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Review

Polycyclic aromatic hydrocarbons in marine mammals: A review and synthesis

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ABSTRACT

Most marine mammal species and populations are listed as endangered, threatened, or depleted under the Endangered Species Act and the Marine Mammal Protection Act. Organic contaminants such as polycyclic aromatic hydrocarbons from anthropogenic activities are part of the threat to marine mammals. The evaluation of the potential bioaccumulation of these compounds by marine mammals is a tool for adoption of policies to reduce polycyclic aromatic hydrocarbons discharges to the marine environment, where important players such as the oil and gas industries, maritime transport and sewage companies operate. This review seeks to present a bibliographic survey covering all published peer reviewed works of the contents of polycyclic aromatic hydrocarbons in biological tissues of marine mammals. It intended to compare the sampling protocols, procedures for preservation of the tissues, and the analytical method applied to quantify the polycyclic aromatic hydrocarbons, no to criticize any of them but to review the data and discuss how they can be compared.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of semi-volatile organic compounds with at least two fused benzene rings, which may have branches of aliphatic chains (alkyl-PAHs). Petroleum is a rich source of PAHs, most of the PAHs in crude oil are low molecular weight (LMW) compounds, composed of two or three fused aromatic rings (Neff, 1979). The abundance of PAHs in petroleum usually decreases with increasing molecular weight. Higher molecular weight (HMW) four- to six-ring PAHs, some of which are known or suspected mammalian carcinogens, are much less abundant in crude oils than they are in most pyrogenic PAHs assemblages. The PAHs assemblages produced by pyrolysis of organic matter are complex, and dominated by four-, five-, and six-ring PAHs.

PAHs are widely distributed in the environment and can be found in all compartments: atmosphere, water, sediment and biota. The physical-chemical properties of these compounds and their distribution and transformation processes, such as evaporation, dissolution, sedimentation, photo-oxidation and biodegradation, determine the fate of PAHs in the environment (Neff, 2002; Stout et al., 2002; Stout and Wang, 2007).

The number and position of the aromatic rings of the PAHs affect their physical and chemical properties, behavior in environment and their interactions with biota (UNEP/IOC/IAEA, 1992). These compounds have high melting and boiling points, low vapor pressure and low water solubility. In addition, they are hydrophobic and lipophilic (Skupińska et al., 2004; Bjorseth, 1983). The hydrophobicity of PAHs results in high affinity for the organic fractions and therefore PAHs tend to adsorb to particulate organic material and accumulate in sediments (Chu and Chan, 2000; Mcelroy et al., 1989).

When released into the marine environment, PAHs partially evaporate, and dissolve in seawater, and a major part tends to quickly adsorb to suspended materials and sediments (Lourenço et al., 2016). The absorption of PAHs by marine organisms occurs mainly from the soluble and particulate fractions through contact with the gills or dietary exposure (Lourenço et al., 2016). However, PAHs absorption depends on their bioavailability and the physiology of the organisms (Meador et al., 1995). In the vertebrates most of the PAHs absorbed are efficiently biotransformed by enzymes that increase their solubility in water allowing their excretion, while in invertebrates the metabolic capability is lower (Jonsson et al., 2004; Meador et al., 1995). Therefore, vertebrates more easily metabolize and excrete PAHs than invertebrates and, for this reason; PAHs are not biomagnified in marine food chains

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(Broman et al., 1990; Hylland, 2006; Wan et al., 2007; Takeuchi et al., 2009).

Several PAHs, especially the HMW, are classified as toxic, some of which present mutagenic and carcinogenic properties: benz[*a*]anthracene, chrysene, dibenz[*a,h*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-c,d]pyrene, benzo[*ghi*]perylene (Neff, 1979). Consequently, the evaluation of these PAHs has been incorporated into monitoring programs of environmental agencies (USEPA, 1993; USEPA, 1995; Webster et al., 2017).

PAHs can be formed through different processes and are classified according to their origin (Yunker et al., 2002). PAHs can be synthesized by some bacteria, plants or fungi (Bakhtiari et al., 2010; Wilcke et al., 2003) and can still be formed from diagenetic reactions that involve the recent transformation of organic matter (Venkatesan, 1988; Wakeham et al., 1980). However, anthropogenic activities are largely responsible for the release of PAHs in the marine environment (McElroy et al., 1989; NRC, 2003).

