SUMMARY

EXPLANATION OF CONDUCTED RESEARCH

Mercury (Hg) is one of the most dangerous metals found in the environment. As a mutagenic neurotoxin, it negatively affects the cardiovascular system and central nervous system causing permanent damage to brain cells (Splading et al., 2000; Rutkiewicz et al., 2011). It is also classified as an endocrine disruptive compound (Zhu et al., 2000). The World Health Organization (WHO) has placed mercury alongside with dioxins and other heavy metals on the list of top 10 chemicals of major public health concern (WHO, 2010). Due to global transport and deposition in areas far from emission sources, the United Nations Environmental Programme (UNEP) has declared mercury a harmful substance of global significance (UNEP, 2013). In order to solve the problem of environmental mercury contamination the Minamata Convention was adopted in 2013. Its objective is to protect human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds (Council Decision (EU) 2017/939).

Mercury is introduced to the marine environment mainly as inorganic form Hg^{2+} or Hg^{0} (Driscoll et al., 2013). With the participation of microorganisms (Berman and Bartha, 1986) and during chemical methylation (Weber, 1993), it can be transformed into methylmercury (MeHg) and in this form can easily enter the marine trophic chain (Evers et al., 2007; Scheuhammer et al., 2007). Methylmercury is the form of Hg that bioaccumulates most efficiently in marine organisms. Additionally, due to its toxicity, this form of mercury is of primary concern for human and wildlife health (Eagle-Smith et al., 2018; Hsu-Kim et al., 2018). Predatory marine mammals' mercury exposure occurs mainly through food consumption. Biomagnification process lets these animals accumulate mercury at levels that often exceed thousands of times those observed in the surrounding environment (Das et al., 2003; Das et al., 2008; Bossart, 2006; Scheuhammer et al., 2007). Dietary absorbed methylmercury is distributed with blood to internal organs, mainly kidneys and liver. The latter is responsible for demethylation processes during which methylmercury is transformed into less toxic forms (National Research Council, 2000). Mercury elimination takes place every day with urine and faeces. (Nigro et al., 2002). In case of phocids, mercury can additionally be incorporated into the claws and fur (Habran et al., 2013; Cossaboon et al., 2015; Grajewska et al., 2019). The ability of methylmercury to penetrate the blood-brain and blood-placenta barriers is extremely dangerous for mammals (WHO, 2012). The proper

development of an offspring may be disturbed even at the prenatal stage especially that its nervous system is extremely vulnerable to the presence of mercury.

The presented dissertation discussed intake routes of mercury to the grey seals (**Publication 1**, **Publication 2**). However, the alimentary route is the most important for adults, placental and lactational transfer of mercury to a pup during the period of foetal development and maternal care was also studied. Recently observed increase in the mortality of grey seal pups (Harding et al., 2007), potentially caused by marine pollution, including trace metals, indicates the significance of such research. Considering that juvenile individuals are particularly sensitive to changes occurring in the environment it can be assumed that their condition and health in the first period of life have a major impact on their future fate. Therefore, the investigation of maternal transfer of toxic substances seems to be crucial.

As food consumption provides constant mercury intake, the efficiency and elimination rate may have a major impact on maintaining seal's mercury body burden at level that does not endanger its life and health. A part of the dissertation was devoted to the most important mercury elimination routes for seals (**Publication 3**), including the excretion with faeces and incorporation into fur during molting. Furthermore, the problem of transferring mercury to the next generation was introduced as cleansing mechanism for the seals' females which gives them the opportunity to eliminate additional mercury load (**Publication 1**, **Publication 2**).

Mercury toxicity is determined by its chemical form (WHO, 2010). In case of both intake and elimination of mercury, the knowledge in what extent these most toxic forms involving processes occur, can provide important information about mercury transformation inside the body. Therefore, besides total mercury (THg), measurements of organic mercury, which is predominantly methylmercury (MeHg) in seal organisms, were included in the analyses (Hg_{ORG}; **Publication 1**). The purchase of the new equipment and the implementation of the methodology for determining methylmercury allowed for later including this form in the research (**Publication 2**, **Publication 3**).

