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Investigations of Thyroid and Stress Hormones in Free-Ranging and Captive Harbor Porpoises (*Phocoena phocoena*): A Pilot Study

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Abstract

Increasing anthropogenic activities in the marine environment are potential threats to the health of harbor porpoises by causing unnatural continuous stress to the animals. The present study was a first assessment to investigate stress and thyroid hormones of free-ranging and captive harbor porpoises. In addition, hormone levels were measured to compare those with concentrations of polychlorinated biphenyl (PCB) congeners, p,p'-dichlorodiphenyltrichloroethane (DDT), and p,p'-dichlorodiphenyldichloroethylene (DDE) in the blood of a limited number of animals (10 free-ranging and 7 captive individuals). Samples of seven captive harbor porpoises were taken at the Dolphinarium Harderwijk (the Netherlands) and the Fjord & Bælt Centre in Kerteminde (Denmark). Blood from 29 free-ranging harbor porpoises was collected within the frame of tagging projects on animals accidentally caught in pound nets in Denmark. Median levels of the catecholamines adrenaline, noradrenaline, and dopamine were higher in free-ranging harbor porpoises compared to the captive animals. The median value of adrenocorticotropic hormone (ACTH) for captive animals from the Fjord & Bælt Centre was almost three-fold lower than in the free-ranging harbor porpoises. The median values of T4 were similar in both captive and free-ranging harbor porpoises (9.3 to 10.8. µg/dL). T3 levels of the investigated harbor porpoises varied widely between individuals. No significant correlation between any investigated hormones and investigated organochlorine compounds was observed. The study provides first results on stress hormones other than cortisol. Further investigations are needed to improve the understanding of stress hormones and the influence of anthropogenic stressors on the health of harbor porpoises.

Key Words: harbor porpoise, *Phocoena phocoena*, catecholamines, Baltic Sea, North Sea, pollutants, adrenocorticotropic hormone, thyroid hormones

Introduction

The harbor porpoise (*Phocoena phocoena*) is a small cetacean species inhabiting coastal waters in the temperate and subarctic regions of the northern hemisphere. The populations of harbor porpoises in the North and Baltic Seas are under increasing pressure from different anthropogenic activities such as offshore construction, ship traffic, fisheries, and the military. To secure the survival of small cetaceans in these regions, the Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish, and North Seas (ASCOBANS) was created and signed by several European countries in 1992. Under this agreement, signatory countries are obliged to perform research in the field of small cetacean protection.

Several studies on harbor porpoises in the North and Baltic Seas have shown that animals are suffering more often from infectious diseases when compared with individuals from Greenland, Norway, and Iceland (Jepson et al., 2000; Siebert et al., 2001, 2006; Wünschmann et al., 2001; Jauniaux et al., 2002; Siebert & Weiss, 2009). Apart from by-catch (Vinther & Larsen, 2004), the decline in health of harbor porpoise populations is often attributed to environmental pollution as one stressor (e.g., Aguilar & Borrel, 1995; Bruhn et al., 1995; Beineke et al., 2005). Therefore ASCOBANS, the International Council for the Exploration of the Sea, and the International Whaling Commission recommended conducting research programs that would elucidate the effects of pollutants on small cetacean populations.

Several studies suggested relations between pollutant body burden and lesions in the endocrine system of harbor porpoises, belugas (*Delphinapterus leucas*), and gray seals (*Halichoerus grypus*) (De Guise et al., 1994; Lund, 1994; Lair et al., 1997; Das et al., 2006). Organochlorines like polychlorinated biphenyls (PCBs); p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT); and its metabolite, p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) are part of a wide range of environmental contaminants suspected to interfere with endocrine functions in marine mammals (Aguilar & Borrel, 1995; Bruhn et al., 1995; Brouwer et al., 1999; Gregory & Cyr, 2003). Studies performed on laboratory and domestic animals as well as marine mammals showed a correlation between the intake of organochlorines and changes in hormone levels (Byrne et al., 1987; Brouwer et al., 1989; Chiba et al., 2001; Skaare et al., 2001; Haave et al., 2003; Braathen et al., 2004; Oskam et al., 2004; Sørmo et al., 2005; Zimmer et al., 2009). Organochlorines are lipophilic compounds. Marine mammals, which have well-developed fat depots, may accumulate high amounts of those contaminants (Gregory & Cyr, 2003).