Anthropogenic PAHs present in the marine environment come mainly from the direct introduction of oil and derivatives or from the incomplete burning of fossil fuels (NRC, 2003). The relative concentrations of parent and alkyl-PAHs can be used to distinguish their origin (Yunker et al., 2002). The diagnostic ratios for identifying sources of PAHs (petrogenic, pyrolytic, and diagenetic) are well established for sediment (Yunker et al., 2002) and there are also some studies that assess the origin of PAHs in organisms such as bivalves and fish (Huckins et al., 1993, Shigenaka and Henry Jr, 1995; Neff et al., 2006; Durell et al., 2000; Durell et al., 2006; Lourenço et al., 2015, 2016, 2018; Fontenelle et al., 2019). However, this literature review showed that the use of diagnostic ratios for PAHs source identification in mammals is incipient, not only because PAHs are assimilated, metabolized and excreted rapidly, but also because these processes rates are most likely different among the compounds (Neff, 2002), altering the known distribution patterns.

As discussed, and referenced in detail below, a few studies reported the content of organic compounds in biological tissues (liver, fat, skin, kidney, brain, lung, among others) in carcasses of marine mammals (e.g., Mazzariol et al., 2011; Weijs et al., 2016), however the carcasses state of decomposition were rarely described. Additionally, when the state of decomposition was reported, the descriptions have not followed the codes indicated by international protocols (Geraci and Lounsbury, 1993, 2005). The state of decomposition of the animal can affect the concentrations of contaminants in tissues, either by microbial degradation of the compounds or by the introduction of external contaminants, if the animal's body is wounded. The most recent studies showed a trend to analyze samples of blood (e.g., Formigaro et al., 2014) or tissues obtained by biopsies (e.g., Noren and Mocklin, 2012, Fossi et al., 2014), which can be collected from living organisms. The advantage of working with living organisms is the possibility to obtain information about the health status of the animal and improve the sampling distribution by sex, age, sexual maturation, and other parameters, which is not always possible with random sampling, such as obtaining from carcasses.

Regarding the analytical methods described, there is no consensus in any of the analytical steps (i.e. extraction, pre-treatment or instrumental analysis). In general, the same organic extract is used for organohalogenates and PAHs analysis. These works described different methods of organic extraction (Soxhlet, microwave assisted extraction, accelerated solvent extraction, pressurized fluid extraction, ultrasound extraction, and Turrax® homogenizer), using different organic solvents (n-hexane, dichloromethane, pentane, acetonitrile, methanol, benzene, and acetone) and different methods to cleanup the organic extracts (silica, alumina or florisil column chromatography, alkaline digestion, and gel permeation). Different organic solvents have different polarities, what influence not only the efficiency of extraction of the PAHs but also the amount of lipids extracted, affecting the final reported results, especially when results are presented on a lipid weight basis.

For PAHs, the following identification and quantification methods were mentioned: high performance liquid chromatography (HPLC) coupled to mass spectrometer (MS) or to a fluorescence detector, gas chromatography (GC) coupled to MS, and more recently in triple-quadrupole mass spectrometry (TQMS). The method of quantification can also bias the result. Methods that rely on fluorescence detection are much less specific than those relying on mass spectrometry and the false positives can be produced.

It is noteworthy that most of the works are focused on the organohalogenates compounds, and the PAHs were treated as accessory data. When reported, PAHs analyzed comprised only the 16 priority PAHs of the United States Environmental Protection Agency (USEPA) (i.e. naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno(1,2,3-c,d)pyrene, dibenz[*a,h*]anthracene, benzo(*ghi*)perylene) and a few works included the alkylated PAHs (e.g. C1- to C4-naphthalenes, C1- to C3-fluorenes, C1- to C3-dibenzothiophenes, C1- to C4-phenanthrenes-anthracenes, C1- and C2-fluoranthenes-pyrenes, and C1- and C2-chrysenes), but in a different number of compounds. The results were reported in different basis. Some studies reported the concentrations normalized by the lipid weight (lw), others in wet weight or fresh weight (ww, fw), and others in dry weight (dw) or even normalized by the extractable organic material (EOM) or by some kind of oil. In most studies, the lipid and the moisture contents were not mentioned. Even the results reported in the same basis need to be compared sparingly as the solvents used for extraction were different. All of these variables can make the comparison of results unfeasible.