In coastal regions of seas and oceans faeces and fur can constitute a potential source of environmental mercury contamination (Cossaboon et al., 2015). The form of mercury offloaded in animal excreta should be determined, as bioavailable mercury (Hg (II) or MeHg) can be easily re-incorporated at the base of marine food web (Jędruch et al., 2018).

Separation of the labile and stable forms of mercury (**Publication 3**) allowed to assess the potential top-down mercury enrichment and internal recycling of this element in seals' habitats.

Selenium, which can partly reduce mercury toxicity by forming $(CH_3Hg)_2Se$ complexes (Berry and Ralston, 2008; Correa et al., 2014; Das et al., 2016) was included in the analyses as an additional parameter (**Publication 1**, **Publication 2**). In case of mammals, this process may be particularly important because of this element's high availability in the marine environment (Correa et al., 2014). Moreover, the combination of mercury and selenium considerably affects the organ distribution and the rate of mercury elimination from the body (Gailer, 2007; Khan and Wang, 2009).

AIMS OF THE RESEARCH

Due to the difficulties related to sample collection, research on marine mammals has rarely been conducted. Little is known about the effects of nutrition, condition or physiological state on trace elements concentrations in marine mammals (Habran et al., 2011). The aim of the presented research was to increase the knowledge and provide new information on mercury intake and elimination processes in a Baltic grey seal, and to verify the following hypotheses:

1. Adverse effects of maternal transfer of mercury to a grey seal pup may be reduced by protective mechanisms observed during prenatal and postnatal development.
2. Fur and faeces of seals can be considered as an important component of mercury circulation in local ecosystems.

The hypotheses were verified by achieving the following research objectives:

1. Evaluation of mercury exposure via alimentary route (**Publication 2, Publication 3**).
2. Evaluation of a grey seal selected mercury elimination processes (**Publication 3**):
   a) comparison of the effectiveness of mercury elimination from a grey seal via faeces and fur
   b) estimation what portion of mercury eliminated from a seal gets into the environment as bioavailable mercury compounds.
3. Description of the intergenerational transfer of mercury and selenium (**Publication 1**, **Publication 2**):
   a) determination the role of the placenta in mercury (organic and inorganic) and selenium transport with a female blood into an offspring during foetal development
   b) examination of the maternal transfer of mercury and selenium with mother's milk during nursing
   c) characteristic of the changes in mercury and selenium blood concentrations in the first months of pup's life which are represented by the three subsequent stages – nursing, postweaning fast and fish diet.

COLLECTED MATERIAL AND CHEMICAL ANALYSIS

The grey seal (*Halichoerus grypys grypus*; Fabricius, 1791) is the most numerous of the three seal species found in the Baltic Sea. According to Helsinki Commission (HELCOM 2013) it is a protected species in all Baltic countries, with population estimated to 30,000 individuals since 2014. The research was carried out in cooperation with the Professor Krzysztof Skóra Hel Marine Station, the Institute of Oceanography, University of Gdansk field unit. The mission of the Hel Marine Station is to actively protect Baltic seals and their habitats. Currently, the most important task is to reduce excessive mortality of these animals. The sealarium, operating as a part of the Hel Marine Station, deals with the rehabilitation and treatment of sick and weakened pups found on the Baltic coast. In the years 2001-2018, the sealarium also implemented a grey seal restitution programme, under which the group of seals inhabiting the unit were breeded. The postnatal development of the pups born at the Station was analogous to the wild animals. First, they remained under maternal care (nursing) for a period of about 21 days. During this time, they lost lanugo and gained new fur. Then they went through a two-week postweaning fast and adapted to life in water. After this, they were taught how to hunt fish. After mastering the skills required to survive in the wild and gaining the proper body mass, they were released into the waters of the southern Baltic Sea.

