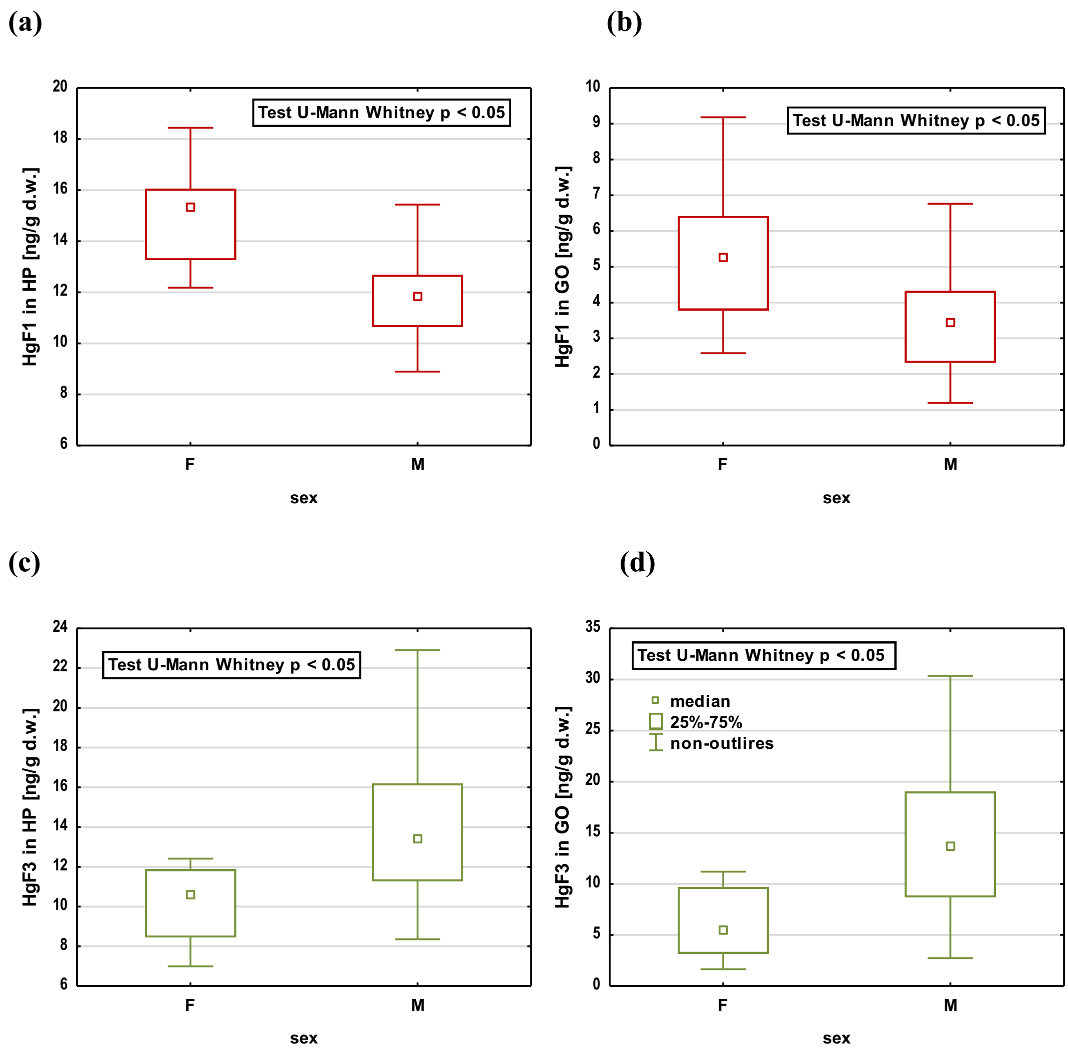

Fig. 6. Effect of sex on hepatopancreas (a) and gonads (b) halogenated Hg levels and hepato-pancreas (c) and gonads (d) mercury sulfide levels in *E.sinensis* (F-female: M-male).

the factors favoring the acquisition of new areas by this alien species. In the world literature, the problem concerning the elimination of xenobiotics together with gametes released from the gonads in invertebrates is poorly recognized. As far as fish are concerned, the opposite process is observed compared to the present study. Males show some of the highest gonadosomatic indices (GSIs) among fish, with testes accounting for 10 to 15 % of body weight when the fish are in their mature state, and androgens have been linked to increased rates of Hg detoxification in other vertebrates, which they eliminate along with genetic material during reproduction (Madenjian et al., 2015).

Therefore, it can be assumed that in *E. sinensis* there is a process of elimination of Hg from the body to the carapace. Of the soft tissues analysed, redistribution of Hg was observed from the gills to: carapace ($r = 0.7$), merus ($r = 0.6$) and claws ($r = 0.4$), all of which were statistically significant ($P < 0.05$). An analogous process of Hg transfer was observed from the fleshy walking leg muscles to the carapace ($r = 0.6$), merus ($r = 0.6$) and claws ($r = 0.4$). From the hepatopancreas, mercury was directly transferred to the carapace ($r = 0.6$ and $P < 0.05$). The inverse relationship between the fraction of mercury in complexes with organic matter (HgF2) in the carapace and its content in the hepatopancreas ($r = -0.6$) may indicate that, in addition to demethylation of Hg in this organ, the hepatopancreas eliminates organic Hg complexes directly into the carapace. The more important processes of the elimination of Hg from the body to the carapace and merus are probably related to the moulting of the crab *E. sinensis*. The number of moults varies during the life of the crab. In the first year of life, crabs moult up to 6–8 times, in the second year 4–5 times and in the third year 2–3 times. Older crabs only moult once a year (Panning, 1938).

The Hg concentration in the pincers of males was statistically significantly lower than in females (Fig. 5d). A distinguishing feature between males and females is the size of the pincers. In females they are small, with very few cuticular outgrowths, whereas the pincers of males are more massive and luxuriantly covered with mittens (Gollasch, 2006). Hence, there may be a more efficient dilution of Hg in the biomineralisation of the pincers in male *E.sinensis*. Furthermore, bioenergetics modelling results from previous studies indicated that growth dilution would explain any portion of this observed difference in Hg concentrations between the sexes, the opposite of what is observed in fish, among others (Madenjian et al., 2015). As the process of moulting during the life of the Chinese mitten crab occurs more frequently than reproduction, this crab may have adapted through evolution to detoxify Hg neurotoxin from the body into the carapace. The natural crab's exoskeleton composite consisting of highly mineralised chitin-protein fibres arranged in a twisted plywood or Bouligand pattern may favour efficient Hg binding in its structures (Chen et al., 2008).

3.4. Spatial and temporal Hg changes in E.sinensis

In the present study, an attempt was made to determine the temporal and spatial variability of total mercury concentrations in the foot leg muscles (FWL) of *Eriocheir sinensis*. The observed temporal and spatial trends are in line with the general characteristics of the change in Hg concentrations within the southern Baltic defined for the atmosphere, terrestrial as well as marine ecosystem (Jędruch et al., 2021; Jędruch et al., 2023). The decrease in Hg emissions to the environment in the study area over the last years may also have a role in reducing bioaccumulation and biomagnification of the neurotoxin in the trophic network, including the Chinese mitten crab. Spatial variability in the Hg concentration in crab FWL was observed in 2019. Statistically significantly higher concentrations were measured in crabs caught in the eastern part of the Vistula Lagoon near the Russian border than in the western part (Fig. 7a). However, it may be difficult to determine environmental changes using this crab species as a biomonitor due to its high mobility and living in transitional zones. Being a catadromous species, Chinese mitten crab is perfectly adapted, both morphologically (long walking legs) and physiologically (effective metabolism outside water) for long migrations, during which it can travel even up to 20 km per day (Olthof 1936; Panning, 1938; De Giosa and Czerniejewski, 2011).

This species is also very resistant to drying (Fialho et al., 2016). Thanks to its strong, muscular walking legs ending with a sharp dactylus, the Chinese mitten crab can also climb vertical surfaces, effectively overcoming physical barriers present in the path of its migration (Peters and Panning, 1933). Thus, determining spatial changes in Hg within a single floodplain may be difficult to interpret. However, given its mobility, it may be a vector of change for the entire southern Baltic region just like fish or grey seals. Previous studies have indicated that spatial variation within a single body of water may only be characteristic of a particular region, as in three areas of San Francisco Bay, Hg concentrations in crabs varied statistically significantly, while within Puck Bay (southern Baltic) researchers observed no such variation in *E. sinensis* (Boszke et al., 2003; Hui et al., 2005).

There was an exponential negative trend in the change in metal concentration from 2011 (median = 343.0 ng/g) at FWL to 2020 (median = 250.0 ng/g) (Fig. 7b). The decrease in the Hg concentration over the analysed period was 30 %, and this variation was statistically significant ($P < 0.05$; Fig. 7b). Molecular studies indicate that *E. sinensis* in the Vistula Lagoon probably originates from Germany, where it lives in rivers that may be more polluted (Czerniejewski et al., 2012). Consequently, crabs migrating towards the south-eastern Baltic are already burdened with a certain pool of Hg in their tissues. Recent studies

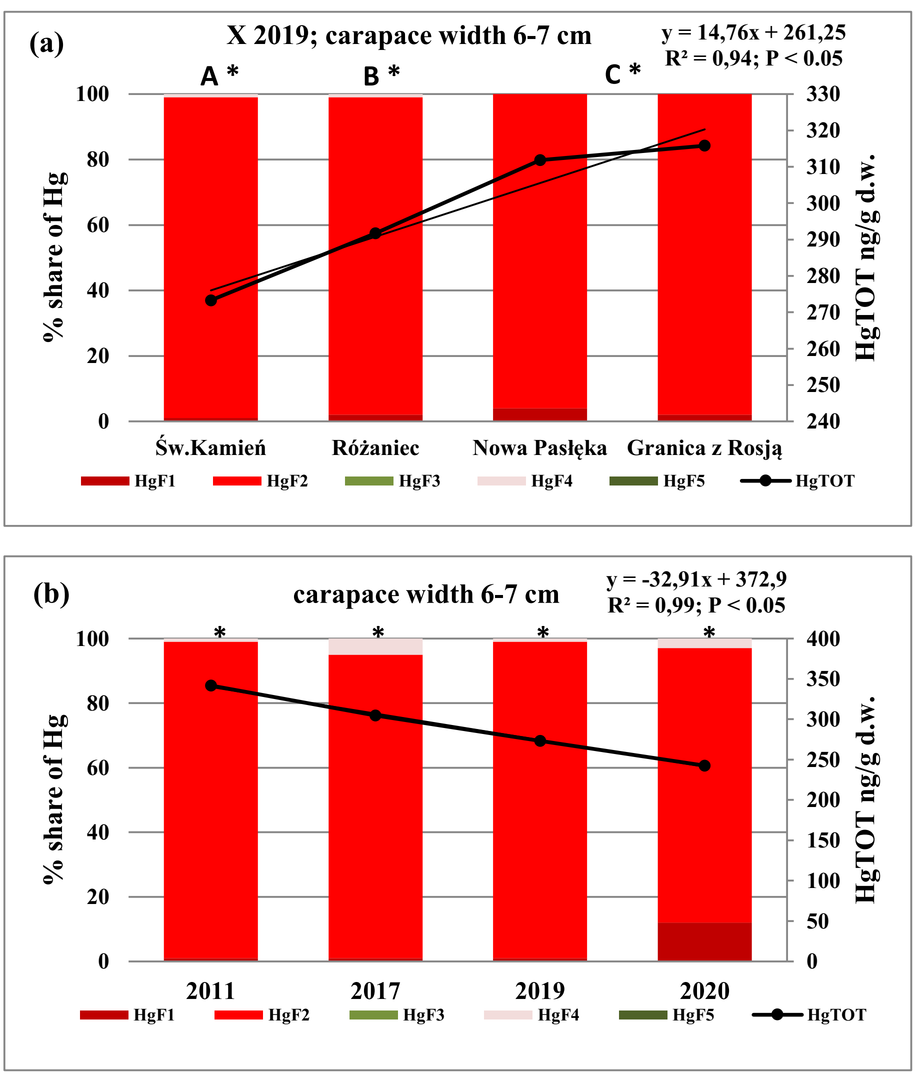

Fig. 7. Spatial (a) and temporal (b) variability of total mercury concentration (HgTOT) and its forms in *E.sinensis* fleshes walking legs. A regression line was used to determine the trend of variation in Hg concentration (y) between regions A, B, C and between the years studied (x). Asterisks indicate Hg values for which $P < 0.05$ between regions (a) and years (b).