Description of the quality assurance and quality control of the analytical methods are scarce or poorly described. Commercial standard reference material (SRM) for PAHs in tissues of marine mammals is not available. Recently, Huncik et al. (2019) produced an interlaboratory study to investigate the potential for false-positive identification of polycyclic aromatic hydrocarbons in blubber sampled from marine mammals. In this study, the SRM 1945 (Organics in Whale Blubber, NIST), a reference material for chlorinated compounds, was amended with PAHs. The false positives for PAHs observed in the study were close to the quantification limits and may arise from low-level laboratory contamination. Unfortunately, this study did not evolve into the production of an SRM.

In general, no correlations were found between biotic parameters, such as sex and age of the animal, and the content of PAHs in tissues. The relatively high concentrations observed were usually associated with the influence of urban and industrialized areas or eventually with oil spill or oil by-products.

2. Tissue collection and storage procedures

Most works reported the sampling of tissues in animals found dead, naturally stranded or incidentally captured. In the last years, the number of studies using biopsies has increased, as part of an effort to develop non-lethal techniques to study marine mammals and other organisms (Noren and Mocklin, 2012).

A protocol for sampling tissues from marine organisms was initially proposed by Geraci and Lounsbury (1993) and later reviewed (Geraci and Lounsbury, 2005). This protocol established that the tissues must be preserved at −80 °C or in a freezer with liquid nitrogen vapor at −150 °C, which reduces the loss of volatile compounds and prevents sample degradation (Pugh et al., 2010).

The system of carcasses codes proposed by Geraci and Lounsbury (1993) provided specific guidelines for whales, pinnipeds, dolphins, and sirenians and can be synthesized as: code 1 (live animals), code 2 (dead animals: carcass in good condition), code 3 (carcass in reasonable condition), code 4 (decomposed carcass), code 5 (mummified carcass or skeletal remains). For the analysis of organic contaminants the protocol suggests to carry out the analysis only in animals in codes 1 (via biopsies) and code 2 (carcass in good conditions). Despite the existing

protocols, few published works described the carcasses codes and the procedures for sampling and storage the tissues.

3. Polycyclic aromatic hydrocarbons in marine mammals

Few works reporting concentration of PAHs in tissues of marine mammals were published, probably because the upper trophic levels of aquatic organisms have high ability to metabolize PAHs (hydroxylation) producing hydrosoluble species that enable their excretion (Varanasi et al., 1989; Collier and Varanasi, 1991). Bioaccumulation studies in different marine organisms have shown that PAHs with similar molecular weight and similar octanol-water partition coefficient (Kow) have their concentrations decreased with an increase in trophic level. Therefore, it is assumed that PAHs do not biomagnify through the trophic web (Nakata et al., 2003; Wan et al., 2007; Takeuchi et al., 2009).

4. PAHs concentrations in cetaceans

The knowledge about the effects of PAHs on whales, dolphins, and porpoises, collectively known as cetaceans is scarce. There are few published papers describing concentrations of PAHs in tissues of wild cetaceans. Table 1 summarizes these works, which are described in detail below.

The first report of PAHs in tissues of free-ranging cetaceans occurred in 1988 when Martineau et al. (1988) analyzed the diolepoxide benzo[*a*] pyrene bounded to the DNA extracted from the brain tissue of three stranded beluga whales (*Delphinapterus leucas*) in St. Lawrence Estuary, Canada. The PAHs extraction was performed with alkaline digestion and the quantification with HPLC/Fluorescence detector. The concentrations of PAHs (from 69 to 206) were reported in ng of tetrol (resulting from binding to DNA via the anti-BaPDE metabolite of benzo[*a*]pyrene (BaP) per g of DNA. The authors concluded that the animals had been exposed to BaP and had metabolized it to the diolepoxide BaP. Later, Hellou et al. (1990) studied PAHs in the muscle tissue of the beluga whales (*Delphinapterus leucas*), sperm whales (*Physeter macrocephalus*), minke whales (*Balaenoptera acutorostrata*), white-sided dolphin (*Lagenorhynchus obliquidens*), and harbor porpoise (*Phocoena phocoena*) stranded in the Newfoundland-Labrador, the same estuary where Martineau et al. (1988) performed the first PAHs experiments. The authors mentioned that most samples were frozen (−20 °C) soon after death, although the sperm whale has been dead for 1 week before sampling. The method for PAHs analysis involved alkaline digestion of animal tissue, hexane solvent partitioning and extract purification using silica and alumina chromatography columns. Total PAHs concentrations were determined in terms of a Venezuelan crude oil and chrysene equivalents on dry and wet basis through HPLC-UV/fluorescence detector. For the whales, the total PAHs (16 PAHs) expressed on dw, in Venezuelan oil equivalents, ranged from 0.26 to 0.69 μg g^{-1}, and from 0.21 to 0.34 μg g^{-1} in chrysene equivalents. For the dolphin, the total PAHs concentration ranged from 1.14 to 5.50 and from 0.52 to 1.21 μg g^{-1} (dw) for Venezuelan oil and chrysene equivalents, respectively.