Although stress reactions occur naturally throughout an individual's life due to, for example, social competition, breeding behaviour, or environmental demands, anthropogenic factors such as vessel traffic, noise, fishing, and chemical pollution can act as significant stressors and negatively impact the health of cetaceans (St. Aubin & Dierauf, 2001; Eskesen et al., 2009). During stress reactions, neurologic, endocrinologic, and immunologic changes take place, and if the animal is unable to adapt to the stressful event, deleterious effects or death may occur (Moberg, 1987; St. Aubin & Dierauf, 2001).

Knowledge about the influence of chemical stressors on the endocrine status in marine mammals is still scarce (St. Aubin & Dierauf, 2001). Moreover, most previous studies concentrated on estimating cortisol as the sole hormonal indicator of stress. In the present study, adrenocorticotropic hormone (ACTH), catecholamines (adrenaline, noradrenaline, dopamine), and the thyroid hormones triiodothyronine (T3) and thyroxine (T4) were studied. Data on cortisol obtained parallel to this study were already published in Eskesen et al. (2009) for free-ranging harbor porpoises and in Desportes et al. (2007) for harbor porpoises in human care. These results are cited here for statistical analysis in search of relationships with blood organochlorine concentrations. Cortisol is an endogenous glucocorticoid produced in the cortex of adrenal glands under control of the pituitary ACTH. It has a broad spectrum of metabolic effects mainly associated with stress reactions (St. Aubin & Dierauf, 2001; Feldman & Nelson, 2004). Catecholamines are associated with a fast "fight-or-flight" reaction as their levels increase rapidly in response to the stressor (Henry, 1992; St. Aubin & Dierauf, 2001). Thyroid hormones, represented by T3 and T4, are produced in the thyroid gland under control of the pituitary gland. T3 and T4 are important regulators of metabolism, tissue growth, development, and differentiation (Feldman & Nelson, 2004; Zoeller & Rovet, 2004; Miller et al., 2009). Changes in thyroid hormones are part of the stress response (St. Aubin & Dierauf, 2001; Chrousos, 2009). Plasma concentrations of many hormones, like ACTH, cortisol, or T3 and T4, show some periodic (e.g., diurnal) variation (Kraft & Duerr, 1995; Feldman & Nelson, 2004; Castillo et al., 2009).

The aim of this pilot study was to investigate stress hormone and thyroid hormone profiles in the blood of harbor porpoises to enable an assessment of the endocrinium in wild harbor porpoises and its changes in relation to anthropogenic stress. The evaluation of the potential correlation with organochlorine blood burden was investigated as one stressor. Examined animals included both free-ranging and captive harbor porpoises. Repetitive sampling in captive animals provided an overview of individual hormone level variations. To measure organochlorine body burden, blood concentrations of PCBs, p,p'-DDT, and p,p'-DDE were investigated.

Materials and Methods

The Harbor Porpoise Sampling

For the present study, 36 living harbor porpoises were sampled. The majority of animals ($n = 29$) were by-caught in pound nets by Danish fishermen and sampled before tagging and release (Eskesen et al., 2009; permits from the Danish Forest and Nature Agency [SN 343/SN-0008] and Ministry of Justice [1995-101-62]). The broad spectrum of parameters to be analyzed required fairly large sample volumes, but it was not always possible to obtain a sufficient amount of blood. Due to this limitation, not every animal was investigated for the full hormone profile or pollutant burden.