indicate that conditions in rivers in the northern German region are, however, improving (Ewers et al., 2023). Consequently, over the years, the observed trend of decreasing xenobiotic Hg concentrations in muscle may be due to an improvement in the quality of the habitat of *E.sinensis*. In addition, according to the IMGW Environmental Assessment, in 2010/2011 the sum of negative daily mean temperatures (total cold) was the highest $\sum|\text{-Tp}| = 260$; the first ice appeared on 1.12 and the last on 3.02, while the total number of days with ice was 33. In contrast, in 2019/2020 $\sum|\text{-Tp}| = 30$, and the total days with ice in the Vistula Lagoon was 10. Based on the change in thermal conditions in the Vistula Lagoon, we can conclude that the period of winter stagnation of organisms is shortening, so that lower Hg concentrations are observed. In seasonal studies on another group of invertebrates (bivalves), an increase in organismal Hg concentrations was observed during the winter stagnation period in the study area (Wilman et al., 2023b, 2023c). The shortened period of stagnation may also result in this crab preparing later for reproduction and migration.. Shortening this period from year to year may affect the organisms' behavior and reduce vital functions in winter (anabiosis) so that they can possibly more efficiently metabolize toxins and detoxify them from their body, including mercury. According to European studies of this species, adult mitten crabs begin to migrate downstream in late fall and winter, with the males descending to brackish waters first, and the females following within a month of the males (Anger, 1991). The crab itself is unlikely to prey on fish, mainly eating them out of nets or consuming those found dead. Reduced access to food from higher trophic levels may have resulted in an increase in the proportion of plants in the diet of *E.sinensis,* hence the lower Hg concentrations and an increase in the proportion of HgF1 in 2020.

3.5. Human risk health assessment

The Global Mercury Assessment 2018 suggested that the lack of good quality and nationally representative data in many geographical regions poses obstacles to reducing human Hg exposure (UNEP, 2019). In this study Hg levels were measured at 154 ± 66 ng/g w.w. in Chinese mitten crab from Vistula Lagoon (Poland). Common edible crabs worldwide have high Hg levels in their muscles (Sunderland et al., 2018). For example, the red snow crab *Chionoecetes japonicus* in Japan has a mean Hg level of 200 ng/g ww (wet weight basis) and a maximum Hg level of 540 ng/g (Kakimoto et al., 2019). The king crab *Pseudocarcinus gigas* in Australia has a total Hg level of 220 ± 100 ng/g ww (Turoczy et al., 2001). Other studies have produced a high total of Hg levels, such as the red king crab (50 ± 24 ng/g w.w.) in Norway (Julshamn et al., 2015). In China, measurements of Hg levels in crabs are relatively rare, besides the reported Hg levels of 110 ± 76 ng/g w.w. for marine crabs (Swimming crab and Mud crab) and 53 ± 26 ng/g w.w. for freshwater crabs – Chinese mitten crab (Liu et al., 2020).

The permissible concentration of Hg in food is 500 ng/ g w.w. and, according to EFSA, the permissible weekly intake (TWI) from food is 4 μg Hg per kilogram body weight for the average body weight of a European adult (70 kg) (Walpole et al., 2012). Consumption of 1.95 kg of *E. sinensis* crab meat from the Vistula Lagoon at an average concentration of 154 ng/g w.w. would deliver an acceptable weekly dose of the xenobiotic to the human body. t The maximum measured Hg values (220 ng/g w.w.), consumption of 1.37 kg of *E.sinensis* crab meat from the Vistula Lagoon would deliver an acceptable weekly dose of the xenobiotic to the human body. In comparison, the average Hg concentration in the muscle of flounder (a predatory, benthic fish commercially consumed in the southern Baltic region) in October 2019 from the study area was 51 ± 38 ng/g m.m (Marine Environment Research Programme of the Bay of Puck 2019–2021, MIR-PIB). As a result, the ingestion of 2.6 kg of fish muscle would deliver an acceptable weekly dose of the xenobiotic to the human body. The means that for the TWI limit values in clam *Mytilus trossulus* from the southern Baltic to be exceeded, the mussel consumption per person would have to increase around 50-fold and reach 4.2 kg per week (Jędruch et al., 2019). In the China region (Zhejang Province) the weekly intake of *E. sinensis* meat should not exceed 3.2 kg (Dongren et al., 2018).

Seafood is a source of energy and protein with high biological value, and contributes to the intake of essential nutrients, such as iodine, selenium, calcium, and vitamins A and D, with well-established health benefits. Seafood also provides n-3 long-chain polyunsaturated fatty acids (LCPUFA), and is a component of dietary patterns associated with good health (EFSA). Available data suggest a large variation in the amount of fish and other seafood consumed across European countries and age groups, as well as in the type of seafood and species eaten, although data from European surveys are difficult to compare, as the type of seafood consumed is largely unknown in some countries, and data are particularly scarce for infants. To protect against inter alia neurodevelopmental toxicity of Hg and methylmercury and achieve the benefits of fish consumption (effect of fish/seafood consumption during pregnancy on functional outcomes of children's neurodevelopment and on cardiovascular diseases in adults), which are associated with 1–4 fish servings per week, fish/seafood species with a high content of mercury in the daily diet should be limited (EFSA). Since Hg concentrations in *E. sinensis* did not exceed acceptable levels in fish and seafood, it could be safe to consume. However, the concentration of Hg in Chinese mittens' crab meat was higher than in fish or clams in the southern Baltic region, so its inclusion in the diet should be limited to 1–2 servings per week in diets with reduced consumption of fish or other seafood.

4. Conclusions

The Hg concentration determined in the crab *E.sinensis* was at a high level compared to other invertebrates or fish in the southern Baltic region. Biometric parameters did not determine the concentration of Hg and its forms in whole individuals of *E.sinensis*. The present study showed that mercury accumulation of the crab organism largely occurred via the gills and then via the oral route. Distribution of Hg in the crab organs was most strongly related to the trophic origin of mercury, while halide-associated mercury and semilabile forms of Hg from the filtration process were redistributed into the crab exoskeleton. The greatest bioaccumulation of mercury was in crab muscles, where the highest concentrations of the metal were measured.

Male crabs, compared to females, were characterized by a higher Hg burden of internal organs such as the liver and pancreas and gonads. This is probably related to feeding on food from higher trophic levels (zoonotic) as indicated by the lower proportion of halogenated and semilabile forms of Hg in the organs of males, compared to females. The concentration of Hg in carapace elements was closely related to the type of mineralization of the exoskeleton tissue formation. The pincers of males had lower xenobiotic Hg concentrations compared to females, which may indicate metal dilution in the larger, more massive pincers of the male individuals.

The elimination of Hg from the muscles and from the hepatopancreas into the carapace was one of the important detoxification processes of the crab's body. In this way, the crab effectively removes toxic Hg during the growth process (moulting), thus protecting its body from the neurotoxin. Consequently, a lower Hg load is biomagnified, making the muscle tissue of the woolly crab fit for human consumption. Consequently, mercury concentrations did not exceed the acceptable standard set for Hg in fish and seafood.

The observed trend of decreasing Hg concentrations over the years in *E.sinensis* is similar to that in abiotic and biotic environmental elements in the southern Baltic region. Due to the crab's high mobility, it may serve as a biovector of environmental change more for whole water bodies than for regional estuaries or lakes.

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CRediT authorship contribution statement

Bartłomiej Wilman: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Data curation. **Monika Normant-Saremba:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation. **Agata Rychter:** Writing – review & editing, Conceptualization. **Magdalena Bełdowska:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Included in my doctoral dissertation entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

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Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body

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ABSTRACT

Mercury is a potent neurotoxin. The toxicity of Hg depends on the form in which it occurs, which consequently determines its bioavailability to the organism. The processes of distribution and elimination of Hg in organisms from lower trophic levels are still poorly understood. Therefore, in the present study, great point was paid separately to the soft tissues and hard tissues, which may play an important role in the detoxification of the crab's body from neurotoxicant. The study was conducted on the non-native species: Harris mud crab, collected in 2020. Concentrations of total mercury and its forms were carried out using a Direct Mercury Analyzer, DMA-80 (Milestone, Italy). Sex did not determine the Hg concentration of its forms in *R. harrisii*. Organism size influenced on crab body processes: biodilution of Hg during growth in summer and bioaccumulation of Hg before stagnation in autumn. The distribution of Hg in the crab's soft tissues and walking legs was related to the trophic origin of the mercury, while halide-bound mercury and semilabile forms and HgS were redistributed into the crab's carapace. This protects the soft tissue of crabs against the toxic effect of Hg. Consequently, a smaller load of Hg in the crabs can be introduced to the circulation or biomagnified in the food web in the region where *R. harrisii* is an important link of the trophic chain.

1. Introduction

Mercury (Hg) is a highly toxic pollutant present in terrestrial and aquatic ecosystems throughout the world. This is due to the characteristic properties of this element: volatility, reactivity, persistence and high toxicity especially of its organic forms (Kabata-Pendias and Mukherjee, 2007). To date, Hg and its compounds have not been shown to have any positive physiological functions in living organisms. Instead, it is known to be toxic in any form. Mercury has been shown to have neurotoxic, mutagenic, cytotoxic, nephrotoxic and allergenic properties, and can also disrupt the muscle system and many enzymes and proteins (Bakir et al., 1973; Olivieri et al., 2002; Chakraborty, 2017; Ajsuvakova et al., 2020). Mercury deposited on the surface of land, sediment or water can undergo microbial methylation. This process produces one of the most toxic forms of mercury, methylmercury (MeHg), which can then bioaccumulate in the trophic web (Gworek and Rateńska, 2009). Aquatic ecosystems are particularly sensitive to mercury contamination, where Hg biomagnifies at every level of the trophic chain (Ouédraogo and Amyot, 2013). Globally, the consumption of fish and seafood is the main route of entry of Hg into the human body. For this reason, the issue of mercury is mainly addressed in the context of marine and ocean contamination, as it is from these bodies of water that the majority of consumed fish products originate (Cheng et al., 2013; Mania et al., 2012).

Research on mercury in the sea mainly focuses on abiotic samples (water and sediments) and organisms at the higher rungs of the trophic pyramid. The transformation of mercury in the first links of the trophic web is poorly recognised. This is an important knowledge gap, as at this stage the metal bioaccumulates most from the environment (Boening, 2000; Scheuhammer et al., 2007). Nowadays, when the inflow of metals to the lagoon is limited, the remobilisation of Hg from the sediment is becoming more and more important. As a result of several biotic and abiotic processes, mercury has become remobilised into the marine environment (Reckermann et al., 2022). In this way, it can be bioaccumulated in benthic organisms and biomagnified in the subsequent links of the trophic chain, thereby representing the greatest threat to marine mammals, water birds and humans (Jackson, 1998; Chakraborty et al., 2019). One of the common zoobenthic organisms potentially

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exposed to mercury is the Harris mud crab *Rhithropanopeus harrisii*, an American species which was introduced to the Baltic Sea. It is now very widespread and holds the status of a non-indigenous species in 21 countries around the world (Roche and Torchin, 2007). *R. harrisii* become an important element of the food web of the Baltic Sea, being a prey for birds and benthic fish that have strong jaws and are not good swimmers: the perch, the eel, the round goby, flounder or shorthorn sculpin (Filuk and Żmudziński, 1964; Bacevičius and Gasiūnaitė, 2008; Puntila, 2016). While this species is not an energy-rich food having the lowest energy value among the benthic crustaceans from the Gulf of Gdansk (Wiszniewska et al., 1998), its advantage as prey is high availability (abundance) as well as low chance to escape.