Two years later, Law and Whinnett (1992) examined PAHs in the muscle tissue from twenty-six *Phocoena phocoena* from UK waters. The muscle samples were collected from animals stranded or entangled in fishing nets and stored at −20 °C. The samples were subjected to alkaline digestion and extracted with pentane. The cleanup was performed using an alumina chromatograph column. The PAHs were quantified using a GC–MS. The concentrations of the PAHs (9 PAHs) were reported in Ekofish oil equivalent and in chrysene equivalent. The concentrations of total PAHs ranged from 0.11 to 0.56 μg g^{-1} (ww) chrysene equivalents, and 0.47 to 2.4 μg g^{-1} (ww) Ekofisk crude oil equivalents. The highest concentrations were found in a juvenile male porpoise taken from the Isle of Man, Irish Sea. Specific PAHs detected in the samples were 2–4 ring compounds (naphthalenes, phenanthrenes, anthracene, fluoranthene and pyrene), but no higher molecular weight PAHs (benzanthracenes, benzofluoranthenes, benzopyrenes or perylene) were detected. Naphthalenes were the most abundant compound class. Similar concentrations of PAHs were observed in porpoises of all ages, from neonatal animals to 8 years old, and in both sexes. The authors suggested that the low concentrations observed occurred because the vertebrates possess inducible enzyme system capable of metabolizing PAHs.

Only after 7 years a new work describing the concentrations of PAHs in cetaceans was published. Holsbeek et al. (1999) analyzed PAHs in blubber of four *Physeter macrocephalus* stranded on the Belgian and Dutch coasts during the 1994/95 winter. All whales stranded alive and were sampled within 24 h after death. It was not mentioned how the samples were preserved. The blubber samples were 6 h Soxhlet extracted with n-hexane:acetone (3:1, v:v) and the organic extracts eluted on Florisil, silica gel and alumina columns. Quantitation was performed using a HPLC-UV/Fluorescence detector. The total PAH (15 PAHs), in dw, varied from 76.8 to 140.7 ng g^{-1}. Phenanthrene showed the highest concentration (42 ± 80 ng g^{-1}) and PAHs with 5–6 aromatic rings were not detected.

In the 21st century, studies of hydrocarbons in cetacean tissues have become more frequent. Marsili et al. (2001, 2002) analyzed PAHs in the blubber of 23 fin whales (*Balaenoptera physalus*) and 25 striped dolphins (*Stenella coeruleoalba*) in the Ionian and the Ligurian Seas, Italy. Samples of subcutaneous blubber were obtained from free-ranging whales using biopsy darts during the summers of 1993 and 1996. It was not informed how the samples were preserved. The blubber samples (0.1 g) were 5 h Soxhlet extracted with a mixture of KOH/methanol. PAHs were recovered using a liquid-liquid extraction with cyclohexane and subsequently dissolved in benzene. The benzene extract was purified in a chromatographic column packed with Florisil. Fourteen PAHs were analyzed by HPLC/fluorescence detector. In the blubber of *B. physalus* and *S. coeruleoalba* the total PAHs ranged, in ww, from 228.6 to 83,662 ng g^{-1} and from 199.4 to 198,368 ng g^{-1}, respectively. Naphthalene was the most ubiquitous compound, what was related to its major bioavailability in water. The authors described no significant differences between the concentrations of PAHs when comparing *B. physalus* in relation to sex; neither as a whole population was considered, nor by separating data by sampling year. Likewise, no significant differences were found between the Ligurian and Ionian populations of *S. coeruleoalba*. Samples collected in 1993 presented higher concentration of PAHs than those found for the samples from 1996 what was attributed to the incident of the tanker Haven, which spilled about 144,000 tons of crude oil in the Ligurian Sea in the early 90s.