The handling and sampling of the free-ranging harbor porpoises was carried out during the satellite tagging operation on board the fishermen's boats after the harbor porpoises had been lifted out of the pound nets. The remaining seven harbor porpoises were captive animals from the Fjord & Bælt Centre, Kerteminde, Denmark ($n = 3$) (Desportes et al., 2007; permit from the Danish Ministry of Justice [1998-561-110]) and from Dolphinarium Harderwijk, the Netherlands ($n = 4$). The harbor porpoises were kept for rehabilitation (in Harderwijk) or research programs (both facilities). The age classification was performed according to body length and reproductive hormone levels.

Blood Sampling

Blood samples from the harbor porpoises collected between 1997 and 2002 were obtained in two ways. In the first method, the animal was taken out of water, and sampling was performed when restrained. The blood samples were obtained from the vena cava of the fluke for all the free-ranging harbor porpoises and for the two captive individuals using the method described by Eskesen et al. (2009). In the second method, blood sampling was conducted at poolside under voluntary husbandry behaviors for trained animals (Desportes et al., 2007). During the study period, blood was sampled at least once a month for routine medical checks and up to four times per month for shorter periods during specific research projects.

Hormone Status Analysis

Blood was collected in S-Monovetten® KE (Sarstedt), centrifuged immediately after the sampling at 2,500 U/min for 15 min, and serum separated and frozen in 1 ml aliquots. Within the next few days, serum hormone levels were measured by radio-immunoassay following standard techniques in a commercial laboratory in Germany (Gemeinschaftspraxis Dr. Kramer und Kollegen, D-21494 Geesthacht) with X-Counter (Wallace, Lisle, Ireland) (Desportes et al., 2007). The minimum detectable level was 2 µg/l, the intra-assay coefficients of variation were 3 to 5%, and the inter-assay coefficients of variation were 4 to 6%. The nonspecific binding was not evaluated, but experience showed that in the standard deviation, identical results were found regardless of whether nonspecific binding was taken into consideration.

Organochlorine Analysis

The lipid fractions from 15 harbor porpoise full blood samples (cells and serum) were investigated for concentrations of 12 individual polychlorinated biphenyl congeners (PCB 92, 95, 99, 101, 118, 138, 149, 153, 170, 177, 180 & 187), p,p'-dichlorodiphenyltrichloroethane (DDT), and p,p'-dichlorodiphenyldichloroethylene (DDE) using standardized methods (e.g., Bruhn et al., 1999; Siebert et al., 2002). Only organochlorine analyses of animals with both pollutant and hormone measurements were included in the current study. The 15 samples consisted of ten blood samples from free-ranging harbor porpoises, two from captive harbor porpoises from the Fjord & Bælt Centre, and three samples from harbor porpoises kept at the Dolphinarium Harderwijk.

Statistical Analysis

When comparing hormone levels between captive and free-ranging harbor porpoises, only one measurement was available for each free-ranging animal, whereas several repeated measurements were performed for captive animals from Fjord & Bælt. For a comparison of the free-ranging with the captive harbor porpoises, the variance within the hormone levels of the three captive animals was not of interest; therefore, a mixed effect model (Pinheiro & Bates, 2000; Zuur et al., 2009) was performed. The animal ID was used as a random effect, with a dummy variable (0 for captive and 1 for free-ranging harbor porpoises) used as a fixed effect. As the data were not evenly distributed, a mixed effect model using the Poisson distribution included in the package lme4 (Bates & Maechler, 2009) of the open source program *R* (R Development Core Team, 2009) was used.

Results

Hormone Status Analysis

The free-ranging porpoises and individuals from Harderwijk were sampled only once, whereas the animals from Fjord & Bælt were sampled between 10 and 49 times each (Table 1). A summary of the hormone levels is presented in Table 1.

Organochlorine Analysis

The sum of PCBs varied between 1.3 and 57 ng/g TG, p,p'-DDE between 1.3 and 6.7 ng/g TG, and p,p'-DDT between 1.2 and 8.5 ng/g TG (Tables 2 & 3).

