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Detection of Carcinogenic Polycyclic Aromatic Hydrocarbons in Stranded Caspian Seals (*Pusa caspica*)

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Abstract

Polycyclic aromatic hydrocarbons (PAHs), which contain many carcinogenic compounds, are a major ingredient of petroleum/oil. PAH pollution of the Caspian Sea, the world's largest lake, is rapidly occurring and may be affecting the endangered Caspian seal (*Pusa caspica*), the only marine mammal in this lake. To analyze the entrance of PAHs into the Caspian Sea food chain and the health status of Caspian seals, we measured 16 carcinogenic PAHs in the liver, kidney, and blubber tissues of ten Caspian seal carcasses from the coastal region of northeastern Iran using gas chromatography-mass spectrophotometry. Of the 16 PAHs investigated, only anthracene, phenanthrene, and naphthalene were identified in nine sampled Caspian seals. Concentrations of anthracene (=84.83 ± 79.86 ppb wet weight [w.w.]), phenanthrene (=31.75 ± 52.22 ppb w.w.), and naphthalene (=25.1 ± 31.57 ppb w.w.) in blubber tissues were higher than in liver and kidney tissues. The concentration of PAHs in tissues was significantly higher in male than in female seals, and we found an inverse relationship between seal age and PAH concentration in tissues. Although no data exist concerning toxic effect concentrations of PAHs in Caspian seals, PAH detection in seal carcasses highlights a potentially stressful condition that may impact the health of Caspian seals and other sea life in this lake. Appropriate strategies for the control of PAH entrance into the Caspian Sea should be sought, and studies for the determination of pathogenic and lethal doses of PAHs in Caspian seals should be pursued.

Key Words: PAHs, Iran, Caspian seals, *Pusa caspica*

Introduction

The Caspian Sea, the largest closed lake in the world, is surrounded by five countries: Russia, Ghazakhstan, Azerbaijan, Turkmenistan, and Iran (Shinsuke et al., 2003). This multinational access, in addition to the high density of passenger and commercial vessels and the influx of contaminated water from the numerous rivers that flow through industrial and agricultural areas, has resulted in the Caspian Sea becoming increasingly polluted (Kaplin & Selivanov, 1995; Effimoff, 2000). Extraction of oil from the lake, especially from the central and northern parts, and the entry of petroleum-based fuels in contaminated water have also contributed to the pollution of the lake (Tolosa, 2004; Mille, 2007).

Polycyclic aromatic hydrocarbons (PAHs) with benzene rings are a highly toxic component of oil. Sixteen compounds of PAHs from oil have been identified as highly toxic and mutagenic for animals (Freeman et al., 1990). Generally, PAHs enter the environment in two ways: (1) human activities (pyrolytic origin) such as extraction, refining, transfer and export of crude oil, anthropogenic oil spills, and through other products; and (2) natural entrance (petrogenic origin) of biological resources through PAH synthesis by plankton, bacteria, algae, and the decomposition of plants (Nasrollahzadeh Saravi et al., 2012). Fortunately, PAHs break down quickly when exposed to light and oxygen (e.g., photo-oxidation), and some PAHs are biodegraded by naturally occurring bacteria (Kanaly & Harayama, 2000; Mrozik et al., 2003). PAHs are divided into low and high molecular weight based on number of benzene rings. Oil spills represent one of the major threats for marine mammals, both in the short and long term. However, high molecular weight (HMW) PAHs have a higher tendency

to accumulate in animals' tissues than low molecular weight (LMW) PAHs. In an aquatic ecosystem, HMW PAHs precipitate faster than LMW PAHs and have a lower chance to accumulate in the bodies of marine animals (Lawal, 2017). Limited studies have shown PAH pollution in Caspian Sea coastal areas of Baku (due to oil extraction), northern Russia and Kazakhstan, and southern Iran (Kardovani, 1995; Tolosa, 2004).

The Caspian seal (*Pusa caspica*), the only species of marine mammal in the Caspian Sea, is at the top of the food chain in this ecosystem. This species has a migratory lifestyle and, due to various factors such as death in fishing nets and reduced reproductive rates in male and female seals because of exposure to pollutants, the health status of this species is poor (Watanabe et al., 2002; Härkönen et al., 2008). Mass mortalities of these seals along the Caspian Sea coast between 1997 and 2000, along with the factors cited above, have resulted in the placement of the Caspian seal on the International Union for Conservation of Nature's (IUCN) *Red List* (Goodman & Dmitrieva, 2016). Considering the increase in Caspian Sea oil pollution in recent years and the migratory lifestyle of Caspian seals, we monitored PAH pollution in the Caspian Sea and their health status by measuring and assessing concentrations of 16 mutagenic PAHs in stranded Caspian seal tissues.

Methods

Sampling

After approval of the study by the Ethics Committee of the Deputy of Natural Environment of Golestan Province (Permit Number 125/7894), ten Golestan and Mazandaran provinces, Iran, were collected during the 2012 to 2015 study period. Stranded Caspian seals were trapped in fishing nets and were found fresh by fisheries. Following biometric analysis and clinical examination, the seals were transferred to the laboratory and necropsied. Liver, kidney, and blubber tissues were sampled to measure PAH concentrations, and a lower canine tooth was extracted to determine seal age (Amano et al., 2000).