The samples were collected from 6 seals (4 females and 2 males) forming a breeding group and 13 pups born at the Marine Station during the research period. The collected material consisted of 30 placentas (**Publication 1**), 153 blood samples, 53 milk samples (**Publication 2**), 95 faeces samples and 36 fur samples (**Publication 3**). Additionally, 29 herrings (*Clupea harengus membras*; Linneaus, 1761) were collected, as this species is the main component of the sealarium seals' diet. A detailed description of the collected material is presented in Table 1.

Total mercury concentration (THg) was measured using the AMA 254 atomic absorption spectrometer (**Publication 1**, **Publication 2**, **Publication 3**). The analysis of organic mercury (Hg_{ORG}) (**Publication 1**) was carried out by extracting this form from a lyophilized sample, and then transferring it to a hydrophobic carrier (Carbonell et al., 2009; Kwaśniak et al., 2012). Mercury measurements in the obtained extracts were made using the AMA-254 analyzer. Methylmercury (**Publication 2**, **Publication 3**) was analysed in samples mineralised (12h) in 30% nitric acid at a temperature not exceeding 60°C, using

atomic fluorescence spectroscopy after prior separation by gas chromatography using the MERX-M analyser in accordance with the methodology of US EPA (2002). Additionally, the mercury fractionation by the thermodesorption method (**Publication 3**), proposed by Jędruch et al. (2018), was performed in the selected samples using the DMA-80 direct mercury analyser. This method enabled separation of five groups of compounds with similar properties, differing in bioavailability in the environment. Three labile: $Hg_{Labile\ 1a}$ (mercury mainly associated with halides), $Hg_{Labile\ 1b}$ (organic mercury, including MeHg), $Hg_{Labile\ 2}$ (mercury sulfate and oxide) and two stable fractions: HgS (mercury sulfide), $Hg_{Residual}$ (residual mercury embedded in minerals) were obtained. Selenium was determined in the samples mineralised under elevated pressure and temperature, using the hydride generation technique in an atomic absorption spectrometer coupled with a Fias 200 (**Publication 1**) and inductively coupled plasma – mass spectrometry (ICP-MS) on a Nexion 300X analyzer (**Publication 2**).

Table 1. Collected samples.

Publication	Sample	Sampling period	Number of samples	Parameter
Publication 1	placenta	2007-2016	30	THg; Hg_{ORG}; Se;
Publication 2	female blood	2014-2017	76	THg; MeHg; Se
	pup blood		73	
	umbilical cord blood		4	
	milk		53	
	herring	2017	29	
Publication 3	adult seal faeces	2016-2017	71	THg; MeHg; Hg fractions
	pup faeces		24	
	adult seal fur		14	
	pup lanugo	2014-2017	13	
	pup fur (new pelage)		9	

CONCLUSIONS

It was estimated that an adult grey seal can take 140 µg of mercury with food daily (**Publication 2**; **Publication 3**). Together, with fur and faeces an animal can expel nearly 50% of this load. Over 90% of this is removed with faeces. Thus, the elimination of mercury with excrements is far more effective than incorporating it into the fur during every year molt (Publication 3). The relatively low share of methylmercury found in faeces (17%) in comparison to the food in which methylmercury constitutes over 80%, may indicate that mercury excreted to faeces is previously transformed inside an animal's body. Almost all mercury in faeces and fur (>95% for faeces and >85% for the hair) is in a labile form, which can be quickly incorporated at the base of the marine food web. This may be relevant especially in coastal areas where seals assemblage to rest and breed (**Publication 3**). Based on the results obtained from grey seals inhabiting the Marine Station, it was calculated that the population of 30,000 individuals deliver to the Baltic Sea through faeces and fur nearly 800 g of mercury annually. This is not a significant load. Mammalian faeces and fur should not be considered as an additional source of mercury contamination. Seals life processes can locally control the time and forms of mercury, but they do not have a significant impact on the total load of mercury that circulates in the ecosystem