Correlations Between Biological Parameters, Hormones, and Contaminants

The blood levels of ACTH, adrenaline, cortisol, dopamine, noradrenaline, T3, and T4 in several harbor porpoises, together with total PCBs, p,p'-DDE, and p,p'-DDT levels found in the same blood samples, are shown in Table 3. The relationship between the investigated organochlorine compounds and age vs all investigated hormones was investigated by use of Spearman's rank correlation coefficient and multiple linear regressions. No significant correlation was observed in any of the tests performed (Tables 4 & 5).

Discussion

Catecholamines

Median levels of the catecholamines adrenaline, noradrenaline, and dopamine were higher in free-ranging harbor porpoises compared to the animals from the Fjord & Bælt Centre. Animals from Harderwijk showed considerably higher levels of catecholamines. Adrenaline, noradrenaline, and dopamine belong to the catecholamines, a group of hormones with related action. They are secreted in situations of emergency ("fight-or-flight" reaction) and thus are known as stress hormones (Doecke, 1994). The lower median levels

Table 1. Summary of the hormone levels measured in free-ranging harbor porpoises from Danish waters and captive harbor porpoises from the Fjord & Bælt Centre, Kerteminde (Freja, Eigil, and Nuka)

Animal(s)		AD	NA	DA	CS	ACTH	T4	T3
Free-ranging porpoises	Mean (SD)	96.6	726.4	114.3	166.8	142.1	13.7	120.0
	Median	65	384	107	153	71.1	10	115
	Max	380	4,709	290	338.6	926.4	75	190
	Min	17	80	34	27.1	5	7.4	68.3
	N	16	16	15	29	15	18	16
Freja	Mean (SD)	100.9	268.4	69.7	65.7	54.4	92.6	171
	Median	64	134	57.5	64.5	26.3	10.8	161
	Max	705	846	218	216	217	908	368
	Min	10	9.7	10	7.5	5	5.9	13.9
	N	15	15	12	44	11	25	22
Eigil	Mean (SD)	59.4	573.5	104.6	80.8	44.2	11.1	174
	Median	57.5	356	64.5	52.8	24.7	9.9	158
	Max	161	2,736	358	793	149	21.1	639
	Min	10	83	30	3	5	6.7	87.3
	N	18	18	14	49	13	26	24
Nuka	Mean (SD)	--	--	--	88.3	--	9.9	--
	Median	--	--	--	92.7	--	9.3	--
	Max	--	--	--	166	--	13.7	--
	Min	--	--	--	22.5	--	7.8	--
	N	--	--	--	10	--	7	--

AD – adrenaline ng/L
NA – noradrenaline ng/L
DA – dopamine ng/L
CS – cortisol µg/L
ACTH pg/L
T4 – thyroxine µg/dL
T3 – triiodthyronine ng/dL
N – number of samples investigated

Table 2. Median, minimum, and maximum levels of ΣPCB, p,p'-DDE, and p,p'-DDT measured in blood samples obtained from wild harbor porpoises and from individuals kept in captivity (Fjord & Bælt Centre, Kerteminde [FBC] and Dolphinarium Harderwijk)

			ΣPCB			p,p'-DDE			p,p'-DDT		
		N	Median	Min	Max	Median	Min	Max	Median	Min	Max
FBC: Freja	ng/g TG	2	4.1E+04	4.0E+04	4.1E+04	6.7E+03	6.7E+03	6.7E+03	1.5E+03	< LOD	1.5E+03
FBC: Eigil	ng/g TG	8	1.3E+04	2.9E+03	6.3E+04	2.4E+03	1.0E+03	8.5E+03	1.7E+03	< LOD	2.4E+03
FBC: Nuka	ng/g TG	1	1.5E+04			1.2E+03			< LOD		
Median FBC	ng/g TG	3	1.5E+04	1.3E+04	4.1E+04	2.4E+03	1.2E+03	6.7E+03	1.6E+03	< LOD	1.7E+03
Wild harbor porpoises from Baltic Sea	ng/g TG	8	1.7E+04	5.2E+03	4.7E+04	2.9E+03	7.6E+02	4.8E+03	4.3E+02	< LOD	6.4E+02
Harbor porpoises from Harderwijk	ng/g TG	4	2.8E+03	2.1E+03	4,8E+03	5.8E+03	1.5E+02	6.9E+02	1.1E+02	7.4E+01	1.8E+02