In spite of the relatively numerous studies on the biology, ecology and genetics of *R. harrisii* in Polish waters (Kujawa, 1957; Szudarski, 1963; Turoboyski, 1973; Rychter, 1997; Normant et al., 2004; Hegele-Drywa and Normant, 2009; Hegele-Drywa et al., 2014) there are no studies that determine the levels of toxic substances, including mercury, in this species. There are also no scientific reports on this subject. This is very important because of Harris mud crab is a component of the diet of many fish consumed by human. In this way *R. harrisii* is potentially new source of mercury into human body. The aim of the present study was therefore to determine the levels of total mercury (THg), and its labile and stable forms, in this non-native species. Mercury in any form is toxic; however, its toxicity varies depending on the concentration and form, which determines its physical and chemical properties (Kim et al., 2004; Sedlar et al., 2015; Jędruch et al., 2018; Wilman et al., 2023a). The studies took into account both the size and sex of the specimens and season of the year, which are essential factors determining biology and physiology of aquatic species affecting probably also bioaccumulation of toxic substances (Wilman et al., 2023b, 2023c). Discussing about Hg accumulation from the crab's body of whole individuals may be inaccurate, as it is mainly the internal organs are the site of the bioaccumulation and biomagnification of mercury (Branco et al., 2007; Kojadinovic et al., 2006). The study was therefore extended to analyze Hg in the exoskeleton, the so-called hard tissues, which are the attachment site for muscles and internal organs (soft tissues). Studies on another group of invertebrates have shown that the clam's shell and crab's carapace plays an important role in the transfer of mercury along the soft tissue-shell (hard tissue) and internal organs-exoskeleton pathway (Wilman et al., 2023b, 2023c; Wilman et al., 2024). The hypothesis tested was that physiological state (season), biometric parameters and sex determine the process of bioaccumulation and elimination of mercury in the southern Baltic crab. And that hard tissue may represent a site of accumulation and temporal deactivation of mercury via a transfer pathway: soft tissue - carapace route in the crab.

2. Materials and methods

2.1. Sampling

The organisms were collected during a cruise of a research vessel of the Marine Fishery Institute in Gdynia – ŁM MIR 2, in the western part of the Vistula Lagoon (N 54°18.791′, E 19°17.019′) in April, August, and October 2020 (Fig. 1; Table 1). The biological material was collected by means of a manual drag (with dimensions: 36 × 21 cm, length of trawl of 60 cm, and net mesh size of 3 × 3 mm) from a depth of 2.5 m, and then sieved through a sieve with mesh of 0.25 mm. Individuals from species *Rhithropanopeus harrisii* were collected manually from the sieve, and

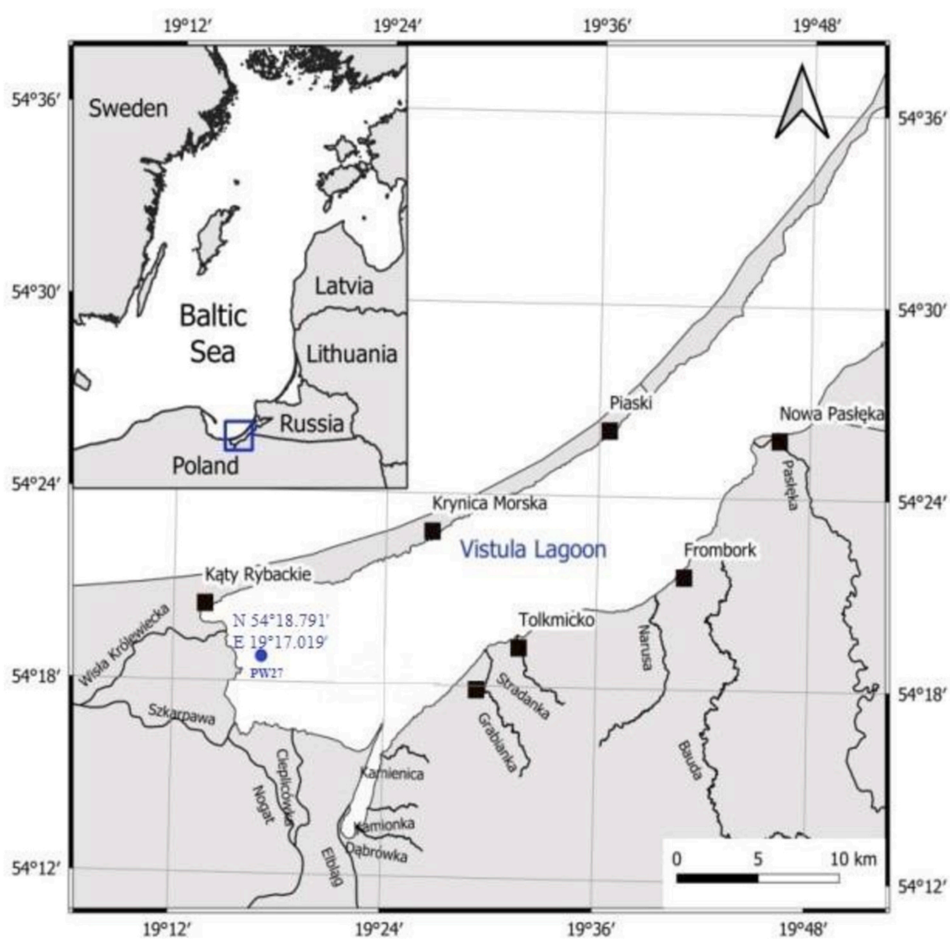

Fig. 1. Study area - Vistula Lagoon (estuary of the southern Baltic Sea).

Table 1
Habitat characteristics of *Rhithropanopeus harrisii* from the Vistula Lagoon (southern Baltic Sea).

Season	Date	Depth [m]	Secchi disc [cm]	Temperature [°C]	O_2 [mg/dm^3]	pH	Salinity [PSU]
Spring	April 2020	2,5	75	13,1	9,85	7,86	4,08
Summer	August 2020	2,4	40	19,8	8,05	7,91	3,60
Autumn	October 2020	2,5	77	10,5	10,73	7,26	4,06

placed in aerated thermoses with lagoon water. After transporting the samples to the laboratory, the organisms were sorted in an aquarium with filtered water for 24 h for purification. After 24 h, the organisms were stored at −20 °C before biological and chemical analyses. During sampling, water was also subject to in situ measurements. Salinity [PSU], water pH and temperature [°C], and Secchi depth were measured (Table 1).

2.2. Biological analyses

Prior to analysis, crabs were weighed and its carapace length was measured (using calipers) for the determination of size classes. Individuals of the species *Rhithropanopeus harrisii* were classified by size into seven classes (I: 0–4.4 mm; II: 4.4–6.4 mm; III: 6.4–8.4 mm; IV: 8.4–10.4 mm; V: 10.4–12.4 mm; VI: 12.4–14.4 mm; VII: 14.4–16.4 mm) and sex based on abdominal structure (Hegele-Drywa et al., 2014; Wilman et al., 2019). A list of the number of organisms used during the analyses is presented in Table 2. A total of 106 organisms were analyzed: (65 females and 41 males) (Table 2). From the whole set, organisms were selected that were large enough (size classes III–VII) to be divided into the soft body (ST) as a whole of the internal organs under the carapace (gonads, hepato-pancreas, gills, heart, digestive system), fleshes walking legs (FWL) and the carapace (C), from the most representative seasons (summer and autumn). An important criterion was the number of individuals per size class, so as to obtain a representative sample mass for further chemical analyses. Hence, in the spring season, individuals of *R. harrisii* were further analyzed in their entirety and individuals from summer and autumn in size classes < III. Before proceeding with further chemical analyses, the *R. harrisii* specimens and their tissues were lyophilized and their dry mass was determined. The wetness of the organisms was determined on the basis of the difference between wet and dry mass. The crabs were then homogenized in an agate mortar. Owing to their low mass, the smallest size class individuals were homogenized jointly in order to obtain sufficient sample weight for mercury analysis (males and females per measuring season).

2.3. Total mercury analysis

Analysis of total mercury (HgTOT) was performed by thermal desorption using a DMA-80 analyzer (Milestone, Italy), according to the method published by Saniewska and Bełdowska (2017). This method does not require prior mineralisation of the sample, which reduces the risk of contamination. Approximately 0.1 to 0.2 g of sample was weighed on quartz boats in triplicate. Determination of the Hg concentration, and the share of individual forms of Hg, was carried out according to the thermal desorption method of Hg compounds, published by Wilman et al. (2023a) for biological material. Samples were weighed in triplicate on quartz boats and the sample mass (0.05–0.1 g), depending on the expected HgTOT concentration. The method was verified by analyzing certified reference materials (mussel tissue SRM-2976 – HgTOT: 61 ng/g d.w., scallop IAEA-452 – HgTOT: 160 ng/g d. w., fish protein DORM-4 – HgTOT: 410 ng/g d.w., dogfish liver DOLT-5 – HgTOT: 440 ng/g d.w.) (sample weight: 0.1–0.2 g), with a mean recovery of 98 % and standard deviation not exceeding 7 %. The limit of detection (LOD) was calculated as the threshold of the standard deviation (SD) of the Hg concentration in the ignited sample boat measured in 10 replicates. In the case of HgTOT analysis, the LOD was 1 pg of Hg.

Table 2
Relation of the number of specimens of Harris mud crab of the respective sex to the defined size classes in the different measurement seasons of 2020 year.

Season + sex/class size		I	II	III	IV	V	VI	VII
Spring	Female	6	1	1	–	–	–	–
	Male	6	3	3	–	–	–	–
	All	12	4	4	–	–	–	–
Summer	Female	17	12	3	4	1	1	–
	Male	4	4	3	1	1	–	–
	All	21	16	6	5	2	1	–
Autumn	Female	5	9	2	1	–	1	1
	Male	3	4	4	1	1	2	1
	All	8	13	6	2	1	3	2
All seasons		41	33	16	7	3	4	2

– missing in the sample.

2.4. Mercury fractionation

The application of the 5-step thermo-desorption method allowed the separation of five groups of compounds with similar properties (Jędruch et al., 2018; Wilman et al., 2023a). The method involved the simultaneous combustion of the same sample at five temperatures: In 175 °C (release of labile forms – HgF1 bound to halides: HgCl, $HgCl_2$, $HgBr_2$); in 225 °C (release of labile forms – HgF2: Hg compounds combined with humic substances, MeHg, as well as other Hg compounds formed with ligands having strong complexing properties ($(CH_3COO)_2Hg$, $Hg(NO_3)_2{\cdot}H_2O$, $Hg(SCN)_2$); in 325 °C (release of stable form – HgF3: HgS); in 475 °C (release of semi-labile forms – HgF4: $HgSO_4$, HgO (red)); in 750 °C (release of residual mercury - HgF5) (Bełdowska et al., 2018; Jędruch et al., 2018; Wilman et al., 2023a). The analyses of the environmental samples were preceded by an examination of the Hg patterns specific to each fraction according to the method of Saniewska and Bełdowska (2017). Seal muscle was used as the pattern for the thermally labile fraction (HgF2), in which MeHg accounted for 98 % of the contribution to the total HgTOT (Jędruch et al., 2018) and the contribution of fraction HgF2 accounted for 98 % of the total Hg fraction in the seal muscle, respectively. The use of Hg standards in this method is important, as there are no certified reference materials for individual forms of Hg in environmental samples. The analysis of the samples was performed in triplicate. The analysis was verified by comparing the sum of Hg concentrations, measured via the 5-step fractionation method with the result of the HgTOT concentration analysis. The mean recovery of the Hg form analysis was 109 %, with standard deviation not exceeding 5 %. The LOD for each fractionation method was calculated from ten replicates of the substrate analysis. The calculated LOD were at the level of 1.3 pg of Hg, as in the case of HgTOT.