Measurement of PAH Concentration

This analysis was performed at the Toxicology Lab, Faculty of Veterinary Medicine, University of Extremadura, Caceres, Spain. To measure tissue concentrations of PAHs, the standard method of Lucas & Zhao (2015) using gas chromatography-mass spectrometry (GC-MS/MS) was applied. The samples were shredded with glass slurry, freeze dried for 72 h, and homogenized using a mortar and pestle. A portion of each freeze-dried sample was weighed into 50-mL centrifuge tubes, and MilliQ water was added to obtain the equivalent to 5 g of fresh sample. Ten milliliters of acetonitrile were added to the tube, and the sample was shaken vigorously by hand for 2 min and centrifuged for 5 min at 5,000 rpm. The supernatant was transferred to a 15-ml centrifuge tube containing 1 g of Agilent Enhanced Matrix Removal-Lipid sorbent (Agilent Technologies, Santa Clara, CA, USA) and placed on a vortex mixer for 60 s. The solution was then centrifuged at 5,000 rpm for 3 min. The supernatant was transferred to a second 15-ml tube containing 2 g of salt (1:4 NaCl: MgSO 4) and was immediately vortexed and centrifuged for 3 min. The top layer containing acetonitrile was evaporated to dryness in a rotatory concentrator, reconstituted in ethyl acetate:n-hexane (1:4), and filtered (0.45 micron) into a 2-ml GC vial.

Obtained extracts (1 µl) were injected into a gas chromatograph-mass spectrometer (SCION GC-MS-Triple Quad MS/MS; Bruker Scientific Instruments, Billerica, MA, USA), and PAHs were separated on a 30-m Agilent J&W DB-5ms (5% diphenyl/dimethylpolysiloxane) capillary column (250 µm id. × 0.25 µm film thickness). For quality control and quantitative purposes, one sample from each matrix (liver, kidney, and blubber) was pre-spiked at 40 ng/g with a certificate standard (DE-PROM 16 EPA Priority PAHs in Toluene from LGC Standards, 100 µg/ml) containing the 16 PAHs considered to be of primary concern by the U.S. Environmental Protection Agency (EPA). Qualification and quantification ions were monitored for each compound at m/z = 102 + 126 + 127 for naphthalene; 126 + 150 + 151 for acenaphthylene; 127 + 151 + 152 for acenaphthene; 139 + 163 + 164 for fluorene; 152 + 176 + 177 for phenanthrene and anthracene; 200 for fluoranthene; 151 + 200 for pyrene; 202 + 226 + 227 for benzo[a] anthracene and chrysene; 250 for benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene; 274 for benzo[g,h,i]-perylene and indeno[1,2,3-cd] pyrene; and 276 + 277 for dibenzo[a,h]anthracene.

Results of each spiked matrix were used for quantitation of matrix-related samples. These matrix-matched samples were chosen to compensate for any signal suppression/enhancement compared to their relative response in pure solvent. Procedural blanks containing reagents only were screened during the analysis of each batch (one per tissue) to ensure that solvents and all the used material were free from PAH residues. To evaluate the linearity of the method, calibration curves were built with concentrations ranging from 4 to 400 µg/L with $r^2 > 0.98$. The limit of quantitation (LOQ) was determined as the lowest concentration of the compounds that can be reliably detected with the signal-to-noise (S/N) ratio higher than 10. In our study, the

LOQ for individual PAHs in samples ranged from 0.5 to 5 ng/g on a wet weight [w.w.] basis.

Data Analysis

Normality and lack of data were evaluated using a Kolmogorov-Smirnov test. The result of this test was higher than 0.05, indicating that the data were normal. The effect of sex on the PAH concentration in sampled tissues was investigated using Student's *t* tests. A linear regression test was used to survey the impact of age on PAH concentration. Also, an ANOVA one-way test was used to survey differences of PAH concentrations in sampled tissues.

Results

Of the 16 PAHs studied, three (i.e., anthracene [Log K_{OW} = 4.54, octanol-air partition coefficient (K_{AO}) = 7.55, number of benzene rings (NB) = 3], phenanthrene [Log K_{OW} = 4.57, K_{AO} = 7.57, NB = 3], and naphthalene [Log K_{OW} = 3.37, K_{AO} = 5.19, NB = 2]) were identified in the tissues of sampled Caspian seals (Table 1; National Center for Biotechnology Information, 2019). We detected PAHs in nine of the 10 sampled seals at different concentrations, and the accumulation pattern of PAHs differed among tissue types. Among blubber tissues, 70% were contaminated with anthracene, 50% with naphthalene, and 40% with phenanthrene. For liver tissues, 80% of samples were also contaminated with anthracene, 80% contained phenanthrene, and 50% contained naphthalene. For kidney tissues, 90% were contaminated with anthracene and 40% with naphthalene. Phenanthrene contamination was not detected in kidney tissues (Table 1). The concentrations of anthracene (=84.83 ± 79.86 ppb w.w.), phenanthrene (=31.75 ± 52.22 ppb w.w.), and naphthalene (=25.1 ± 31.57 ppb w.w.) in blubber tissues were significantly (p = 0.001) higher than in the kidney and liver (Table 2). The concentration of anthracene in all sampled tissues was significantly (p = 0.001) higher than the concentrations of phenanthrene and naphthalene (Table 2). Concentrations of PAHs in liver, kidney, and blubber were significantly higher in male than in female seals (Table 3; Figure 1). A linear regression test indicated a negative relationship between Caspian seal age (using body length as a surrogate for age) and the concentration of PAHs in the tissues (Figure 2).

