Marine Pollution Bulletin 88 (2014) 311–318

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Molecular biomarkers in grey seals (*Halichoerus grypus*) to evaluate pollutant exposure, health and immune status

K. Lehnert [a,b,*], S. Müller [a], L. Weirup [a], K. Ronnenberg [a], I. Pawliczka [c], T. Rosenberger [d], U. Siebert [a]

[a] Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, 25761 Büsum, Germany
[b] Institute for Coastal Research, Helmholtz-Zentrum Geesthacht Centre for Materials and Coastal Research, 21502 Geesthacht, Germany
[c] Hel Marine Station, University of Gdansk, Morska 2, 84-150 Hel, Poland
[d] Seal Centre Friedrichskoog, An der Seeschleuse 4, 25718 Friedrichskoog, Germany

ARTICLE INFO

Article history:
Available online 11 September 2014

Keywords:
Halichoerus grypus
mRNA biomarker
Xenobiotic metabolism
Immune function
Pollution effects
Multiple stresses

ABSTRACT

Grey seals as top-predators bioaccumulate contaminants and can be considered as sentinels of eco-system health. Pups are weaned after a short nursing period, characterised by an enormous lipid transfer and exposure to contaminants. This study established molecular biomarkers of the xenobiotic metabolism and immune system to help assess health and immune status. mRNA transcription of AHR, ARNT, PPARα and cytokine IL-2 and heat-shock-protein HSP70 was measured in blood of grey seal pups and adults in rehabilitation and permanent care using RT-qPCR and compared to rehabilitating harbour seal pups and haematology values. In pups highest levels at admission in xenobiotic biomarker, HSP70 and cytokine transcription may show contaminant exposure via lactation, stress during abandonment and dehydration. The significant decrease may be linked to diet, health improvement and adaptation. Adults showed higher levels and more variation in biomarker transcription and clear species-specific differences between harbour and grey seal pups were found.

1. Introduction

Grey seals (*Halichoerus grypus*) are one of the two pinniped species reproducing in the North and Baltic Seas and characterised by long life spans, large deposits of fatty tissue reserves and relatively short nursing periods. As top predators they can bioaccumulate high levels of contaminants such as organochlorines (Sormo et al., 2003; Debier et al., 2003), trace elements, and metals (Teigen et al., 1999; Das et al., 2003; Kakuschke and Prange, 2007) and may be considered as sentinel species of pollution (Bossart, 2006) and eco-system health in their marine environment (Reddy et al., 2001). Grey seals are recolonizing German North and Baltic Sea areas after a century of hunting and several decades of impaired reproductive rates and other pathological effects caused by environmental pollution (Bergman, 1999; Bergman and Olsson, 1985; Bäcklin et al., 2003; Harding et al., 2007). They increasingly inhabit areas heavily exploited by anthropogenic use, e.g. shipping, construction of offshore wind farms, fisheries, chemical pollution and seismic and military activities (Harwood, 2001; Waterman et al., 2003; Vinther and Larsen, 2004). Grey seal offspring are nursed for only 16–21 days (Bonner, 1981; King, 1983) characterised by the high fat content in the mother milk (up to 60%) and an important mass transfer between the mother and the pup (Iverson et al., 1993; Pomeroy et al., 1996). Dams fast throughout their short lactation period and after weaning pups lose up to 25% weight before they learn to hunt successfully (Mansfield, 1988). Fat-soluble compounds such as e.g. PCBs are mobilised from the blubber (Debier et al., 2002; Schweigert et al., 2002) allowing the transfer of lipophilic xenobiotics to pups (Addison and Stobo, 1993; Beckmen et al., 1999; Debier et al., 2003; Wolkers et al., 2004). Little is known about the effects of the nutritional and physiological status (e.g., fasting, gestation, lactation) on the development of immuno-competence in seal pups and the impact of anthropogenic activities on their health status. This study uses molecular biomarkers of the xenobiotic metabolism because they can be sensitive early warning indicators for environmental pollutants that impact on the cellular level (Gil and Pla, 2001; Kim et al., 2005). The aryl hydrocarbon receptor (AHR) is activated by environmental pollutants and suspected to mediate immune toxic contaminants (Barouki et al., 2007). AHR functions together with its dimerization partner the aryl hydrocarbon receptor nuclear translocator (ARNT) (Beischlag et al., 2008; Fujii-Kuriyama and Kawajiri, 2010; Swedenborg and Pongratz,