2.5. Processing result

Statistical analysis and graphic representation of the obtained results were carried out using STATISTICA 13 (StatSoft) and Microsoft Excel (2008). The analyzed data were characterised by non-normal distribution (Shapiro-Wilk test $P < 0.05$). Therefore, non-parametric tests were used for statistical analyses. In order to determine the significance of differences between the Hg concentration and biometric parameters, the non-parametric U Mann-Whitney and ANOVA Kruskal-Wallis tests were used. The relationships between the analyzed variables were determined on the basis of Spearman's coefficient, with a confidence interval of at least 95 %. $P < 0.05$ was regarded as a statistically significant difference.

The condition index CI (mg cm^{-3}) constituting a good proxy of the physiological state of the investigated crabs (Wołowicz et al., 2006; Pierścieniak et al., 2010; Jędruch et al., 2019) was calculated based on the following formula (Beukema and De Bruin, 1979; Okumuş and Stirling, 1998):

$$CI = STDW\, L^{-3} \quad (1)$$

where STDW is soft tissue dry weight after lyophilising (mg), and L is carapace length (mm) calculated on (mm). The percentage share of HgTOT and mercury forms in the crab body (storage) was expressed as the content in the carapace in relation to the content in the whole body and walking legs, and calculated based on the equation:

$$Storage\ (\%) = 100^{*}(mc^{*}Cc/(mc^{*}Cc + mb^{*}Cb)) \quad (2)$$

where: mc and Cc are dry weight of carapace (g) and concentrations of Hg derivatives in carapace (ng/g d.w.), respectively; mb and Cb are dry weight of crab body (g) and concentrations of Hg derivatives in body (ng/g d.w.), respectively (Graca et al., 2021; Wilman et al., 2023c). The bioaccumulation factor (BAF) was estimated based on the Hg concentration in the crab to Hg concentration ratio in the potential Harris mud crab food (Szefer et al., 1999). Recorded HgTOT concentrations in environmental components collected during 2020 in the same area of Vistula Lagoon the station located the closest to the collection site of the *R. harrisii* specimens, were used: averaged results of HgTOT in potential crab food: Bivalvia: *Dreissena polymorpha*: 21.4 ng/g d.w. (Wilman et al., 2023a, 2023b, 2023c).

3. Results and discussion

3.1. Hg burden in whole organism of *R. harrisii*

The median total mercury concentration (HgTOT) in whole individuals of Harris mud crab from the Vistula Lagoon ($n = 106$) was 22.6 ng/g d.w., with the median HgTOT concentration being statistically non-significantly higher ($p > 0.05$; Fig. 2a) in females compared to males. A similar lack of difference in mercury concentration was found in the crab (*Rhithropanopeus harrisii*) from the Bay of Puck and in other alien zoobenthic species from the Vistula Lagoon i.e. the crab (*Eriocheir sinensis*) and the clams: *Rangia cuneata* and *Dreissena polymorpha* (Wilman et al., 2019; Wilman et al., 2023b; Wilman et al., 2023c; Wilman et al., 2024). The lack of a significant difference in xenobiotic Hg concentrations in males and females may have been due to the same food relationships, as *R. harrisii* forms spatial clusters despite being a vagile representative of macrozoobenthos (Hegele-Drywa and Normant, 2009). In analogy, the food preference of crabs from the Vistula Lagoon is reflected in the percentage of individual forms of Hg in whole individuals of *R. harrisii*, which was unity in males and females (Fig. 2b). In both cases, 95.2 % in HgTOT was accounted for by the HgF2 fraction (Hg in complexes with organic matter), which is mainly of trophic origin and represents a large proportion in zoonotic tissues (Jędruch et al., 2018; Wilman et al., 2023a). In contrast, 2.7 % was the HgF1 fraction (mainly halogenated forms of mercury), which represents a significant proportion in micro and macroalgae and suspended matter (Bełdowska et al., 2018; Wilman et al., 2023a, 2023c). However, as it is the most labile form of Hg its contribution to HgTOT was negligible in *R. harrisii* (Aksentov and Sattarova, 2020; Wilman et al., 2023a). The other forms of Hg accounted for a total of 2.1 % contribution to HgTOT, and their concentrations were negligible (Fig. 2b). The observed percentage, of individual mercury fractions in whole individuals of *R. harrisii* was similar to the determined forms of Hg in whole individuals of the crab *E. sinensis* from the same water body - the Vistula Lagoon (Wilman et al., 2024). In contrast, it differed significantly from the percentage of Hg fractions determined in whole individuals of *R. harrisii* from the Bay of Puck, where HgF1 and HgF2 were 30 % and 67 %, respectively (Wilman et al., 2019). It may indicate that in the Puck Bay, plant food constitutes >50 % of the crab's diet, especially in individuals up to size class 4 where Chlorophyta were found in 50 % of the individuals with filled stomachs (Hegele-Drywa and Normant, 2009) because, as shown in the studies by Bełdowska et al. (2018) and Wilman et al. (2023c), HgF1 forms a large share of HgTOT in microalgae, thereby confirming the uptake of HgF1 with the consumption of microalgae by young crabs. The BAF was estimated on 0.8 level for *R. harrisii* from Puck Bay – no bioaccumulation effect (Wilman et al., 2019). However, in the Vistula Lagoon, the Harris mud crab has established its occurrence classifier in, among others, clams: *Dreissena polymorpha*. It probably uses this area as a refugia (shelter plus food base). The BAF coefficient estimated for *R. harrisii* from the Vistula Lagoon was 1.05, indicating bioaccumulation of Hg compared to crabs from the Puck Bay area. Thus, it is likely that a higher

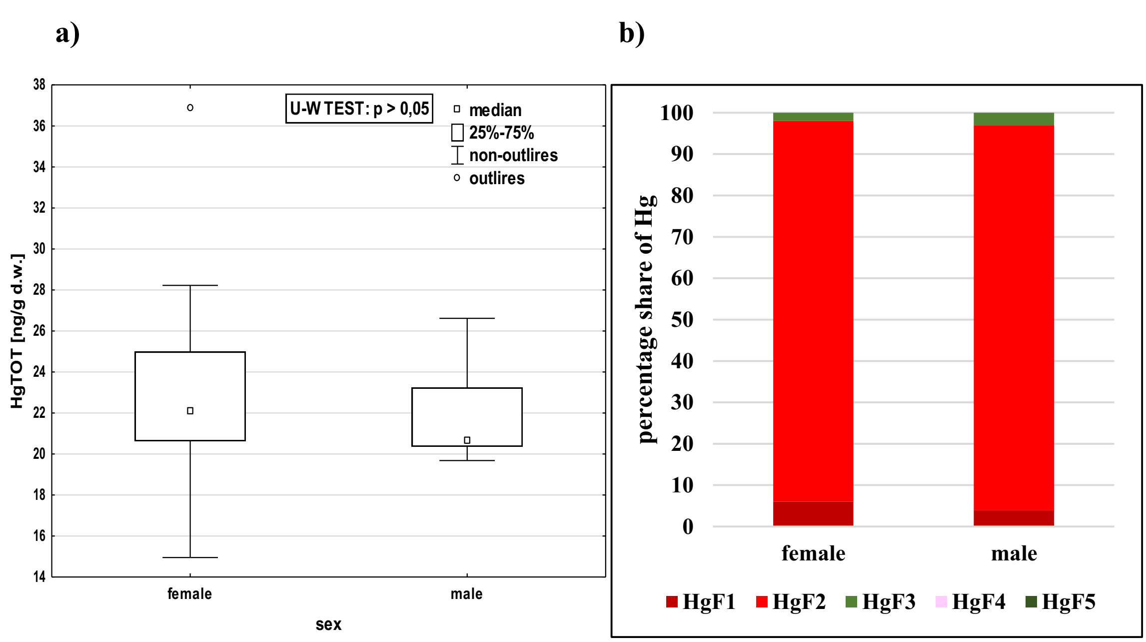

Fig. 2. Effect of sex on the concentration of total mercury (HgTOT) (a) and its forms (b) measured in whole specimens of *R. harrisii* (female and male).

proportion of trophic (animal) mercury is reflected in the percentage fraction of Hg in crabs from the Vistula Lagoon (Fig. 2b). As the total mercury concentration did not differ statistically significantly between male and female Harris mud crabs, also in individual measurement seasons, the median Hg concentration in all individuals was considered in further considerations (Fig. 2a). In most crab species, the reproductive process occurs immediately after the female's moulting period (the carapace is then soft). However, the American crab is one of the few species in which females copulate regardless of the hardness of the carapace (Turoboyski, 1973).

Regarding the seasonal variation of mercury concentration, the lowest Hg concentration was determined in samples from spring, where individuals were characterised by the highest condition factor (Fig. 3a, c). An inverse relationship was observed in the summer and autumn seasons, where crabs in poorer condition were characterised by higher HgTOT concentrations (Fig. 3a, c). The concentration of the xenobiotic Hg in whole individuals in summer was the highest of all measurement seasons (Fig. 3a). Analogous studies conducted on another group of invertebrates, namely bivalves, showed an inverse relationship, where organisms in poorer condition had lower Hg concentrations (Jędruch et al., 2019; Wilman et al., 2023b, 2023c). As a result of the high energy demand for growth and respiration, the mussels loosed on condition, but the growth process is so intense that the process of Hg dilution in the tissues prevails over accumulation (Peltier et al., 2009; Tran et al., 2020). It is likely that other processes may be taking place during the growth phase of the crabs that determine the accumulation of Hg in the tissues. The proportion of individual forms of mercury, did not differ significantly in whole individuals of *R. harrisii* between measurement seasons (Fig. 3b). Considering the individual seasons, statistically significant differences were observed only for the concentration of the halide-bound fraction (HgF1), and the highest proportion of this fraction was determined in summer: 8 %, where in the other seasons it was