Discussion

Detection of PAHs in sampled Caspian seals indicated that PAHs entered the Caspian Sea food chain. Also, previous studies have indicated pollution with just anthracene, phenanthrene, Dibenz

Table 1. PAH concentrations (ppb w.w.) in the tissues of Caspian seals (*Pusa caspica*) based on age and sex; A = anthracene, PH = phenanthrene, N = naphthalene, L = liver, K = kidney, B = blubber, F = female, and M = male.

Sample number	1	2	3	4	5	6	7	8	9	10	
Sex	F	F	M	F	F	M	M	M	M	M	
Age	26	17	16	11	19	9	10	20	6	26	Average
A(BT)	0	56.93	82.44	95.86	44.22	194.55	174.69	0	199.68	0	79.86 ± 84.83
A(LT)	0	6.68	7.34	8.56	6.35	10.42	10.05	7.15	14.27	0	4.40 ± 7.08
A(KT)	0	12.04	12.49	12.24	9.33	17.93	13.06	12.01	27.30	10.24	7.91 ± 11.64
PH(BT)	0	0	0	47.93	0	87.34	27.48	0	154.75	0	52.22 ± 31.75
PH(LT)	0	5.83	7.13	8.64	5.71	9.56	8.31	5.72	12.68	0	3.96 ± 6.35
PH(KT)	0	0	0	0	0	0	0	0	0	0	0
N(BT)	0	26.53	0	47.93	0	58.23	28.46	0	89.85	0	31.57 ± 25.1
N(LT)	0	0	0	7.42	0	8.88	0	0	10.49	0	4.37 ± 2.67
N(KT)	0	0	0	4.08	3.90	8.16	3.58	0	8.53	0	3.40 ± 2.82

Table 2. Results of ANOVA test on concentration of PAHs (ppb w.w.) in different tissue samples

	Blubber	p	Liver	Kidney
Anthracene	84.83 ± 79.86**	0.001	7.08 ± 4.40*	11.64 ± 7.91*
Phenanthrene	31.75 ± 52.22**	0.001	6.35 ± 3.96*	0*
Naphthalene	25.1 ± 31.57**	0.01	2.67 ± 4.37*	2.82 ± 3.40*

*Nonsignificant difference; **Significant difference ($p > 0.05$)

Table 3. Results of Student's *t* tests between sex of sampled seals and concentration of PAHs (ppb w.w.) in tissue samples; phenanthrene contamination was not detected in kidney tissues.

PAH concentrations in liver, kidney, and blubber tissues	*p*	*t* value
Concentration of anthracene in blubber tissue	0.00*	0.46
Concentration of anthracene in liver tissue	0.03*	0.08
Concentration of anthracene in kidney tissue	0.04*	3.70
Concentration of phenanthrene in blubber tissue	0.00*	3.58
Concentration of phenanthrene in liver tissue	0.04*	2.28
Concentration of naphthalene in blubber tissue	0.00*	5.95
Concentration of naphthalene in liver tissue	0.01*	3.65
Concentration of naphthalene in kidney tissue	0.03*	8.83

*Significant difference ($p < 0.05$)

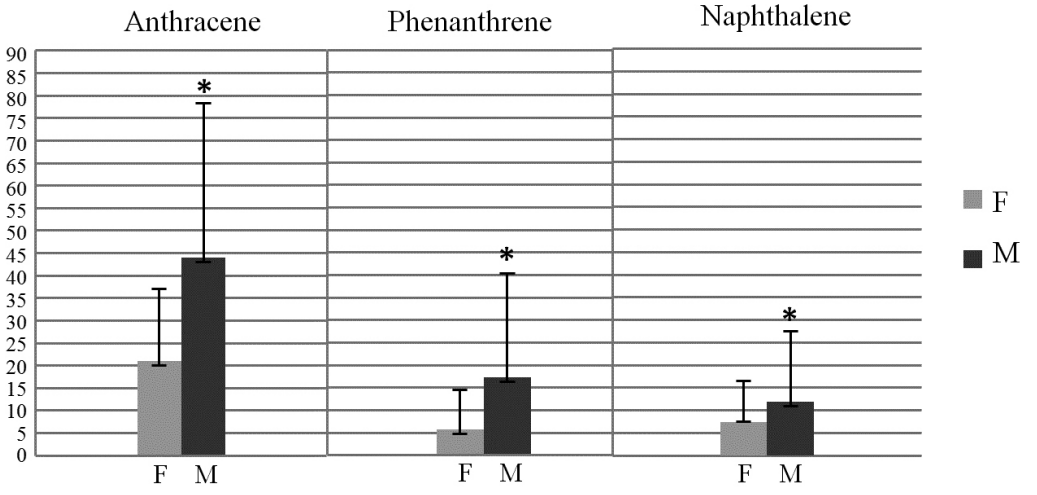

Figure 1. Mean (SD) PAH concentrations (ppb w.w.) in male and female Caspian seals (*Pusa caspica*); F = female and M = male.