* Corresponding author at: Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, 25761 Büsum, Germany.
E-mail address: kristina.lehnert@tiho-hannover.de (K. Lehnert).

http://dx.doi.org/10.1016/j.marpolbul.2014.08.025
0025-326X/

2010; Chopra and Schrenk, 2011). A third marker of the xenobiotic metabolism used is the peroxisome proliferator-activated receptor (PPARα), which functions as a transcription factor, regulates mRNA expression and induction of detoxifying enzymes (van Raalte et al., 2004). Further this study applies the cytokine IL-2 as it correlates with inflammatory disease in marine mammals (Fonfara et al., 2008; Boyman and Sprent, 2012). Heat-shock-protein HSP70 was analysed as it is essential in measuring immune reactions and stress (De Maio, 1995; Chen and Cao, 2010).

The aim of this study was to measure mediators of the pollutant- and stress related immune response in grey seals. By using blood samples a minimally invasive sampling method is developed to determine baseline levels of various markers (Kakuschke et al., 2005) and it was the first time that ARNT, AHR & PPARα were established in grey seals. Transcription levels of biomarkers from a recent study on harbour seal pups in rehabilitation (Weirup et al., 2013) were compared to the results to account for species-specific differences. Baseline data of indicators for pollutant exposure and environmental stressors in the grey seal metabolism are important for the establishment of biomarkers to evaluate the impact of anthropogenic activities on vulnerable seal species.

2. Materials and methods

2.1. Blood sampling/blood status

North Sea, Germany: Abandoned grey seal pups ($n = 8$), found on the coast of Schleswig Holstein in winter 2010/2011 and 2011/2012, fostered at the Seal Centre Friedrichskoog, Germany, were investigated. The pups, determined by their weight, size, navel and canine development, were between two and eight days old, when admitted to the seal centre and when blood samples were taken for the first time. This study compares blood samples from the admission and from the final examination before the seal's release back into the North Sea at an age of about three months. Seals were fed a mixture of a compensatory milk product (Immulak, by Vet Concept) and minced North Sea herring, where the share in herring was gradually increased until pups solely fed on whole fish. Antibiotics, anti-inflammatory drugs, anti-emetics and antacids were given depending on individual fitness.

Baltic Sea, Poland: Blood samples were obtained from grey seal pups ($n = 8$) which were captive born (3 individuals) or wild rehabilitated (5 individuals) at Hel Marine Station, Poland, in spring 2010. The age of wild pups was estimated to be about ~5 days (2 individuals), 9 days, 12 days and >3 weeks judging by presence and absence of lanugo. Blood samples were taken at final examination in June 2010 before their release into the Baltic Sea, at an age of about three months. The feeding schedule consisted solely of Baltic Sea herring, first minced, later of whole fish. In addition, final examination samples of two captive born grey seal pups from June 2012 and samples of five adult grey seals (age 5–24 years) which are in permanent human care in the station from May and October 2011 were analysed. Antibiotics and anthelmintics were given depending on individual fitness.