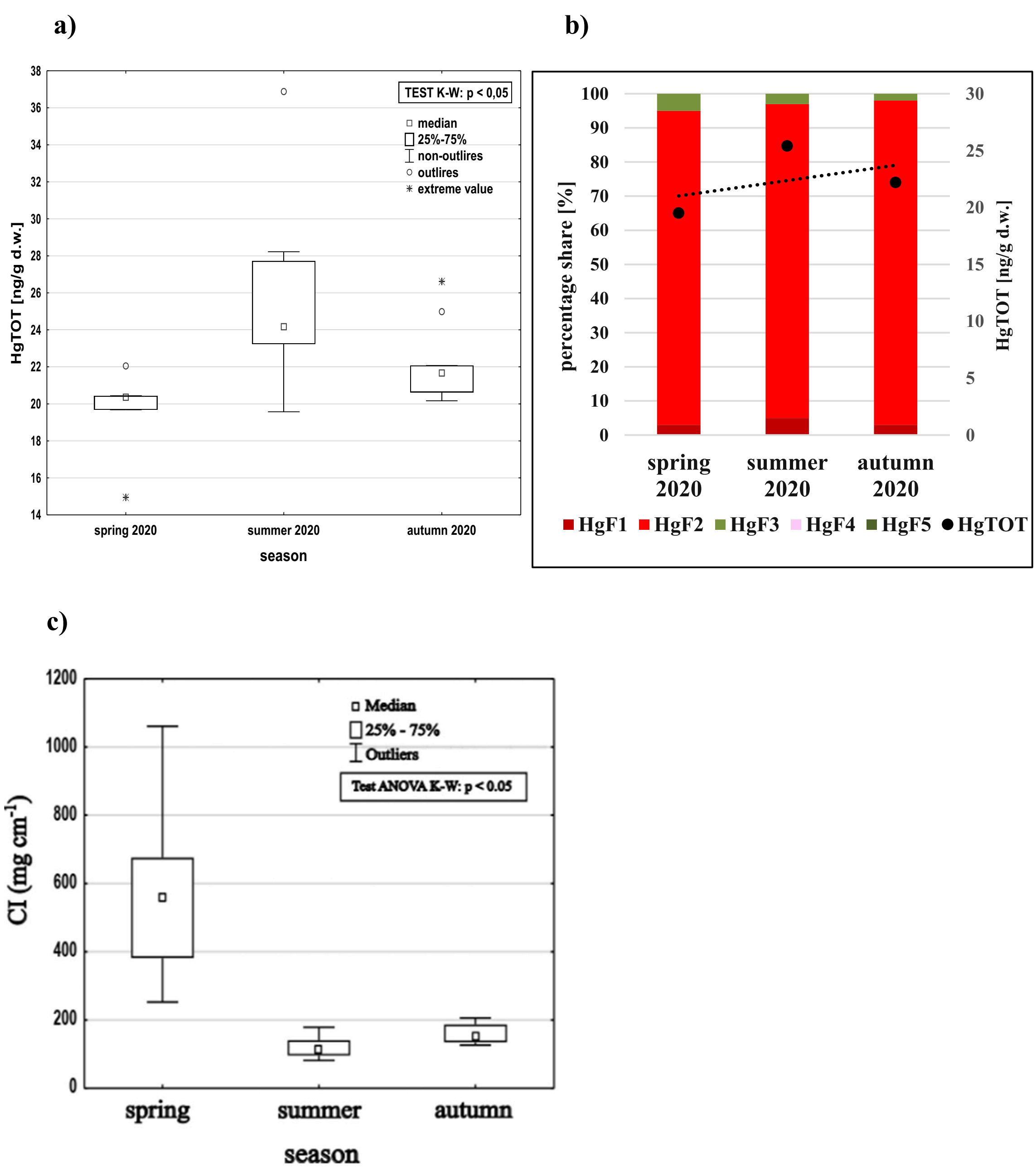

Fig. 3. The seasonal variation of total mercury concentration (HgTOT) in *R. harrisii* (a), the percentage of labile and stable Hg forms (b) and the seasonal condition of the crabs (c).

practically at a level of up to 2 % (Fig. 3b). During the growing season, the American crab, as a representative of omnivorous zoobenthos, enriches its diet with plant food. Along with plant food, the *R. harrisii* obtain the mineral substances necessary to rebuild the carapace after moulting. Smaller individuals grow in size and therefore moult more frequently, so their demand for plant food is high at this life stage (Hegele-Drywa and Normant, 2009; Wilman et al., 2019).This food base is source of HgF1 fraction (mainly halogenated forms of mercury), which is an important form of Hg in micro- and macroalgae and suspension (Bełdowska et al., 2018; Wilman et al., 2023b, 2023c).

Changes in Hg concentration in subsequent size classes as well as within a given class can be additionally related to the availability and quality of food and water temperature which stimulates development (metabolism and reproduction), i.e. to the season of the year. In spring, the smallest individuals of *R. harrisii* were determined among all measurement seasons, and HgTOT concentrations and the proportion of individual mercury forms were not statistically significantly different between size classes (Fig. 4a). The good condition, low Hg concentration and decreasing trend of Hg concentration in successive size classes may indicate that the crabs were probably preparing for reproduction during this period and their growth rate was slower, as the low water temperature was a factor inhibiting these processes (Fig. 4a; Table 2). In summer, on the other hand, an increase in Hg concentration up to size class 3 and a statistically significant decrease in individuals in ≥ size class 4 was observed (Fig. 4b). In summer, the water temperature already met the thermal optimum for reproduction (Table 2). However, no females with eggs were observed during this period. Furthermore, in warmer water invertebrates have an accelerated metabolism and use more energy for growth (Rao and Khan, 2000). As a result, larger, older crabs were characterised by an inversely proportional correlation of Hg concentration to condition factor across size classes ($r = -0.73$; $P < 0.05$), which may indicate biodilution of metal through growth with an unequivocal loss of energy for the growth process. In addition, between late spring and autumn, observe the hatching of ochre larvae, which are the main food component during this period for the crab, and a higher biomass of the mussels *Dreissena polymorpha* or *Rangia cuneata*. The higher proportion of zoonotic food in the diet of the crab, can probably be confirmed by an increase in the concentration of the HgF2 fraction in successive size classes (Fig. 4b), where, with age and size, their first pair of legs transformed into pincers increases and they become more predatory. This confirms the assumption that in the diet of larger (older) individuals there is more food of animal origin, i.e. that they are more predatory than smaller (younger) individuals (Wilman et al., 2019). Which could also have influenced the observed higher Hg concentrations in the crabs during this season (Fig. 3a). In autumn, an increase in Hg concentrations was observed in subsequent size classes (Fig. 4c). The low water temperature, would indicate that the crabs were preparing for winter stagnation during this period, slowing down their metabolism and growth rate, thus bioaccumulation of mercury was observed (Fig. 4c; Table 2). Additionally, organisms decrease their metabolism under stressful conditions and thus crabs have valve-closure for preventing stressor exposure, which effected in lower Hg accumulation in *R. harrisii* body than in summer period and bad condition index (Fig. 3a, c).

3.2. Accumulation of Hg in crab tissues

Discussing about Hg accumulation from the crab's body of whole individuals may be inaccurate, as it is mainly the internal organs are the site of the bioaccumulation and biomagnification of mercury (Branco et al., 2007; Kojadinovic et al., 2006). The median concentration of total mercury was statistically significantly different in different parts of the

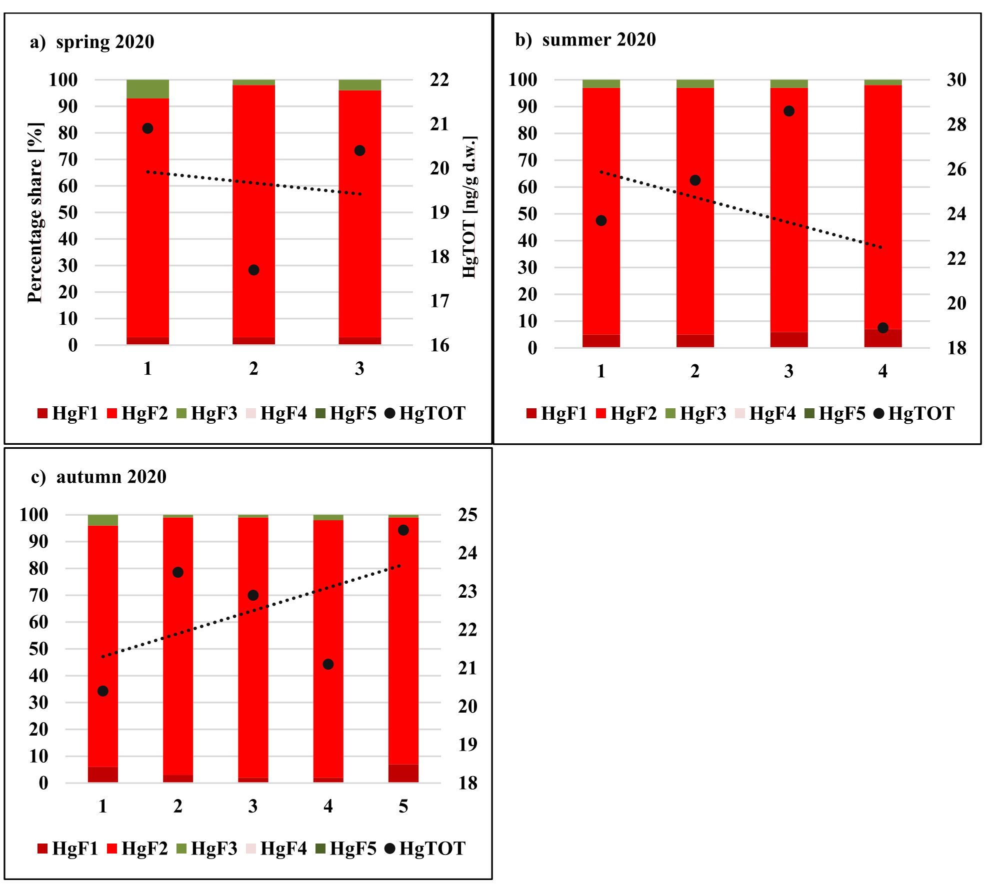

Fig. 4. Variability in the percentage of mercury forms in successive size classes of *R. harrisii* during the season: a) spring; b) summer; c) autumn.

Harris mud crab body ($p < 0.05$) (Fig. 5). A similar pattern of body burden in *R. harrisii* was observed between measurement seasons (Fig. 5a, c). The highest median values of total mercury concentration were found in the soft body (viscera: gonads, hepato-pancreas, gills, heart, digestive system) (57.4 ng/g d.w.). Crabs have an open circulatory system. The hemolymph is pumped by the heart and forced directly into the body cavity, which washes over the organs thus supplying nutrients and oxygen as well as hazardous substances (Barreto et al., 2009; Hoogenboom et al., 2015). Some tissues, such as the hepatopancreas, are considered target organs for metal accumulation (Yılmaz and Yılmaz, 2007). In general, metal accumulation in the hepatopancreas may be due to the abundance of metallothionein proteins in these tissues compared to gills and muscle tissue (Sen and Semiz, 2007). Almost twice the median HgTOT concentration was obtained in the walking legs (27.6 ng/g d.w.). In the carapace, the median HgTOT concentration was almost four times lower (7.3 ng/g d.w.) compared to the walking legs. Sulphydryl groups contain in muscles, included in many amino acids and peptides, are crucial for toxic mercury to form biological ligands with it by a direct interaction of Hg cations with SH groups, e.g. cysteine (Ajsuvakova et al., 2020). In contrast to the carapace, which is highly mineralised and saturated with inorganic compounds such as calcium carbonate (Cribb et al., 2009). The highest percentage of mercury fraction was determined for the HgF2 fraction in all tissues of the organisms studied (Fig. 5b, d). This indicates a bioaccumulation process for this form of metal in the American crab. This may be related to the fact that methylmercury (which belongs to the group of compounds of the HgF2 fraction) can bind to cysteine groups in the organism (Gupta et al., 2018). In contrast, 1.8 % was the HgF1 fraction (mainly halogenated forms of mercury), which is an important form of Hg in micro- and macroalgae and suspension (Bełdowska et al., 2018; Wilman et al., 2023c). However, because it is the most labile form of Hg and has a lower affinity for mercury toxicity target groups, its contribution to