In 2003, Nakata et al. (2003) reported concentrations of PAHs in the blubber samples of finless porpoises (*Neophocaena phocaenoides*) stranded along the Ariake Sea and the Yatsushiro Sea coasts, Japan, in 1999–2001. After collected, the samples were stored at −20 °C. The PAHs were Soxhlet extracted with dichloromethane/hexane (8:1) and the organic extract was subjected to acetonitrile solvent partitioning to remove lipid, followed by a cleanup procedure with silica gel column. The PAHs were analyzed using a GC–MS. The PAHs concentrations (14 PAHs) were lower than the detection limit (<0.04 ng g^{-1} ww), what was attributed to the metabolism of PAHs in higher trophic level animals, such as the *N. phocaenoides*.

Leung et al. (2005) studied PAHs in blubber samples of Indo-Pacific humpback dolphins (*Sousa chinensis*). The samples from living *S. chinensis* in the estuarine northwestern waters of Hong Kong ($n = 5$) were taken using biopsy darts. In addition, blubbers from the same species were sampled from dolphins stranded in Xiamen ($n = 4$) and Zhuhai ($n = 1$), China. The samples were stored at −20 °C. PAHs were extracted from 0.2 g of subcutaneous blubber using K-Ultra-Turrax homogenizer and dichloromethane. The organic extract was purified in a silica gel column and the PAHs ($n = 15$) were quantified using GC–MS. Concentrations of total PAHs, in ww, were highest in the Xiamen sample (mean 6751 ng g^{-1}), followed by the Hong Kong biopsy samples (mean 3275 ng g^{-1}), and were lowest in the Zhuhai sample (2762 ng g^{-1}). In the Hong Kong and Zhuhai samples, the most dominant chemical among

Table 1
Summary of the works that analyzed PAHs in marine mammals. * studies in which PAHs were treated as accessory data.

Specie	Tissue	Concentration of PAHs	n PAHs	Extraction method	Cleanup	Quantification Instrument	Site	Reference
Delphinapterus leucas	Brain	26–206 ng of tetro g^{-1} DNA (dw)	1	Alkaline digestion/ chloroform - phenol	not informed	HPLC/ fluorescence	St. Lawrence Estuary, Canada	Martineau et al. (1988) *
Delphinapterus leucas	Muscle	0.21–0.34 μg g^{-1} chrysene eq. (dw)	16	Alkaline digestion/ hexane	Silica and alumina	HPLC-UV/ fluorescence	Newfoundland-Labrador, Canada	Hellou et al. (1990)
Physeter macrocephalus								
Balaenoptera acutorostrata								
Lagenorhynchus obliquidens		1.14–5.50 μg g^{-1} chrysene eq. (dw)						
Phocoena phocoena								
Phocoena phocoena	Muscle	0.11–0.56 μg g^{-1} chrysene eq. (ww)	9	Alkaline digestion/ pentane	Alumina	GC–MS	United Kingdom	Law and Whinnett (1992)
Physeter macrocephalus	Blubber	76.8–140.7 ng g^{-1} (dw)	15	Soxhlet/n-hexane - acetone	Florisil, silica and alumina	HPLC-UV/ fluorescence	Belgian and Dutch coasts	Holsbeek et al. (1999) *
Balaenoptera physalus	Blubber	228.6–83,662 ng g^{-1} (ww)	14	Soxhlet/KOH - methanol	Florisil	HPLC-UV/ fluorescence	Ionian and Ligurian Seas, Italy	Marsili et al. (2001, 2002)
Stenella coeruleoalba		199.4–198,368 ng g^{-1} (ww)	14					
Neophocaena phocaenoides	Blubber	<0.04 ng g^{-1} (ww)	14	Soxhlet/ dichloromethane - hexane	Silica	GC–MS	Ariake and Yatsushiro Sea, Japan	Nakata et al. (2003)
Sousa chinensis	Blubber	2761.6–6751 ng g^{-1} (dw)	15	Turrax/ dichloromethane	Silica	GC–MS	Xiamen and Zhuhai, China	Leung et al. (2005)
Balaenoptera physalus	Blubber	2000 ng g^{-1} (dw) 5000 ng^{-1} (dw)	16	Not informed	Not informed	HPLC/ fluorescence	Ligurian Sea, Italy, and Gulf of California, Mexico	Fossi et al. (2010) *
Tursiops truncatus	Blubber	<10–9140 ng g^{-1} (lw)	45	Dionex ASE/ Dichloromethane	Not informed	GC–MS	Charleston, USA	Fair et al. (2010) *
		<10–1200 ng g^{-1} (lw)	45				Indian River Lagoon, USA	
Neophocaena phocaenoides	Blubber	4.8–432 ng g^{-1} (lw)	16	Soxhlet/ dichloromethane - hexane	GPC	GC–MS	South and Yellow Seas, Korea	Moon et al. (2011)
Physeter macrocephalus	Skin	1000 and 6000 ng g^{-1} (EOM)	15	Soxhlet/KOH - methanol	Florisil	HPLC-UV/ fluorescence	Pacific Ocean	Godard-Codding et al. (2011) *
Balaenoptera acutorostrata	Liver blubber	<LQ to 518 ng g^{-1} (lw) 66 to 555 ng g^{-1} (lw)	16	Soxhlet/ dichloromethane - hexane	GPC	GC–MS	Korean coastal water	Moon et al. (2012)
Delphinus capensis	Liver blubber	7.2 to 230 ng g^{-1} (lw) 63 to 269 ng g^{-1} (lw)						
Sousa chinensis	Blubber	12,552–86,711 ng g^{-1} (lw)	14	Soxhlet/KOH - methanol	Florisil	HPLC-UV/ fluorescence	Queensland, Australia	Cagnazzi et al. (2013) *
Orcaella heinsohni	Blubber	8682–61,921 ng g^{-1} (lw)						
Orcinus orca	Plasma	257–1910 ng g^{-1} (lw)	16	SPE	Not informed	GC-TQMS	Animals in captivity	Formigaro et al. (2014)
Balaenoptera physalus	Blubber	4772–30,114 ng g^{-1} (lw)	16	Soxhlet/KOH - methanol	Florisil	HPLC-UV/ fluorescence	Gulf of California, Mexico	Mazzariol et al. (2011) *, Fossi et al. (2014) *
Balaenoptera edeni								
Tursiops truncatus								
Delphinus capensis								
Physeter macrocephalus								
Orcinus orca								
Balaenoptera musculus								
Tursiops truncatus	Blubber	2526–35,864 ng g^{-1} (lw)	16	Soxhlet/ dichloromethane	GPC	GC-TQMS	Canary Islands	García-Álvarez et al. (2014a)
Tursiops truncatus	Blubber	10.2 to 162.8 ng g^{-1} (lw)	16	Soxhlet/ dichloromethane	GPC	GC-TQMS	Canary Islands	García-Álvarez et al. (2014b) *
Physeter macrocephalus	Fat	136.47 to 551.46 ng g^{-1} (lw)	14	Soxhlet/KOH - methanol	Florisil	HPLC-UV/ fluorescence	Adriatic coast, Italy	Marsili et al. (2014)
	Liver	796.16 to 891.84 ng g^{-1} (lw)						
	Muscle	513.41 to 3747.43 ng g^{-1} (lw)						
Sousa sahulensis	Blubber		16		Silica	GC–MS		