2.2. Blood status

As described by Dierauf and Gulland (2001) the blood was taken from the epidural intravertebral vein and collected in ethylenediaminetetraacetic acid (EDTA) tubes. Samples taken for haemogram profiles at the Seal Centre Friedrichskoog, Germany were measured with ScilVet ABC™ Animal Blood Counter (Scil Animal Care Company GmbH, D68519 Viernheim, Germany). Polish haemogram

Table 1
Differential haematology profile of red blood cells (RBC), their derivatives haemoglobin (HGB), haematocrit (HCT) and white blood cells (WBC) and their derivatives lymphocytes (LYM), monocytes (MO) and granulocytes (GRA); from grey seals of the Hel Marine Station, Poland (P1_pup – P15_adult) and the Seal Centre Friedrichskoog, Germany (G1_pup – G8_pup) at admission (Admis (1)) and final examination (Final ex (2)).

Animal-ID	Admis. (1)/Final ex. (2)	Sampling date	RBC (10^6/mm^3)	HGB (10^6/mm^3)	HCT (10^6/mm^3)	WBC (10^3/mm^3)	LYM (10^3/mm^3)	MO (10^3/mm^3)	GRA (10^3/mm^3)
P1_pup	2	13.06.2010	5.02	18.6	50.8	11.3	5.5	–	–
P2_pup	2	13.06.2010	4.7	20.0	54.1	7.9	–	–	–
P3_pup	2	13.06.2010	4.5	17.9	48.1	8.4	–	–	–
P4_pup	2	13.06.2010	5.6	22.0	57.4	12.2	4.7	–	–
P5_pup	2	13.06.2010	4.1	16.8	45.8	7.3	–	–	–
P6_pup	2	13.06.2010	5.0	19.8	53.0	11.5	–	–	–
P7_pup	2	13.06.2010	5.1	20.2	53.3	11.7	–	–	–
P8_pup	2	13.06.2010	5.0	20.2	52.5	13.8	13.1	–	–
P9_pup	2	03.06.2012	–	–	–	–	–	–	–
P10_pup	2	03.06.2012	4.3	15.0	45.1	5.1	–	–	–
P11_adult	1	15.05.2011	3.9	17.3	46.4	9.0	–	–	–
P12_adult	1	15.05.2011	3.8	17.5	46.5	9.8	–	–	–
P13_adult	1	15.05.2011	3.9	17.8	47.3	6.7	–	–	–
P13_adult	2	15.10.2011	3.9	17.0	47.0	6.4	–	–	–
P14_adult	1	15.05.2011	3.2	15.3	41.1	7.1	–	–	–
P14_adult	2	15.10.2011	4.2	19.0	52.0	7.3	–	–	–
P15_adult	1	15.05.2011	3.5	15.5	42.4	6.9	–	–	–
P15_adult	2	15.10.2011	4.0	18.5	47.6	6.9	–	–	–
G1_pup	1	30.10.2010	4.5	17.9	49.2	9.3	2.2	0.2	6.9
G1_pup	2	07.03.2011	4.2	18.0	48.3	12.6	1.5	0.4	10.7
G2_pup	1	30.10.2010	4.6	17.0	46.6	10.7	2.3	0.3	7.2
G2_pup	2	07.03.2011	4.6	18.0	49.7	9.8	1.7	0.5	7.6
G3_pup	1	18.12.2010	3.7	14.8	39.0	11.5	1.1	0.3	10.1
G3_pup	2	07.03.2011	4.5	16.9	47.3	12.1	2.0	0.6	9.5
G4_pup	1	11.12.2011	4.2	17.2	46.4	10.4	2.5	0.3	7.6
G4_pup	2	14.03.2012	3.7	15.6	41.9	7.0	0.5	0.1	6.4
G5_pup	2	08.03.2012	4.6	16.9	47.6	8.2	1.6	0.3	6.3
G6_pup	2	03.04.2012	4.0	17.3	44.8	9.3	2.6	0.1	6.6
G7_pup	1	16.12.2011	3.9	16.6	44.2	11.5	3.1	0.4	8.0
G7_pup	2	08.02.2012	3.9	15.8	43.2	10.6	1.4	0.4	8.8
G8_pup	2	08.03.2012	4.2	17.2	47.6	12.1	1.4	0.4	10.3