(a,h) anthracene, fluoranthene, fluorene, and naphthalene in the Caspian Sea (Nasrallahzadeh Saravi et al., 2012; Eskandarpour et al., 2014). Absence of the other 13 surveyed PAHs in Caspian seals' tissues might be related to their elimination in Caspian seals by metabolization or absence of them in Caspian seals' habitat (Meador et al., 1995b). Nasrallahzadeh Saravi et al. (2012) studied the sediment and muscle tissue of fish in the southern Caspian Sea near Astara, White River, Tonekabon, and Amir Abad, Iran. They measured an anthracene concentration of 7.6 ± 4 ppb w.w. and a dibenzene concentration of 66.6 ± 75 ppb w.w. in sediment. Benzo fluoranthene and benzo pyrene were detected in the muscle tissues of several Caspian Sea fish species, including the Caspian kutum (*Rutilus frisii kutum*) with concentrations of 530 and 96.6 ppb w.w. and the leaping mullet (*Chelon saliens*) at 80 and 176 ppb w.w., respectively. In another study on fish flour (fish meal) of Caspian Sea sprat (*Clupeonella cultriventris caspia*), naphthalene, fluorine, and anthracene concentrations were measured at 24.66 ± 15.52, 1.32 ± 1.54, and 1.1 ± 1.92 ppb w.w., respectively (Eskandarpour et al., 2014). Absence of the other studied PAHs in sampled Caspian seals might be due to the short time that Caspian seals have been observed along the shores of Iran (Härkönen et al., 2008). As surveyed tissues in previously mentioned studies of fish are not similar with the tested tissues in our study, we cannot compare obtained results. However, compared to the PAH concentration in some of these fish species, the higher concentrations in sampled

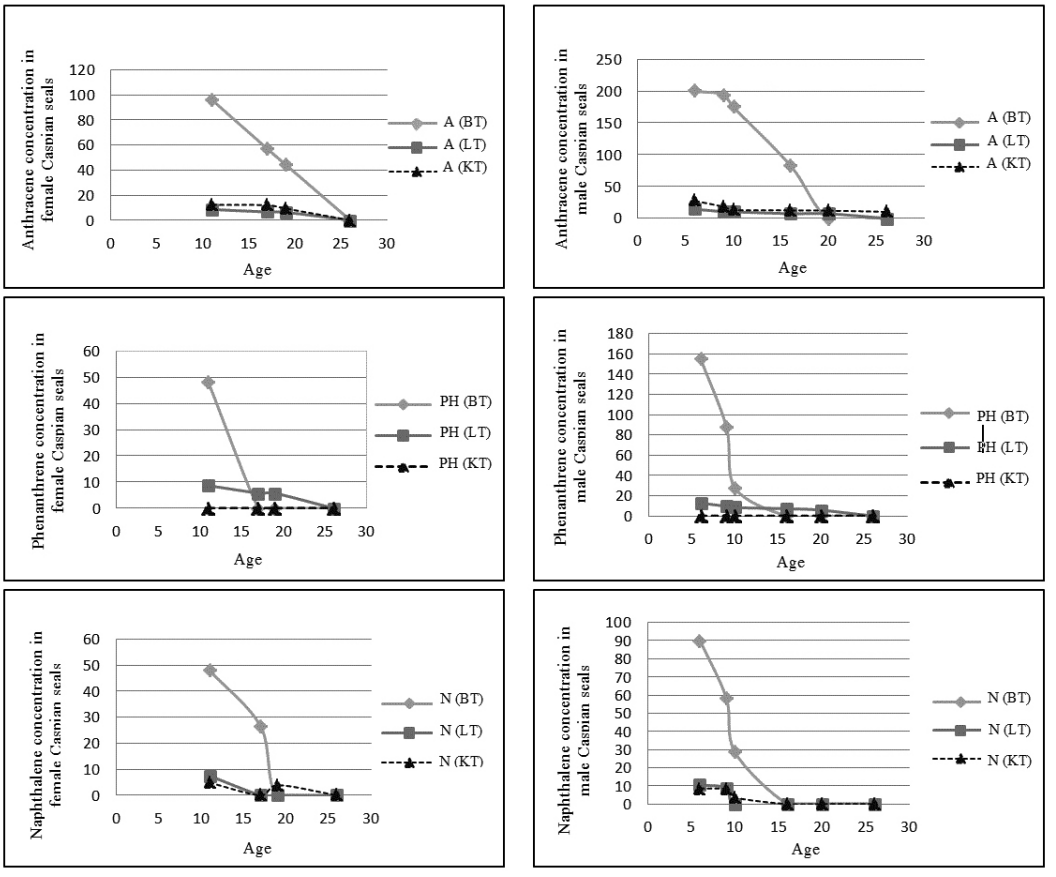

Figure 2. Results of a linear regression test on the relationships between PAH concentrations (ppb w.w.) and age (year) of male and female Caspian seals. A = anthracene, N = naphthalene, PH = phenanthrene, BT = blubber tissue, LT = liver tissue, and KT = kidney tissue.