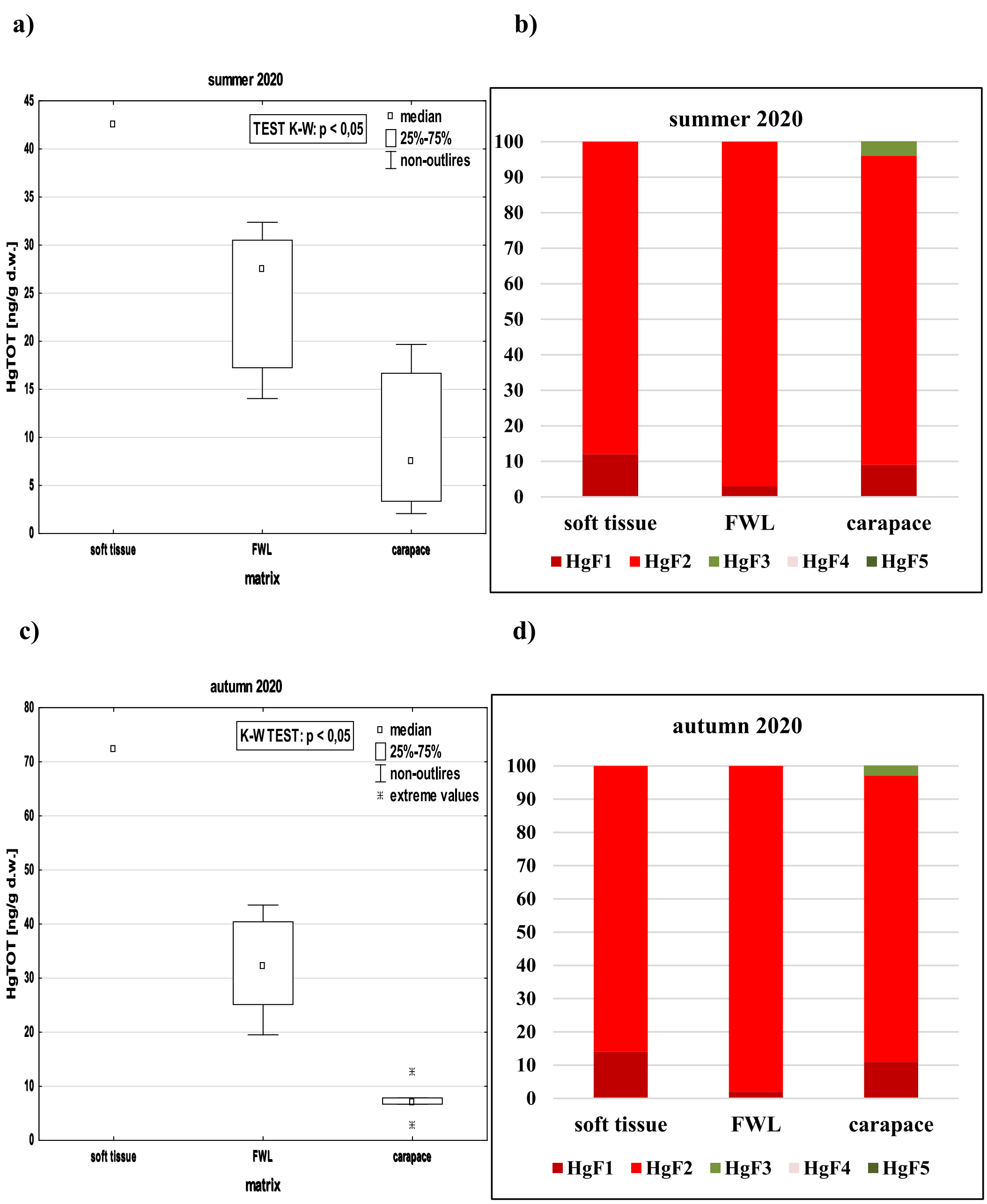

Fig. 5. Concentration of total mercury HgTOT in individual *R. harrisii* tissues in season: (a) summer and (c) autumn, and the percentage of labile and stable forms of Hg in isolated tissues: summer (b) and autumn (d).

HgTOT was negligible in *R. harrisii* (Aksentov and Sattarova, 2020; Wilman et al., 2023a). In the case of the soft tissue, the HgF1 fraction accounted for approximately 10 % of the total HgTOT (Fig. 5b, d). As shown in the studies by Bełdowska et al. (2018) and Wilman et al. (2023c), HgF1 forms a large share of HgTOT in microalgae, thereby confirming the uptake of HgF1 with the consumption of microalgae by young crabs (Wilman et al., 2019). Although it is an omnivorous species, and can move freely on the bottom, as many other brachyurans it prefers food which does not require much energy to catch: plants, detritus or sessile benthos (Kidawa et al., 2004; Hegele-Drywa and Normant, 2009; Wójcik et al., 2015) which contains more halogenated Hg forms (Jędruch et al., 2019; Wilman et al., 2023a). A stable form of mercury, HgS, was only determined in the crab carapace (Fig. 5b, d). This was a fraction directly correlated with HgF1 ($r = 0.80$, $P < 0.05$). Presumably, inorganic forms of mercury are redistributed into the carapace along with other inorganic compounds required for carapace mineralisation during the growth process, analogous to *Eriocheir sinensis* from the same reservoir (Schofield et al., 2003; Wilman et al., 2024).

The season and size of *R. harrisii* individuals were important factors influencing the bioaccumulation of Hg in the crab body and its redistribution (Fig. 6). During the summer season, bioaccumulation of mercury was observed in the muscles of the walking legs, which was mainly related to the fraction of mercury of trophic origin (Fig. 6a). There was a concomitant bioaccumulation of Hg in the carapace and an increase in the proportion of halogenated and sulphide forms of Hg in subsequent size classes, as confirmed by the correlation coefficients (Fig. 6b; Table 3). The results presented here confirm that older larger crabs become more predatory and feed on food from higher trophic levels, and the fact that their growth rate is highest during the summer, where, in the process of moulting growth, they eliminate a certain pool of Hg from the body and its biodilution occurs due to the formation of new exoskeleton tissues (Fig. 6b). A similar relationship was observed in the Harris mud crab from the Bay of Puck and the Chinese mitten crab from the Vistula Lagoon and the Puck Bay (Boszke et al., 2003; Wilman et al., 2019, 2024). During the autumn season, the opposite trend was observed: a decrease in Hg concentration in the walking legs in successive size classes and bioaccumulation of Hg in the carapace (Fig. 6c, d). Fleshes mass during the autumn season was on average 4 times greater than in individuals from the summer season. This may indicate that in preparation for winter stagnation, they were gaining weight and a dilution of Hg in the muscle mass of the legs was observed. Concentration of metals increases with age and size, when the growth of the organism becomes slow in comparison to the rate of accumulation of metals (Roditi and Fisher, 1999). The twofold higher Hg concentration in the soft tissue in autumn compared to summer (Fig. 5a, c), may indicate that the body partially efficiently redistributes Hg compounds into the carapace during the period of increased Hg accumulation in the body (Table 3). Hence, an increase in Hg concentration was observed in successive size classes in the carapace of the Harris mud crab in autumn

Table 3
Correlation coefficient between the concentration of HgTOT and its forms in fleshes walking legs and the concentration of these compounds in the carapace.

	FWL	carapace			
		HgTOT	HgF1	HgF2	HgF3
Summer	HgTOT	−0.50*	–	0.80*	–
	HgF1	–	–	–	0.55*
	HgF2	−0.60*	−0.75*	–	−0.59*
	HgF3	–	0.69*	–	–
Autumn	HgTOT	−0.70*	–	0.70*	–
	HgF1	–	–	–	0.60*
	HgF2	−0.75*	−0.85*	–	−0.69*
	HgF3	–	0.75*	–	–

[*] In table contains values for which only $P < 0.05$.

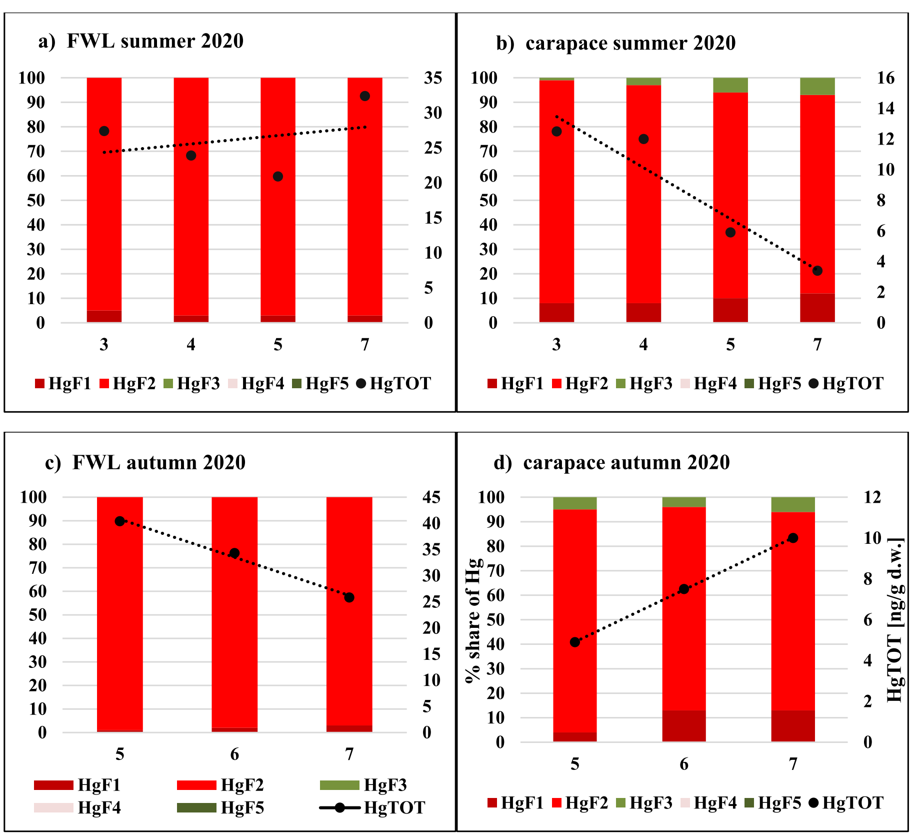

Fig. 6. Effect of organism size on Hg accumulation in fleshes in summer (a) and biodilution in the carapace in summer (b) and the opposite process in autumn: in fleshes (c) and in the carapace (d) in *R. harrisii*.

(Fig. 6d). An increase in the proportion of inorganic forms of mercury (HgF1 and HgS) in the crab carapace was observed in both measurement seasons, with a decrease in the proportion of mercury forms in complexes with organic matter - HgF2 (Fig. 6b, c). As indicated by studies in other crab species, with crab size (age), their ability to demethylate mercury increases as a result of a higher body burden of Hg neurotoxin (Hui et al., 2005). As shown in a study by Wilman et al. (2024) as a result of this process, inorganic forms of Hg are then redistributed into the carapace of crabs. Which could indicate analogous processes for the obtained Hg fractions in the present work.

3.3. Storage of Hg crab's body

However, our previous work indicates an important role for hard tissues in the redistribution of Hg from soft tissues and its temporal accumulation via soft tissue-shell transfer in bivalves, or internal organs to carapace in the case of the crab (Wilman et al., 2023b, 2023c; Wilman et al., 2024). The mass of accumulated total mercury and all its labile and stable was similar in the soft tissue and carapace but less in the walking legs (Fig. 7). A similar dependency was observed in particular measurement seasons (Fig. 7). In every cases of tissues type, 97.2 % in HgTOT was accounted for by the labile fractions, which is mainly of trophic origin and represents a large proportion in animal tissues as well as detritus (Jędruch et al., 2018; Wilman et al., 2023c). These Hg compounds are easily transported inside the organism's cells – they are adsorbed and absorbed more completely than inorganic salts because they are more lipid-soluble and because they bind to sulfhydryl groups (Broussard et al., 2002). They also undergo bioaccumulation and magnification in the trophic chain (Cavoura et al., 2019). In contrast, a large proportion of mercury halides and 100 % of the HgS from the