Table 3. Summary of the results of organochlorine analyses and hormone measurements of the same blood sample in free-ranging and captive harbor porpoises

Animal[1]	Age[2]	Sex[3]	ΣPCB ng/g TG[4]	p,p'-DDE ng/g TG	p,p'-DDT ng/g TG	ACTH	AD	CS	DA	NA	T4	T3
Free ranging												
P.p.1	I	2	5.7E+04	2.2E+03	1.2E+02	203.6	75	86.4	140	806	68.3	75
P.p.2	N	2	4.4E+04	4.6 E+03	1.9E+02	--	--	338.6	--	--	118.7	9.9
P.p.3	N	2	1.4E+04	2.3E+03	1.9E+02	--	--	214.6	--	--	110.4	8.5
P.p.4	N	2	1.3E+04	3.0E+03	4.3E+02	12.7	141	130	198	714	198.8	--
P.p.5	N	2	1.9E+04	4.3E+03	6.4E+02	--	--	153	--	--	184.2	11.9
P.p.6	N	1	4.9E+03	7.1E+02	5.5E+01	124	43	153.7	151	422	141.7	11.8
P.p.7	I	2	1.7E+04	2.1E+03	3.6E+02	105	40	197	55	111	87.8	8.1
P.p.8	N	1	1.5E+04	2.5E+03	1.7E+02	926.4	27	82.5	59	283	113.2	7.5
P.p.9	N	2	4.7E+04	2.6E+03	4.3E+02	238.7	244	233.5	109	346	126.2	16.5
P.p.10	N	1	2.7E+04	4.2E+03	5.2E+02	14.2	17	78.7	34	115	94.8	7.5
Captive												
Eigil 21 June 1999	I	2	2.9E+03	1.3E+03	8.5E+01	29	36	--	46	1210	162	18.8
Freja 21 June 1999	I	1	4.0E+04	6.7E+03	1.5E+03	11.3	65	--	63	525	189.38	10.8
H1	N	2	4.7E+03	4.9E+02	1.8E+02	4	320	122	263	590	145.85	7.8
H2	N	1	2.7E+04	3.5E+03	1.6E+03	8.1	1,692	--	1,700	15,908	--	--
H3	A	1	2.2E+03	1.5E+02	7.4E+01	14	956	--	138	7,322	--	--

[1] P.p. – wild harbor porpoises caught in Danish waters, H – captive porpoises from Dolphinarium Harderwijk
[2] N – neonate, I – immature, A – adult
[3] 1 – female, 2 – male
[4] TG – triglyceride
NA – noradrenaline ng/L
DA – dopamine ng/L
CS – cortisol µg/L
ACTH pg/L
T4 – thyroxine µg/dL
T3 – triiodthyronine ng/dL
N – number of samples investigated