Caspian seals may be due to higher concentrations of fat in Caspian seal tissue and their greater longevity, allowing for longer bioaccumulation.

Unfortunately, there are few similar studies on carcinogenic PAH contamination in marine mammals. Marsili et al. (2001) measured total PAHs in blubber tissues of fin whales (*Balaenoptera physalus*) and striped dolphins (*Stenella coeruleoalba*) and found high levels of contamination in these Mediterranean cetaceans. Marsili et al. (1997) surveyed PAH concentration in liver tissues of South American sea lions (*Otaria flavescens*) in the Plata Sea, Argentina. They detected naphthalene (=194 ± 54.07 ppb w.w.), anthracene (=0.30 ± 2.270 ppb w.w.), and phenanthrene (=27.1 ± 21.75 ppb w.w.) in sampled tissues. Many factors, such as level and duration of animal PAH exposure, sex and age of sampled animals, species' ability to metabolize PAHs, and the differences in volume of PAHs' entrance in the animals' habitat, can result in detection of higher concentrations of naphthalene and phenanthrene and a lower concentration of anthracene in sampled sea lions as compared with sampled Caspian seals (Meador et al., 1995b). Also, Hellou et al. (1991) examined PAH concentration in the muscle tissue of harp seals (*Phoca groenlandica*) in the northwestern Atlantic Ocean. Those specimens were contaminated with phenanthrene, anthracene, and fluorene, and PAH concentration was measured between 10 and 31 ppb lipid w.w. As we did not examine muscle tissues in sampled Caspian seals, we cannot compare our results with the results of Hellou et al. (1991) on harp seals.

Of the three PAHs detected (i.e., naphthalene, phenanthrene, and anthracene), anthracene occurred at the highest concentrations in sampled tissues while phenanthrene had the lowest concentrations. A different trend in PAH concentrations has been documented in fish species of the Caspian

Sea—for example, Eskandarpour et al. (2014) reported higher concentrations of naphthalene than anthracene in Caspian Sea sprat (*Clupeonella cultriventris caspia*). Similarly, Kannan & Perrotta (2008) detected naphthalene in kidney tissues in higher concentrations compared to other PAHs in 81 adult female sea otters (*Enhydra lutris*) from the California coast. Many factors, such as differences in the source of PAHs in sampling areas, chemical condition of the sampling area, number of PAH benzene rings, and tendency for bioaccumulation of PAHs in different species, could lead to these differences in findings (Meador et al., 1995b).

As mentioned, tissue accumulation patterns of detected PAHs differed among Caspian seal tissue types. The number of PAH benzene rings is an important variable associated with bioaccumulation of PAHs in Caspian seal tissues. With increases in the number of rings, the hydrophobic and lipophilic properties of PAHs increase. When PAHs enter Caspian seal bodies, they can accumulate in several tissues, but especially in blubber. Microbial degradation of PAHs decreases with increases in the number of PAH benzene rings, so PAHs with more benzene rings can accumulate to higher concentrations in tissues (Landrum, 1989; Landrum & Robbins, 1990; Meador et al., 1995a; Meador, 2003). It follows that a higher tissue concentration of anthracene than naphthalene could be explained by the higher number of anthracene benzene rings. A higher anthracene tissue concentration than phenanthrene, which has the same number of benzene rings, might be explained by a higher rate of entry of anthracene into the Caspian Sea.

The higher PAH concentrations in blubber tissues may be explained by the hydrophobic and lipophilic characteristics of PAHs. Eskandarpour et al. (2014) found that bioaccumulation of PAHs in Caspian Sea sprat was directly related to the concentration of fat in sampled tissues, with higher concentrations detected in tissues with higher levels of fats (Eskandarpour et al., 2014). In contrast, there was no correlation between tissue PAH concentrations and the amount of fat in harp seal tissues (Hellou et al., 1991).

We found the lowest PAH concentrations in liver tissues. Most of the PAHs are absorbed through the digestive system and transferred to the liver for detoxification by enzymes, including the P450 family and microsomal enzymes (Engelhardt, 1982; Addison & Brodie, 1984; Marsili et al., 1997; Lee et al., 2005). This detoxification process could lead to lower concentrations of PAHs in liver tissues than blubber or kidney.

A negative correlation between PAH concentration in tissues with the age of sampled Caspian seals was found, indicating the influence of age as a confounding factor on PAH concentrations in our seals. Similarly, studies of northern pike (*Esox lucius*) and Caspian Sea sprat revealed that animal age can affect PAH bioaccumulation rate with similar trends (Salimi et al., 2011; Eskandarpour et al., 2014). As age increases, PAH concentration in tissues can decrease due to an increase in PAH metabolism. Hellou et al. (1991) showed that PAH concentration was higher in younger harp seals than older seals. They also found that the activity of the metabolizing enzymes, including P450 family enzymes, on PAHs increases with age and can lead to a decrease in PAH concentrations in tissues by age (Hellou et al., 1991). However, Harris et al. (2011) surveyed hydrocarbon concentrations and patterns in blood samples of 29 live-captured sea otters from British Columbia, Canada, and reported similar hydrocarbon concentrations among different age classes.