The placenta plays an important role in the transfer of mercury to an offspring as it is responsible for the exchange of substances between a female and a foetus. It was determined that it is a place of accumulation for inorganic forms of mercury, and does not constitute a barrier to the most toxic organic forms of mercury including methylmercury (**Publication 1**). Systematic decrease of total and methylmercury concentration in the blood of females during gestation, which reduced mercury levels by about 40% just before delivery, suggested not only the effective elimination of mercury from a female, but also, together with the highest, almost 100%, methylmercury content in total mercury in umbilical cord blood, proved intoxication of a pup (**Publication 2**). It was not clearly stated whether the placenta is a barrier to selenium. Lower than in females' blood, the concentration of selenium in umbilical cord blood indicated a smaller role of the placenta in the intergenerational transfer of selenium (**Publication 2**). Due to the high Se:Hg ratio in placental tissue, it cannot be ruled out that the selenium present there may have limited the transport of toxic mercury to the foetus (**Publication 1**).

Over the postnatal period of maternal care and nursing, females did not purify their organisms from toxic mercury. The gradual weight loss, caused by female starvation and the progressive mobilization of energy reserves, resulted in an increase of total mercury and methylmercury concentration in their blood during lactation (**Publication 2**). The results did not reveal statistically significant differences between the mercury levels in the females' blood before and during lactation. It indicates that the transfer of mercury across the placenta effectively reduced the level of total mercury and methylmercury in the blood, and eliminated the effect of short-term lactation-induced increases. However, milk production decreased selenium concentration in the blood of lactating females. The most prominent transfer of selenium with milk occurred in the first days of lactation (**Publication 2**). During the 21-day cycle, pups received approximately 335 μg of mercury, including 15 μg of methylmercury, and 16 mg of selenium with milk. Therefore, milk appears to be an insignificant source of mercury, but a substantial source of selenium (**Publication 2**).

The presence of mercury in lanugo reflects the incorporation of mercury during a foetal period. (**Publication 3**). Transport of mercury to the pelage is probably the most efficient way to eliminate methylmercury for an unborn pup. Continuous exposure to mercury in prenatal life, resulted that at no later stage of pup's development, was there such a high blood concentration of total and methylmercury as on the day of parturition (**Publication 2**). As the consequence of high concentration of the toxin in the body, immediate removal of mercury with faeces started after birth (**Publication 3**). The first excrements were characterized by the lowest total mercury concentration with the highest share of methylmercury. Effective methylmercury transformation into less-toxic forms was evident in the next days of pups' life. Despite the small amounts of mercury delivered with milk, the concentration of mercury in the faeces began to increase and often exceeded the levels in the faeces of adult seals. Elimination and rapid weight gain (1.51 kg per day) observed during nursing led to a reduction in mercury concentration in pups' blood (**Publication 2**). The reverse trend was described for selenium. Its concentrations started to increase in the following days of nursing, which confirmed the significant role of milk as a source of this element (**Publication 2**).

Relatively fast fur replacement (just over a dozen days after birth) created the opportunity to eliminate the extra load of mercury from the pup's body (**Publication 2, Publication 3**). Although the lanugo, unlike the new hair, increased during the period of

placental methylmercury exposure, mercury concentrations in lanugo and the newly formed pelage remained at a similar level (**Publication 3**). In consequence, pups which were released to the Baltic Sea after about 3 months of life in the Hel Marine Station, despite eating fish, had lower total mercury and methylmercury concentration in blood than on the day they were born. Therefore, it can be concluded that the natural development of the grey seal allows for effective elimination of mercury transferred to the pup with blood through the placenta (**Publication 2**). However, this does not diminish the potential threat that methylmercury transported via this route can pose to the developing foetus.