(*continued on next page*)

Table 1 (*continued*)

Specie	Tissue	Concentration of PAHs	n PAHs	Extraction method	Cleanup	Quantification Instrument	Site	Reference
		<LQ - 82 ng g^{-1} (lw)		Soxhlet/n-hexane - acetone			Queensland, Australia	Weijs et al. (2016) *
Sousa chinensis	Blubber	17.6 to 6080 ng g^{-1} (ww)	16	Soxhlet/ dichloromethane - hexane	GPC	GC–MS	Pearl River estuary, China	Gui et al. (2018)
Tursiops truncatus	Lung	162 ng g^{-1} (ww)	50	Turrax/ dichloromethane	GPC	GC–MS	Port Fourchon Island, USA	Stout et al. (2018)
Physeter microcephalus	Blood, muscle, liver, lung, placenta, heart, mammary gland, ovary, umbilical cord, epidermis, and blubber	21.2–3112 ng g^{-1} (ww)	16	Soxhlet/ dichloromethane - hexane	GPC	GC–MS	South China Sea	Zhan et al. (2019) *
Sousa chinensis	Brain	367 ± 261 ng g^{-1} (ww)	16	Soxhlet/ dichloromethane - hexane	GPC	GC–MS	Pearl River Estuary, China	Sun et al. (2020) *
	Blubber	4513 ± 3366 ng g^{-1} (ww)						
Cystophora cristata *Pusa hispida* *Phoca vitulina* *Pagophilus groenlandicus*	Muscle	0.10 to 0.80 μg g^{-1} (dw)	16	Alkaline digestion/ hexane	Silica and alumina	HPLC-UV/ fluorescence	Newfoundland-Labrador, Canada	Hellou et al. (1990)
Phoca largha and Pusa hispida	Blubber	<LQ - 48 ng g^{-1} (ww)	45	ASE/ dichloromethane	GPC	GC-TQMS	Bering Strait, Alaska	Stimmelmayr et al. (2018)
	Feces	10 ng g^{-1} (ww)						
	Hair	40 ng g^{-1} (ww)						
	Kidney	0.7–3.7 ng g^{-1} (ww)						
	Liver	<LQ - 18 ng g^{-1} (ww)						
	Lung	<LQ - 5.8 ng g^{-1} (ww)						
	Muscle	0.2–7.9 ng g^{-1} (ww)						
	Skin	10–280 ng g^{-1} (ww)						
	Stomach	4.4–12 ng g^{-1} (ww)						
	Trachea	3.3 ng g^{-1} (ww)						