The higher average age of sampled females (18.2 y old) than males (14.5 y old) may be one explanation for our finding showing lower PAH concentrations in tissues of female Caspian seals, highlighting the putative biotransformation of PAHs by P450 enzymes. This result supports findings of Addison et al. (1973) and Muyer et al. (1988) in harp and ringed (*Phoca hispida*) seals, respectively. Those authors suggested that this difference could be due to higher activity of P450 family enzymes in females resulting in a lower PAH concentration in female tissues. Eskandarpour et al. (2014) explained that such results can occur due to maternal transfer of PAHs in fish through egg laying. However, studies by Hellou et al. (1991) and Marsili et al. (2001) on harp seals and cetaceans (i.e., fin whales and striped dolphins), respectively, showed no relationships between sex and PAH tissue concentrations.

Elimination of organochlorine pesticides in female Caspian seals through giving birth has been described, but there is no information about offloading of PAHs in seals via that mechanism (i.e., maternal transfer; Subramanian et al., 1987; Tanabe et al., 1987; Nakata et al., 1995). Thus, additional studies are needed on this topic.

Molecular ratios of PAHs have been used to identify their sources in the environment. According to Hajizadeh et al. (2010), if the ratio of phenanthrene to anthracene is > 10, the origin of these pollutants is considered petrogenic, but if the ratio is < 10, their origin is considered pyrolytic. Nasrollahzadeh Saravi et al. (2012), who detected PAHs in leaping mullet and Caspian kutum in the southern part of the Caspian Sea (i.e., Mazandaran and Golestan), reported that the origin of PAHs in that region of the Caspian Sea is more pyrolytic. Nemati Varnosfaderany et al. (2014) and Baniemam et al. (2017) reported both pyrolitic and petrogenic origins of PAHs in the

southern Caspian Sea (Iranian coastal regions). In the present study, the ratio of PAHs with low molecular weight (phenanthrene) to those with a high molecular weight (anthracene) also indicated that the PAH compounds in sampled Caspian seals may have pyrolytic sources. Nevertheless, Kannan & Perrotta (2008) concluded that the detection of PAHs with a predominance of di- and tri-cyclic PAHs over tetra- and penta-cyclic PAHs (as we also detected in Caspian seals) suggest petrogenic sources.

In conclusion, it appears that Caspian seals are exposed to PAHs of both petrogenic and pyrolytic origins in the Caspian Sea. Our results show that PAHs eventually accumulate in the lake's food chain and enter Caspian seal tissues. Because PAHs' retention rate times are low in the environment (i.e., high elimination rate), their accumulation in Caspian seals indicates that the emission or inflow rate of PAH entering into the Caspian Sea ecosystem is much higher than their rate of removal (e.g., burial rate). Of course, given the migratory lifestyle of Caspian seals, it is not possible to identify the exact route and location of PAH contamination of Caspian seals (Anyakora et al., 2005).

No ecotoxicological risk assessments have been conducted to derive the toxic effect of concentration and/or safe level thresholds of PAHs on Caspian seals. PAH accumulation in tissues could have negative effects on the health status of this species, even at very low concentrations. Appropriate strategies for limitation of PAH entrance into the Caspian Sea should be considered in countries bordering the Caspian Sea. Further studies are needed to determine pathogenic and lethal doses of PAHs in Caspian seals.

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Literature Cited

Addison, R. F., & Brodie, P. F. (1984). Characterization of ethoxyresorufin O-de-ethylase in grey seal *Halichoerus grypus*. *Comparative Biochemistry and Physiology Part C: Comparative Pharmatology*, *79*, 261-263. https://doi.org/10.1016/0742-8413(84)90196-8

Addison, R. F., Kerr, S. R., Dale, J., & Sergeant, D. E. (1973). Variation of organochlorine residue levels with age in Gulf of St. Lawrence harp seals (*Pagophilus groenlandicus*). *Journal of the Fisheries Research Board of Canada*, *30*, 595-600. https://doi.org/10.1139/f73-106

Amano, M., Miyazaki, N., & Petrov, E. A. (2000). Age determination and growth of Baikal seals (*Phoca sibirica*). In A. Rossiter & H. Kawanabe (Eds.), *Advances in ecological research* (Vol. 31, pp. 449-462). Amsterdam, Netherlands: Elsevier. https://doi.org/10.1016/S0065-2504(00)31024-8

Anyakora, C., Ogbeche, A., Palmer, P., & Coker, H. (2005). Determination of polynuclear aromatic hydrocarbons in marine samples of Siokolo Fishing Settlement. *Chromatography*, *10*, 323-330. https://doi.org/10.1016/j.chroma.2004.10.014