of catecholamines in the captive harbor porpoises from the Fjord & Bælt Centre might be explained by the fact that the animals became accustomed to the repeated procedure of sampling in captivity. For free-ranging porpoises, capture is a stressful situation. While animals in the Fjord & Bælt Centre were already adapted to captivity over a long period, blood from individuals in Harderwijk was taken during the rehabilitation process, which can be considered a new situation with increased stress for these animals. On the other hand, Romano et al. (2004) observed a significant increase in blood adrenaline, noradrenaline, and dopamine concentrations in captive belugas and bottlenose dolphins (*Tursiops truncatus*) exposed to a loud sound. St. Aubin & Dierauf (2001) measured adrenaline and noradrenaline blood levels in free-ranging belugas from Somerset Island chased for 5 to 15 min, captured, and later held in captivity for 5 d. Both hormone levels were very high at the time of capture. But whereas adrenaline decreased in the following days, noradrenaline remained very high. To explain this observation, the authors pointed out that high noradrenaline levels are more closely associated with muscular activity and discharge from the sympathetic nervous system than states of alarm and anxiety. The values of the catecholamines measured in this study are in the range of those found in free-ranging marine mammals (Hardee et al., 1982; Doecke, 1994) and comparable to those in captive belugas (Thomas et al., 1990).

Previous investigations showed that cortisol levels in samples taken on the same porpoises in the Fjord & Bælt Centre under voluntary conditions were lower when compared to levels taken under nonvoluntary sampling (Desportes et al., 2007). Similar differences were also described by other authors (St. Aubin & Dierauf, 2001; Romano et al., 2004). For the catecholamines investigated in this study, more samples obtained under voluntary conditions need to be analyzed before

Table 4. Spearman's rank correlation coefficients between PCBs, p,p'-DDE, p,p'-DDT, age, and hormones: adrenaline, ACTH, cortisol, dopamine, noradrenaline, T3, and T4

Correlated data		Regression coefficient	Value of significance	N
PCB	ACTH	0.51	0.06	14
	Adrenaline	-0.41	0.15	14
	Cortisol	0	1.00	15
	Dopamine	-0.33	0.25	14
	Noradrenaline	-0.46	0.12	13
	T3	-0.4	0.10	18
	T4	0.11	0.68	17
DDE	ACTH	0.27	0.35	14
	Adrenaline	-0.45	0.11	14
	Cortisol	0	1.00	15
	Dopamine	-0.34	0.23	14
	Noradrenaline	-0.37	0.21	13
	T3	-0.2	0.43	18
	T4	-0.13	0.61	17
DDT	ACTH	-0.36	0.21	14
	Adrenaline	0.13	0.66	14
	Cortisol	0.01	0.96	15
	Dopamine	0.11	0.70	14
	Noradrenaline	-0.05	0.86	13
	T3	0.21	0.41	18
	T4	-0.08	0.75	17
Age	ACTH	0.18	0.54	14
	Adrenaline	-0.17	0.55	14
	Cortisol	-0.34	0.21	15
	Dopamine	-0.43	0.13	14
	Noradrenaline	0.14	0.65	13
	T3	-0.04	0.89	18
	T4	0.26	0.31	17

statistical analyses for similar differences can be conducted. Generally, protocols to avoid stress from sampling should be explored to improve the reliability and comparison of the data sets.

Adrenocorticotropic Hormone

The measured levels of ACTH varied significantly. The median value for captive animals was almost three-fold lower than in the free-ranging harbor porpoises. Both values lie in the range of those found in dogs, cats, or humans (Doecke, 1994; Feldman & Nelson, 2004; Castillo et al., 2009). ACTH is a pituitary hormone responsible for the stimulation of cortisol secretion in the adrenal cortex. Under stress, ACTH is released in a pulsatile manner (Mimoto et al., 2001). It can be surmised that the capture in nets and resulting longer handling time were responsible for higher ACTH levels in by-caught harbor porpoises. Studies on a response to exogenous ACTH were performed on captive bottlenose dolphins, indicating that animals having increased levels of cortisol due to the handling procedure did not show further increases after ACTH stimulation (Thomson & Geraci, 1986). However, two of the bottlenose dolphins died in the following days. The authors suggest that cortisol reservoirs were depleted due to pre-existing stress; thus, a further increase in ACTH was not effective.