the 15 PAHs was naphthalene, which accounted for about 33.6% and 27.5% of the total PAHs. For the Xiamen sample, dibenzo(1,2,5,6)perylene was predominant, accounting for nearly 21% of the total.

In 2010, Fossi et al. (2010) published a work reporting concentration of PAHs in blubber biopsied samples of fin whales (*Balaenoptera physalus*) from the Pelagus Sanctuary (Ligurian Sea, Italy) and the Gulf of California, Mexico. The sampling was performed during the summer of 2008. The mean concentration of PAHs ($n = 16$) in Pelagus Sanctuary and in the Gulf of California, estimated from the histogram presented by the authors, was 5100 ng g^{-1} and 2000 ng g^{-1} dw respectively. No details regarding the sample preservation or method of analysis were described but that PAHs were quantified with HPLC/fluorescence system. The highest PAHs concentrations in blubber of *B. physalus* in Ligurian Sea were 8 times lower than the concentrations found previously by Marsili et al. (2001, 2002) in the same region. However, this comparison may be affected by analytical procedure, which was not fully informed in this last work.

In 2010, Fair et al. (2010) evaluated PAHs in blubber biopsy samples collected from 29 wild bottlenose dolphins (*Tursiops truncatus*) during 2003–2005 in Charleston, South Carolina (CHS), and the Indian River Lagoon (IRL), Florida (USA). Samples were immediately preserved in liquid nitrogen vapor container and latter stored at −80 °C. Samples were solvent extracted with dichloromethane using accelerated solvent extraction (Dionex, ASE) and analyzed with a GC–MS. The PAHs analytes included 45 PAHs parent and alkylated homologs further than 12 PAHs metabolite compounds (9-phenanthrol, 2-phenylphenol, 1-naphthol, 2-naphthol, 9-hydroxyfluorene, 1-hydroxypyrene, 1-hydroxy-BAP, 3-hydroxy-BAP, 1-hydroxychrysene, BAP-trans-4,5-dihydrodiol, BAP-trans-7,8-dihydrodiol and BAP-trans-9,10-dihydrodiol). Results were reported in ng g^{-1} lw. PAHs mean concentration in samples ($n = 17$) from CHS dolphins was 3010 ng g^{-1} and ranged from below detection (10 ng g^{-1}) in five samples to 9140 ng g^{-1}. PAHs mean concentration found in IRL dolphins ($n = 11$) was 1316 ng g^{-1} and ranged from below detection in six samples to 1200 ng g^{-1}. Naphthalene and its homologs accounted for 100% of the total PAHs concentrations. The authors suggested that the low concentrations occur because marine mammals have mixed function oxidase enzyme systems necessary for detoxification and elimination of petroleum hydrocarbons and therefore these compounds are not highly accumulated and sequestered in tissues.

Moon et al. (2011) assessed the occurrence and accumulation of PAHs in finless porpoises (*Neophocaena phocaenoides*) from Korean coastal waters. Fifty-two blubber samples were collected from *N. phocaenoides* found entangled in fishing nets along the South Sea and the Yellow Sea, Korea, during May–August in 2003. Finless porpoise samples were divided into four groups, immature males, immature females, mature males and mature females, based on their sex and growth. All the samples were stored at −20 °C until extraction. Blubber from finless porpoises was analyzed for 16 USEPA - Priority PAHs. Approximately 1–2 g of blubber samples were extracted with a mixture of dichloromethane and hexane using a Soxhlet apparatus. The extracts were subjected to GPC connected sequentially to a cartridge packed with 0.5 g of silica gel for cleanup and the PAHs were analyzed by GC–MS. The total concentrations of 16 PAHs ranged from 4.8 to 432 (mean: 160) ng g^{-1} lw. The lipid contents of all blubber samples were 79 ± 7.0%. The 2–4 rings PAHs were detected in almost all samples, while 5–6 rings PAHs were not detected in most of the samples. Naphthalene,