Baniemam, M., Moradi, A. M., Bakhtiari, A. R., Fatemi, M. R., & Khanghah, K. E. (2017). Seasonal variation of polycyclic aromatic hydrocarbons in the surface sediments of the southern Caspian Sea. *Marine Pollution Bulletin*, *15*, 478-485. https://doi.org/10.1016/j.marpolbul.2017.01.027

Effimoff, I. (2000). The oil and gas resource base of the Caspian region. *Petroleum Science and Engineering*, *28*, 157-159. https://doi.org/10.1016/S0920-4105(00)00075-9

Engelhardt, F. R. (1982). Hydrocarbon metabolism and cortisol balance in oil-exposed ringed seals, *Phoca hispida*. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *72*, 133-136. https://doi.org/10.1016/0306-4492(82)90219-2

Eskandarpour, A. F., Aryanashja, A. A., & Salimi, L. (2014). Qualitative and quantitative analysis of PAHs (sixteen of important PAHs in EPA list) in tissues and meal of *Clupeonella cultriventris caspia* of Guilan Province Caspian waters (Bandar Anzali). *Journal of Aquatic Physiology and Biotechnology*, *105*, 51-56. (In Persian)

Freeman, D. J., & Cattell, F. C. R. (1990). Wood burning as a source of atmospheric polycyclic aromatic hydrocarbons. *Environmental Science and Technology*, *24*, 24-39. https://doi.org/10.1021/es00080a019

Goodman, S., & Dmitrieva, L. (2016). *Pusa caspica*. In International Union for Conservation of Nature (Ed.), *The IUCN red list of threatened species*. Gland, Switzerland: IUCN.

Hajizadeh, N., Abbasi, A., Ramini, S., Moghaddam, M., & Rahmani, I. (2010). The concentration and origin of petroleum hydrocarbons in sediments of Anzali port. *Ecology*, *6*, 99-106.

Härkönen, T., Jüssi, M., Baimukanov, M., Bignert, A., Dmitrieva, L., Kasimbekov, Y., . . . Goodman, S. J. (2008). Pup production and breeding distribution of the Caspian seal (*Phoca caspica*) in relation to human impacts. *AMBIO: A Journal of the Human Environment*, *37*(5), 356-361. https://doi.org/10.1579/07-R-345.1

Harris, K. A., Nichol, L. M., & Ross, P. S. (2011). Hydrocarbon concentrations and patterns in free-ranging sea otters from British Columbia, Canada. *Environmental Toxicology and Chemistry*, *30*, 2184-2193. https://doi.org/10.1002/etc.627

Hellou, J., Upshall, C., Ni, I. H., Payne, J. F., & Huang, Y. S. (1991). Polycyclic aromatic hydrocarbons in harp seals (*Phoca groenlandica*) from the Northwest Atlantic. *Archives of Environmental Contamination and Toxicology*, *21*, 135-140. https://doi.org/10.1007/BF01055568

Kanaly, R. A., & Harayama, S. (2000). Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. *Journal of Bacteriology*, *182*(8), 2059-2067. https://doi.org/10.1128/JB.182.8.2059-2067.2000

Kannan, K., & Perrotta, E. (2008). Polycyclic aromatic hydrocarbons (PAHs) in livers of California sea otters. *Chemosphere*, *7*, 649-655. https://doi.org/10.1016/j.chemosphere.2007.11.043

Kaplin, P. A., & Selivanov, A. O. (1995). Recent coastal evolution of the Caspian Sea as a natural model for coastal response to the possible acceleration of global sea-level rise. *Marine Geology*, *124*, 161-175.

Kardovani, P. (1995). *Iran marine ecosystem (Caspian Sea)*. Tehran, Iran: Ghomse Publication.

Landrum, P. F. (1989). Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. *Environmental Science Technology*, *23*, 588-595. https://doi.org/10.1021/es00063a012

Landrum, P. F., & Robbins, J. A. (1990). Bioavailability of sediment associated contaminants: A review and simulation model. In G. Baudo, J. P. Giesy, & H. Mauntau (Eds.), *Sediments: Chemistry and toxicity of in-place pollutants* (Ch. 8, pp. 237-263). Chelsea, MI: Lewis.

Lawal, A. T. (2017). Polycyclic aromatic hydrocarbons: A review. *Cogent Environmental Science*, *3*(1), 1339841. https://doi.org/10.1080/23311843.2017.1339841

Lee, R. F., & Anderson, J. W. (2005). Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. *Marine Pollution Bulletin*, *50*, 705-723. https://doi.org/10.1016/j.marpolbul.2005.04.036

Lucas, D., & Zhao, L. (2015). *PAH analysis in salmon with enhanced matrix removal*. Santa Clara, CA: Agilent Technologies Inc. Retrieved from https://www.agilent.com/cs/library/applications/5991-6088EN.pdf

Marsili, L., Caruso, A., Fossi, M. C., Zanardelli, M., Politi, E., & Focardi, S. (2001). Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans. *Chemosphere*, *44*, 147-154. https://doi.org/10.1016/S0045-6535(00)00206-X

Marsili, L., Fossi, M. C., Casini, S., Savelli, C., Jimenez, B., Junin, M., & Castello, H. (1997). Fingerprint of polycyclic aromatic hydrocarbons in two populations of southern sea lions (*Otaria flavescens*). *Chemosphere*, *34*, 759-770. https://doi.org/10.1016/S0045-6535(97)00003-9