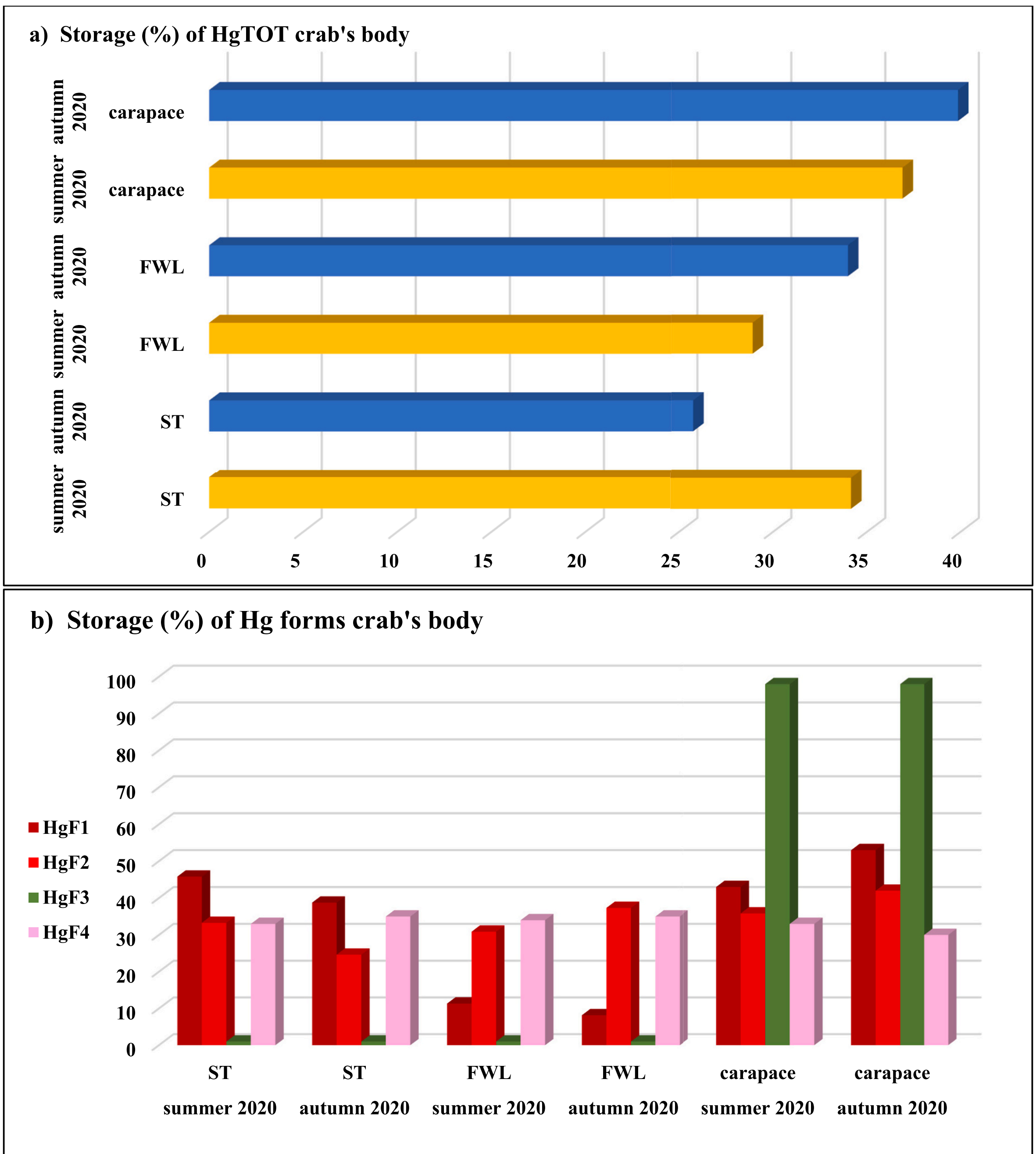

Fig. 7. Storage (%) of total mercury concentration in crab's body (a) and its labile and stable forms (b) (ST-soft tissue; FWL-fleshes walking legs).

whole crab was accumulated in the crab's carapace. In addition to organic compounds, inorganic forms of Cl, Br, F anions or calcium cations are involved in the mineralisation of the carapace, hence it is likely that these forms of mercury are bound to a large extent in the hard tissues (Cribb et al., 2009; Wilman et al., 2024). A similar relationship was also observed for clams shells in the studied southern Baltic region (Wilman et al., 2023b, 2023c). On average in the entire weight of *R. harrisii*, the soft tissue constituted 5 %, walking legs 10 % and the carapace 85 %. The crabs accumulated more mercury in the carapace than in the soft tissue. This protects the soft tissue of Harris mud crabs against the toxic effect of Hg. This was particularly evident during the preparation period for winter stagnation, where Hg transfer to the carapace was observed as a result of the higher neurotoxin load (Fig. 7). Hence, the load of accumulated mercury in the carapace was also highest during this period. And this affects the transfer of toxic mercury in the trophic chain, because birds or benthivores fish that swallow the crabs whole partly excrete the carapace during defecation or with pellets. Presumably, sediment-dwelling organisms have had to adapt to living in an environment with elevated concentrations of toxic substances. In the case of the Harris mud crab, toxic substances and pollutants can be deposited, among other things, in the carapace, which protects the soft tissue from the harmful effects of toxins. In addition, these organisms undergo several moulting (shedding of the carapace) throughout their lives, which allows them to get rid of accumulated pollutants (Turoboyski, 1973). Higher content of mercury in the carapace than in the soft tissue suggests that the exoskeleton constitutes the place of transfer of Hg and its deactivation, analogically to shell in clams, feathers and claws in birds and fur in seals (Scheuhammer et al., 2007; Bustamante et al., 2016; Falkowska et al., 2017; Grajewska et al., 2019; Manceau et al., 2021; Wilman et al., 2023b, 2023c).

4. Conclusions

Sex did not determine the concentration of mercury and its forms in the Harris mud crab. The season of the year, which directly influenced the biological condition of the organisms, determined the processes of Hg distribution in *R. harrisii* and its concentration. An increase in total mercury concentration was observed between spring and summer, indicating that the organisms were preparing for reproduction. In summer, Hg biodilution was observed in the growth process of the crabs. In contrast, Hg bioaccumulation occurred in autumn in preparation for winter stagnation, where the concentration of the xenobiotic increased in successive size classes. The Hg burden on crab organisms varied according to tissue type. Coefficients calculated for the mass of accumulated mercury in the crab body showed that most of the metal is bound in the exoskeleton. It represents the largest percentage as a proportion of the total body mass of *R. harrisii*. Differences in the form of mercury bounding in the crab body were also determined, which was closely related to the functional groups of the compounds involved in soft tissue formation, where mainly labile and semi-labile forms of Hg were bound. In contrast, in the case of the exoskeleton, a large proportion were halogenated Hg and HgS compounds, which can probably be redistributed from the body of the crabs together with other inorganic compounds used in the biomineralization of the carapace during the growth of *R. harrisii*. This protects the soft tissue of crabs against the toxic effect of Hg. Due to all those processes, mercury concentration in Harris mud crab was at a low level. With the assumption that in the future, due to its increasingly frequent occurrence, the organism will become a more and more common component of fish diet, a smaller load of toxic mercury will be introduced to the marine trophic chain into the Vistula Lagoon food chain, at the end of which is a human. It is important, because Hg fulfils no positive function in living organisms, and is toxic even in low doses. Our research has shown that studies in whole individuals give an underestimation of Hg accumulation. Therefore, soft tissue and hard tissue should be considered separately in this type of study.

CRediT authorship contribution statement

Bartłomiej Wilman: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Magdalena Bełdowska:** Writing – review & editing, Supervision, Conceptualization. **Agata Rychter:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Angela Popławska:** Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Statement of co-authors

Individual authors contribution statement has been structured using the Contributors Roles Taxonomy (CRediT) recommended by Brand et al. (2015).

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Author contribution statement

I declare that my contrubution in publication :

Wilman, B., Bełdowska, M., Rychter, A., Popławska, A. 2024. Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body. *Marine Pollution Bulletin*, *209*, 117118. doi: 10.1016/j.marpolbul.2024.117118.

Included in my doctoral dissertation entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- First author, corresponding author
- Collection and preparation of environmental samples for biological and chemical analyses and their implementation
- Processing and interpretation of results
- Preparation of original draft of the manuscript
- Responding to reviews, editing the manuscript

Bartłomiej Wilman

Gdynia, April 7, 2025

Prof. Magdalena Bełdowska, PhD

Faculty of Oceanography and Geography, University of Gdańsk

Department of Chemical Oceanography and Marine Geology

Laboratory of Transformation of Toxic Substances

Author contribution statement

I declare that my contrubution in publication :

Wilman, B., **Bełdowska, M.**, Rychter, A., Popławska, A. 2024. Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body. *Marine Pollution Bulletin*, *209*, 117118. doi: 10.1016/j.marpolbul.2024.117118.

Included in the doctoral dissertation by Bartłomiej Wilman entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- Supervision
- Conceptualization - Ideas
- Writing review and editing
- Resources and funding acquisition

M. Bełdowska

Elbląg, April 14, 2025

Agata Rychter, PhD, Assoc. Prof.

Institute of Technology, State University of Applied Sciences in Elbląg

Author contribution statement

I declare that my contrubution in publication :

Wilman, B., Bełdowska, M., **Rychter, A**., Popławska, A. 2024. Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body. *Marine Pollution Bulletin*, *209*, 117118. doi: 10.1016/j.marpolbul.2024.117118.

Included in the doctoral dissertation by Bartłomiej Wilman entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- Head of research cruises
- Support in the field of biology and ecology of crabs
- Writing review and editing

Signed by / Podpisano przez:

Agata Anna Rychter
Akademia Nauk Stosowanych w Elblągu

Date / Data: 2025-04-14 12:26

Gdynia, April 14, 2025

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Author contribution statement

I declare that my contrubution in publication :

Wilman, B., Bełdowska, M., Rychter, A., **Popławska, A.** 2024. Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body. *Marine Pollution Bulletin, 209*, 117118. doi: 10.1016/j.marpolbul.2024.117118.

Included in the doctoral dissertation by Bartłomiej Wilman entitled: Bioaccumulation and transfer of mercury (Hg) in organisms from lower trophic levels on the case of clams and crabs from the Vistula Lagoon,

Involved:

- Active participation in scientific research cruises
- Assistance with chemical and biological analyses
- Preparation of background material: reference literature, compilation of meteorological data

Appendix B : Author's academic resume

Education:

- **PhD studies (2019 – 2025)**
  University of Gdańsk, Faculty of Oceanography and Geography
  Doctoral School of Natural Science (DSNS)

- **MSc studies (2017 – 2019)**
  University of Gdańsk, Faculty of Oceanography and Geography
  Oceanography, speciality: geological-physical-chemical oceanography

- **BSc studies (2014 – 2017)**
  University of Gdańsk, Faculty of Oceanography and Geography
  Oceanography, speciality: geological-chemical oceanography

Personal achievements

- MEiN scholarship for outsdanding young scientists (2024)

Publications:

Co-authorship of nine scientific publications in journals indexed in the Journal Citation Reports (JCR).

1. **Wilman, B**., Bełdowska, M., Rychter, A., Popławska, A. 2024. Impact of biometric parameters and seasonal condition on mercury (Hg) distribution in Harris mud crab (*Rhithropanopeus harrisii*) body. *Marine Pollution Bulletin*, *209*, 117118. doi: 10.1016/j.marpolbul.2024.117118.
   Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

2. **Wilman B**., Normant-Saremba M., Rychter A., Bełdowska M. 2024. *Total body burden of neurotoxicant Hg in Chinese mitten crab (Eriocheir sinensis) - considerations of distribution and human risk assessment*. *Marine Pollution Bulletin*, *199*, 1–13. https://doi.org/10.1016/j.marpolbul.2024.116028.

   Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

3. **Wilman B**., Bełdowska M., Rychter A., Popławska A. 2023. *Factors determining bioaccumulation of neurotoxicant Hg in the zebra mussels (Dreissena polymorpha): influence of biometric parameters, sex and storage of shell*. *Marine Pollution Bulletin*, *197*, 1–12. https://doi.org/10.1016/j.marpolbul.2023.115718.

Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

4. Jarzynowska M., Saniewska D., Fudala K., **Wilman B**., Balazy P., Płońska P., Saniewski M. 2023. *Mercury and methylmercury in birds and marine mammals inhabiting the coastal zone of the two King George Island's bays: Admiralty and King George Bay (maritime Antarctic). Marine Pollution Bulletin, 193,* 115237. https://doi.org/10.1016/j.marpolbul.2023.115237.

   Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

5. **Wilman B**., Staniszewska M., Bełdowska M. 2023. *Is the inhalation influence on the level of mercury and PAHs in the lungs of the baltic grey seal (Halichoerus grypus grypus)?. Environmental Pollution,* 121083. 100, 9,9. doi: https://doi.org/10.1016/j.envpol.2023.121083.