Thyroid Hormones (T4 and T3)

Although some levels of T4 that were measured in the investigated harbor porpoises varied significantly, the median values were not clearly different between free-ranging and captive porpoises (9.3 to 10.8 µg/dL). Koopman et al. (1995) found comparable T4 values of 11.2 µg/dL blood samples from 31 free-ranging harbor porpoises that were incidentally captured in herring weirs in the Bay of Fundy. Our T4 values were not significantly different from those found in the Bay of Fundy but were lower than in free-ranging belugas from Hudson Bay (19.1 µg/dL) reported by Kirby (1990).

T3 levels of the investigated harbor porpoises varied widely between individuals. Comparable high levels were found in other marine mammals. The T3 values described for the Somerset belugas by Kirby (1990) ranged between 126 and

Table 5. Multiple linear regression analysis between PCBs, p,p'-DDE, p,p'-DDT, age, and hormones: adrenaline, ACTH, cortisol, dopamine, noradrenaline, T3, and T4

	Log ACTH			Log cortisol			Log adrenaline		
	Significance	Regression coefficient	Standard error	Significance	Regression coefficient	Standard error	Significance	Regression coefficient	Standard error
lgPCB	0.22	1.01	0.77	0.52	-0.08	0.12	0.94	0.08	0.98
lgDDE	0.49	-0.56	0.78	0.45	0.12	0.16	0.47	-0.75	1.00
lgDDT	0.12	-0.84	0.49	0.64	-0.15	0.32	0.33	0.64	0.62
Age	0.58	-0.19	0.33	< 0.01	-0.61	0.16	0.86	-0.08	0.43
Sex	0.79	-0.11	0.40	0.14	0.36	0.23	0.62	0.27	0.52
Significance of all factors	0.34	--	--	0.04	--	--	0.36	--	--
	Log dopamine			**Log noradrenaline**			**Log T4**		
	Significance	Regression coefficient	Standard error	Significance	Regression coefficient	Standard error	Significance	Regression coefficient	Standard error
lgPCB	0.75	0.19	0.57	> 0.99	-0.01	0.73	0.03	0.19	0.08
lgDDE	0.48	-0.44	0.58	0.45	-0.59	0.75	0.09	-0.20	0.10
lgDDT	0.46	0.28	0.36	0.29	0.51	0.45	0.18	-0.22	0.16
Age	0.36	-0.24	0.25	0.48	0.23	0.31	0.10	0.22	0.12
Sex	0.88	0.05	0.30	0.55	-0.24	0.38	0.59	0.07	0.12
Significance of all factors	0.58	--	--	0.28	--	--	0.12	--	--
	Log T3								
	Significance	Regression coefficient	Standard error						
lgPCB	0.95	0.00	0.07						
lgDDE	0.44	-0.08	0.09						
lgDDT	0.46	0.11	0.14						
Age	0.87	-0.02	0.11						
Sex	0.43	-0.09	0.11						
Significance of all factors	0.80	--	--						

214 ng/dL. In different seal species (harbor seals [*Phoca vitulina*], harp seals [*Ph. groenlandica*], gray seals), the same author described values in quite a wide range between 10 and 330 ng/dL. Thyroid hormones are not considered to be direct stress hormones, and hence, do not reflect an acute exposure to stress. Harbor porpoises investigated in the present study showed high levels of T3. The reason for this remains unclear. This study is the first to describe a relatively wide range of hormone levels in harbor porpoises. In other species (e.g., livestock), blood levels of ACTH, cortisol, T3, and T4 show characteristic diurnal variation (Kraft & Duerr, 1995; Feldman & Nelson, 2004; Castillo et al., 2009). To measure this variation in domesticated and terrestrial animals, regular and frequent blood sampling is performed throughout the entire day. No effective techniques of such repeated sampling through constant venous access have been developed for marine mammals. This limits the interpretation of hormone levels from a single blood sample. An alternative method to investigate hormone profiles used in humans and domesticated mammals is to monitor hormone levels in urine, feces, or saliva (Aardale & Holm, 1995; Schatz & Palme, 2001).