Meador, J. P. (2003). Bioaccumulation of PAHs in marine invertebrates. In P. E. T. Douben (Ed.), *PAHs: An ecotoxicological perspective* (Ch. 9, pp. 147-171). New York: John Wiley & Sons. https://doi.org/10.1002/0470867132.ch9

Meador, J. P., Casillas, E., Sloan, C. A., & Varanasi, U. (1995a). Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. *Marine Ecology Progress Series*, *123*, 107-124. https://doi.org/10.3354/meps123107

Meador, J. P., Stein, J. E., Reichert, W. L., & Vatanasi, U. (1995b). Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Reviews of Environmental Contamination and Toxicology*, *143*, 79-165. https://doi.org/10.1007/978-1-4612-2542-3_4

Mille, G., Asia, L., Guiliano, M., Malleret, L., & Doumenq, P. (2007). Hydrocarbons in coastal sediments from the Mediterranean Sea (Gulf of Fos Area, France). *Marine Pollution Bulletin, 54*, 566-575. https://doi.org/10.1016/j.marpolbul.2006.12.009

Mrozik, A., Piotrowska-Seget, Z., & Labuzek, S. (2003). Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. *Polish Journal of Environmental Studies*, *12*(1), 11-15.

Muyer, D. C., Norstrom, R. J., & Simon, M. (1988). Organochlorine contaminants in Arctic marine food chains: Accumulation of specific polychlorinated biphenyls and chlordane-related compounds. *Environmental Science Technology*, *22*, 1071-1079. https://doi.org/10.1021/es00174a012

Nakata, H., Tanabe, S., Tatsukawa, R., Amano, M., Miyazaki, N., & Petrov, E. A. (1995). Persistent organochlorine residues and their accumulation kinetics in Baikal seal (*Phoca sibirica*) from Lake Baikal, Russia. *Environmental Science Technology*, *29*, 2877-2885. https://doi.org/10.1021/es00011a026

Nasrollahzadeh Saravi, H., Pourgholam, R., Unesipour, H., & Makhlough, A. (2012). Polyaromatic hydrocarbons (16 PAHs) at the sediments and edible tissue of *Chelon saliens* and *Rutilus frisii kutum* in Caspian Sea. *Journal of Mazandaran University of Medical Sciences*, *22*(94), 79-90. (In Persian)

National Center for Biotechnology Information. (2019). *PubChem*. Retrieved from https//:pubchem.ncbi.nlm.nih.gov/compound/Naphthalene, https://pubchem.ncbi.nlm.nih.gov/compound/Anthracene, and https://pubchem.ncbi.nlm.nih.gov/compound/Phenanthrene

Nemati Varnosfaderany, M., Riyahi Bakhtiari, A., & Guoqiang Chu, Z. H. (2014). Vertical distribution and source identification of polycyclic aromatic hydrocarbons (PAHs) in southwest of the Caspian Sea: Most petrogenic events during the late Little Ice Age. *Marine Pollution Bulletin*, *15*, 152-163. https://doi.org/10.1016/j.marpolbul.2014.07.063

Salimi, L., Motalebi, A. A., Mehdinia, A., & Ghorbani, S. (2011). Qualitative and quantitative analysis of PAHs in *Esox lucius* of Anzali Lagoon. *Journal of Marine Science and Technology Research*, *6*(3), 94-106.

Shinsuke, T., Satoko, N., Tu Binh, M., Nobuyuki, M., & Evgeny, A. (2003). Petrov temporal trends of persistent organochlorine contamination in Russia: A case study of Baikal and Caspian seal. *Archives of Environmental Contamination and Toxicology*, *44*, 533-545. https://doi.org/10.1007/s00244-002-2092-4

Subramanian, A., Tanabe, S., & Tatsukawa, R. (1987). Age and size trends and male-female differences of PCBs and DDE in Dalli-type Dall's porpoises, *Phocoenoides dalli* of northwestern North Pacific. *Proceedings of the 9th-19th NIPR Symposia on Polar Biology*, *1*, 205-216.

Tanabe, S., Loganathan, B. G., Subramanian, A. N., & Tatsukawa, R. (1987). Organochlorine residues in short-finned pilot whale: Possible use as tracers of biological parameters. *Marine Pollution Bulletin*, *18*, 561-563. https://doi.org/10.1016/0025-326X(87)90541-8

Tolosa, I., Mora, S., Sheikholeslami, M. R., Villeneuve, J. P., Bartocci, J., & Cattini, C. (2004). Aliphatic and aromatic hydrocarbons in coastal Caspian Sea sediments. *Marine Pollution Bulletin*, *48*, 44-60. https://doi.org/10.1016/S0025-326X(03)00255-8

Watanabe, I., Kunito, T., Tanabe, S., Amano, M., Koyama, Y., Miyazaki, N., . . . Tatsukawa, R. (2002). Accumulation of heavy metals in Caspian seals (*Phoca caspica*). *Archives of Environmental Contamination and Toxicology*, *43*, 109-120. https://doi.org/10.1007/s00244-001-0055-9