   Impact factor (IF) in a given year: 7.6, 5-year IF: 8.3, MEiN points: 100

6. **Wilman B**., Saniewska D., Pyta H., Wysiecki D., Bełdowska M. 2023. *Mercury fractionation-Problems in method application*. *Marine Pollution Bulletin*, *187,* 114560. https://doi.org/10.1016/j.marpolbul.2022.114560.

   Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

7. **Wilman B**., Bełdowska M., Rychter A., Kornijów R. 2023. *Different pathways of accumulation and elimination of neurotoxicant Hg and its forms in the clam Atlantic rangia* (*Rangia cuneata*). *Science of The Total Environment, 858,* 160018. https://doi.org/10.1016/j.scitotenv.2022.160018.

   Impact factor (IF) in a given year: 8.2, 5-year IF: 8.6, MEiN points: 200

8. **Wilman B**., Bełdowska M., Normant-Saremba M. 2019. *Labile and stable mercury in Harris mud crab (Rhithropanopeus harrisii) from the southern Baltic Sea – considerations for a role of non-native species in the food web*. Mar Pollut Bull 148: 116-122. doi: 10.1016/j.marpolbul.2019.07.064.

   Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

9. Saniewska D., Gębka K, Bełdowska M., Siedlewicz G., Bełdowski J., **Wilman B**. 2019. *Impact of hydrotechnical works on outflow of mercury from the riparian zone to a river and input to the sea*. Mar Pollut Bull 142: 361-376. doi: 10.1016/j.marpolbul.2019.03.059.

Impact factor (IF) in a given year: 5.3 , 5-year IF: 6.1, MEiN points: 100

Summarized metrics:

- **Total IF in a given year: 50.4, total 5-year IF: 59.6, MEiN points: 1,120 (According to the Knowledge Base of the University of Gdańsk)**
- **Number of citations: 59, excluding self-citations: 22, *h*-index: 5 (According to the Scopus database)**

Projects:

Participation in four research projects.

Principal investigator

- Is the level of mercury (Hg) contamination of weevil hens (*Myoxocephalus scorpius*) from the Gulf of Gdansk safe for the potential consumer? (Czy poziom zanieczyszczenia rtęcią (Hg) kur diabła (*Myoxocephalus scorpius*) z Zatoki Gdańskiej jest bezpieczny dla potencjalnego konsumenta?)

Number: 533-0C20-GS30-23
Funding: UGrants-start, University of Gdańsk
Duration: 02.2023 – 02.2024
Budget: 15 000 PLN

Participant

- Primary producers as a key contributor of historical and contemporary mercury in the estuarine trophic network (Producenci pierwotni jako kluczowy nośnik historycznej i współczesnej rtęci w estuaryjnej sieci troficznej)

Number: 2022/45/B/ST10/00368
Funding: National Science Centre, OPUS 23
Principal investigator: Magdalena Bełdowska
Duration: 02.2023 – 01.2027
Budget: 1 999 860 PLN

Principal investigator

- Distribution of mercury (Hg) in the body of the Chinese mitten crab (*Eriocheir sinensis*) (Dystrybucja rtęci (Hg) w organizmie kraba wełnistoszczypcego (*Eriocheir sinensis*))

  Number: 533-0C20-GS69-22
  Funding: UGrants-start, University of Gdańsk
  Duration: 01.2022 – 02.2023
  Budget: 17 000 PLN

Participant

- Benthic organisms as indicators of mercury sources in the Antarctic coastal zone (Admiralty Bay) (Organizmy bentosowe jako wskaźnik źródeł rtęci w strefie brzegowej Antarktyki (Zatoka Admiralicji))

  Number: 2019/33/B/ST10/00290
  Funding: National Science Centre, OPUS 17
  Principal investigator: Dominika Saniewska
  Duration: 02.2020 – 02.2023
  Budget: 993 620 PLN

Conferences:

Authorship and co-authorship of 7 oral presentations and 14 posters presented at 9 scientific conferences, including three international.

Oral presentation:

1. **Bartłomiej Wilman**, Marta Staniszewska, Magdalena Bełdowska, 2023. *Czy wdychane powietrze jest istotnym źródłem Hg i WWA w płucach na przykładzie bałtyckiej foki szarej (Halichoerus grypus grypus)?* XX Sympozjum Młodych Oceanografów, Gdynia, 01.12.2023.

2. **Bartłomiej Wilman**, Monika Normant-Saremba, Magdalena Bełdowska, Agata Rychter, 2023. *Obciążenie organizmu kraba wełnistoszczypcego (Eriocheir sinensis) neurotoksyną Hg - rozważania na temat dystrybucji i eliminacji*. XV Konferencja „Chemia, Geochemia i Ochrona Środowiska Morskiego" Gdynia, 20.10.2023.

3. **Bartłomiej Wilman,** Magdalena Bełdowska, Agata Rychter, 2022. *Wykorzystanie małży Rangia cuneata i Dreissena polymorpha jako bioindykatorów zanieczyszczenia rtęcią (Hg) Zalewu Wiślanego*. VII Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen, panel Ekologia i Ochrona Środowiska, 7-8.04.2022. Łódź

4. **Bartłomiej Wilman**, 2022. *Statystyka w naukach przyrodniczych*. IV Ogólnopolska Konferencja pt.: Matematyka w Okół Nas, 24.05.2022. Elbląg

5. **Bartłomiej Wilman**, Magdalena Bełdowska, Agata Rychter, 2022. *Wybrane drogi detoksykacji małży Rangia cuneata i Dreissena polymorpha z toksycznej rtęci (Hg)*. III Konferencja Polskich Badaczy Morza, 7-8.06.2022. Gdynia

6. **Bartłomiej Wilman**, Dominika Saniewska, Magdalena Bełdowska, Dariusz Wysiecki, 2021. *Nowelizacja 5-cio stopniowej metody termodesorpcji Hg – problemy i rozwiązania*. III Krajowa Konferencja pt.: *Środowisko przyrodnicze jako obszar badań*. 27.05.2021, Poznań

7. **Bartłomiej Wilman**, Magdalena Bełdowska, Monika Normant-Saremba, 2019. *Labile and stabile mercury in Harris mud crab (Rhithropanopeus harrisii) from the southern Baltic Sea – considerations for a role of non-native species in the food web*. International Sopot Youth Conference. 7.06.2019, Sopot

Poster presentation:

1. Ewa Korejwo, Dominika Saniewska, Agnieszka Jędruch, Małgorzata Jarzynowska, Aleksandra Cichecka, **Bartłomiej Wilman**, Piotr Bałazy, Jacek Bełdowski. 2024. *Porównanie stężeń rtęci w organizmach bentosowych z rejonów polarnych*. IV Konferencja Polskich Badaczy Morza. 16-18.09.2024, Szczecin.
2. Wojdasiewicz, A., **Wilman, B**., Zarzeczańska, A., Popławska, A., Kusztal, A., Woźniczka, A., Kornijów, R. (2024). *Mercury in plankton: analysis of the influence of environmental conditions on Hg bioaccumulation in the context of diverse Baltic lagoons*. 16th Internationa Conference On Mercury as a Global Pollutant. Cape Town, RPA.
3. Bełdowska, M., Bełdowski, J., Dziubińska, A., Korejwo, E., Kornijów, R., Panasiuk, A., Sapota, M., **Wilman, B**., Wojdasiewicz, A., Złoch I., Zgrundo, A. (2024). *The role of primary producers in the transfer of mercury into the primary consumers of the*

estuarine trophic web (southern Baltic Sea). 16th Internationa Conference On Mercury as a Global Pollutant. Cape Town, RPA.

4. Korejwo, E., Saniewska, D., Jarzynowska, M., Cichecka, A., **Wilman, B**., Bełdowski, J. (2024). *Mercury in the Arctic and Antarctica: the role of benthic organisms*. 16th Internationa Conference On Mercury as a Global Pollutant. Cape Town, RPA.

5. Jędruch Agnieszka, Bełdowski Jacek, Bełdowska Magdalena, Jakacki Jaromir, Pazdro Ksenia, Staniszewska Marta, Siedlewicz Grzegorz, Korejwo Ewa, **Wilman Bartłomiej**, Grabowski Miłosz, 2023, Baltic Sea Science Congress 2023.

6. **Bartłomiej Wilman**, Martyna Zakrzewska, Magdalena Bełdowska, Monika Normant-Saremba, 2022. *Czy poziom zanieczyszczenia rtęcią kraba wełnistoszczypcego(Eriocheir sisnesis) z Zalewu Wiślanego jest bezpieczny dla potencjalnego konsumenta?* III Konferencja Polskich Badaczy Morza, 7-8.06.2022. Gdynia.

7. Dorota Pryputniewicz-Flis, Dorota Burska, Magdalena Bełdowska, **Bartłomiej Wilman**, Ryszard Kornijów, 2022. *Wpływ prac pogłębiarskich prowadzonych w podejściu do portu w Tolkmicku na skład chemiczny osadów powierzchniowych Zalewu Wiślanego*. III Konferencja Polskich Badaczy Morza, 7-8.06.2022. Gdynia.

8. Anna Pouch, Paulina Rudnicka, Marta Szubska, Ewa Korejwo, **Bartłomiej Wilman**, Agata Zaborska, Magdalena Bełdowska, Jacek Bełdowski, Piotr Kukliński, Angelina Lo Giudice, Carmen Rizzo, Anton Dohrn, , Ksenia Pazdro, 2022. *Akumulacja zanieczyszczeń organicznych i metali w organizmach bentosowych z Isfjorden (zachodni Spitsbergen).* III Konferencja Polskich Badaczy Morza, 7-8.06.2022. Gdynia.

9. Angela Popławska, Piotr Prusiński, **Bartłomiej Wilman**, Agata Rychter, Ryszard Kornijów, Magdalena Bełdowska, 2022. *Zmiany sezonowe rtęci całkowitej (HgTOT) w organizmach krabika amerykańskiego (Rhitropanopeus harrisii) i w osadach z Zalewu Wiślanego.* III Konferencja Polskich Badaczy Morza, 7-8.06.2022. Gdynia.

10. **Bartłomiej Wilman** i inni, 2022. Hg_{TOT} and Its Fractions in The Basin, Lagoons and Catchment Area of the Southern Baltic. The 15th International Conference on Mercury as a Global Pollutant (ICMGP), 25-29.08.2022. Cape Town, RPA.

11. **Bartłomiej Wilman**, Dominika Saniewska, Magdalena Bełdowska, Dariusz Wysiecki, 2021. *Recalibration of mercury fractionation method using thermodesorption*

technique: problems and solutions. International Sopot Youth Conference. 11.06.2021, Sopot.

12. Angela Popławska, **Bartłomiej Wilman**, Magdalena Bełdowska, Agata Rychter, 2021. *Seasonal variability of mercury concentration (Hg) in soft tissue of zebra mussel (Dreissena polymorpha) in Vistula Lagoon*. International Sopot Youth Conference. 11.06.2021, Sopot.

13. **Bartłomiej Wilman**, Lucyna Falkowska, Magdalena Bełdowska, Marta Staniszewska, 2020. *Labilne formy rtęci i wielopierścieniowe węglowodory aromatyczne w płucach foki szarej (Halichoerus grypus).* XIV konferencja pt.: *Chemia, Geochemia i Ochrona Środowiska Morskiego.* 16.10.2020, Sopot, str.87.

14. Ryszard Kornijów, Krzysztof Pawlikowski, Magdalena Bełdowska, **Bartłomiej Wilman**, Mariusz Zalewski, Aneta Jakubowska, Paulina Tonko, 2020. *Wpływ prac pogłębiarskich przy podejściu do portu w Tolkmicku na środowisko Zalewu Wiślanego*. XIV konferencja pt.: *Chemia, Geochemia i Ochrona Środowiska Morskiego.* 16.10.2020, Sopot, str.99.

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