Pollutant Analysis

Estimated values of the organochlorines PCBs, p,p'-DDT, and p,p'-DDE in the blood of investigated harbor porpoises are presented in Tables 2 & 3. Where possible, the results were expressed in nanograms per gram of blood triglycerides. This unit is considered to be a good marker for the blubber organochlorine burden (Reddy et al., 1998). Triglycerides are said to be the main storage compartment of the investigated organochlorines. Whereas their level in blood might change and be different in different animals, in blubber, triglycerides are more stable and make up about 90% of its mass. As the investigated organochlorines are characterized by a long half-life, their concentration in body triglycerides is considered to be equal. This enables predicting the pollutants' levels in the blubber on the basis of their amounts per unit of blood triglycerides (Reddy et al., 1998).

The blood levels of PCBs, p,p'-DDT, and p,p'-DDE were estimated several times in the blood of harbor porpoises kept at the Fjord & Bælt Centre in Kerteminde. The pollutants' levels in two blood samples taken from one harbor porpoise at monthly intervals were similar. On the other hand, levels of the same pollutants measured in the same animals at other times and in the blood of a different captive animal from the same husbandry facility varied significantly. According to Reddy et al. (1998), postprandial concentrations of organochlorine pollutants may simulate the concentrations under conditions of disease-related fat mobilization caused by physiological or health-related changes. Another variable is food intake as individuals are not always eating the same fish or the same amount of fish throughout the year.

No significant difference was observed for the median levels of ΣPCBs between captive harbor porpoises from the Fjord & Bælt Centre and the by-caught individuals from the Baltic Sea (15,000 ng/g TG and 17,000 ng/g TG, respectively). This may be explained by the fact that animals from Fjord & Bælt were also fed local fish. A similar situation was observed for p,p'-DDE, whereas levels of p,p'-DDT were higher in the captive animals. The concentration of ΣPCBs in harbor porpoises from Harderwijk was approximately six times lower when compared to the Danish animals. Differences in feeding might provide an explanation for this phenomenon. While the harbor porpoises in Harderwijk are fed only fish from open Atlantic waters, the diet of animals from Kerteminde includes local fish.

Comprehensive studies on the hormone status of harbor porpoises with regard to environmental contaminants' body burden have not yet been performed. However, the thyroid gland of dead harbor porpoises from Norwegian waters and the North and Baltic Seas showed more interstitial fibrosis and fewer but larger follicles compared to Icelandic animals, possibly indicating a dysfunction of hormone excretion (Das et al., 2007). The present study investigated stress and thyroid hormone profiles in comparison with organochlorine body burden for the first time. Both hormones and pollutants were measured in the blood. No blubber samples were taken for ethical and health reasons.

No significant correlation was found between PCBs, p,p'-DDT, and p,p'-DDE, on the one hand, and the hormones cortisol, ACTH, adrenaline, noradrenaline, dopamine, T3, and T4, on the other. When adding age and sex as covariables to the comparison of organochlorine pollutants and hormone levels by multiple linear regressions, only age had a significant influence on cortisol/pollutant levels. The absence of other significant correlations in the present study may be a result of the small sample size and varying conditions in different facilities (e.g., lower pollutant levels in fish fed in Harderwijk and higher stress level during rehabilitation).

Conclusions and Recommendations

Increasing anthropogenic activities in the marine environment are potential threats to the health of marine mammals. Unnatural stress for harbor porpoises leading to decreased health status and development of chronic diseases can be expected. Therefore, an assessment of stress levels and an understanding of the influence of stress on the health status of harbor porpoises are urgently needed. The present study was a first attempt, demonstrating that in addition to cortisol, other stress hormones should be examined in order to understand stress situations. It was possible to show that similar investigations are valuable and need to be extended to larger sample sizes. Therefore, further investigations are urgently required.

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