acenaphthylene, fluorene and phenanthrene were detected in over 90% of total tissue samples. Detection frequencies of anthracene, fluoranthene and pyrene in all the samples were over 80%. The most abundant PAHs found was naphthalene, ranging from <1.0 to 280 (mean: 92) ng g^{-1} lw. The proportion of naphthalene was 52 ± 24% of the total PAHs concentrations followed by phenanthrene, 19 ± 13%. The contributions of acenaphthylene, acenaphthene and fluorene collectively accounted for about 20% of total PAHs concentrations. The mean concentrations of PAHs in mature females were lower than those in mature males, but no statistically significant differences were observed. Also, no correlations were found between age and the concentrations of PAHs. The lack of correlations between PAHs, sex and age was attributed to the metabolism and excretion of these compounds by the *N. phocaenoides*.

In 2011, Godard-Codding et al. (2011) extensively studied the expression of CYP1A1 as a biomarker of exposure to organic contaminants, including PAHs, in sperm whales (*Physeter macrocephalus*) skin biopsies in five locations across the whole Pacific Ocean. The samples were taken between 1999 and 2001 and were stored at −20 °C for contaminant analysis. The samples were pooled to obtain enough material to determine PAHs. The samples were prepared as exposed previously by Marsili et al. (2001). The sum of 15 PAHs ranged from 1000 to 6000 ng g^{-1} EOM. The authors found no significant correlations between the proxy CYP1A1 Immunohistochemical (IHC) score and the PAHs in pooled samples, whether analyzed by sex or in both sexes combined.

Moon et al. (2012) analyzed the accumulation of PAHs in hepatic and blubber tissue of twenty-seven minke whales (*Balaenoptera acut o rostrata*) and twenty-four long-beaked common dolphins (*Delphinus capensis*) from Korean coastal water. The samples were retrieved from animals entangled in fishing nets along the Korean coasts in 2006. The authors did not report how the samples were stored. 1 to 2g of the liver and blubber samples were extracted with a mixture of dichloromethane and hexane using a Soxhlet apparatus. The organic extracts were purified through GPC and analyzed using GC–MS. The results were reported in lw and the limit of quantification (LQ) ranged from 1.0 to 3.0 ng g^{-1}. The total concentrations of PAHs in the liver from minke whales and common dolphins ranged from <LQ to 518 ng g^{-1} and from 7.2 to 230 ng g^{-1}. The total PAHs concentrations in blubber of minke whales and common dolphins ranged from 66 to 555 ng g^{-1} and from 63 to 269 ng g^{-1}, respectively. The predominant PAHs compound was naphthalene, which accounted for 39% of the total PAHs in the liver, and 57% of the total PAHs in the blubber of both cetaceans. The second most prevalent PAH was phenanthrene, which comprised 30% of the total PAHs concentrations in the liver, and 21% in the blubber. Acenaphthene and fluorene collectively accounted for approximately 20% of the total PAHs concentrations for both tissues and species. No interspecies differences in the concentrations of PAHs in liver tissue and blubber from *B. acutorostrata* and *D. capensis* were found and the concentrations of PAHs between mature male and female common dolphins were not statistically different. The authors attributed this result to the biotransformation and biodilution effects of these contaminants in the marine food web.

Cagnazzi et al. (2013) exanimated PAHs in blubber biopsy samples of *Sousa chinensis* ($n = 18$) and Australian snubfin dolphin (*Orcaella heinsohni*) ($n = 17$) collected from the Mackay–Whitsundays (MWC) and the Fitzroy River (FRC) catchments of Great Barrier Reef Marine Park, Queensland, Australia. Samples were preserved in liquid nitrogen immediately after collection and latter stored at −80 °C. Fourteen PAHs were analyzed by HPLC with fluorescence detection as described in Marsili et al. (2001). The most abundant PAHs were those with lower molecular weight, which are also the most water-soluble and bioavailable. Among these, naphthalene and pyrene were the most dominant chemicals. The concentrations of total PAHs ranged from 12,552 to 86,711 ng g^{-1} lw for *S. chinensis* and from 8682 to 61,921 ng g^{-1} lw for *O. heinsohni*. The authors mentioned that the total PAHs concentrations recorded in this study are among the higher found in the scientific literature. However, this finding cannot be verified, as the results were reported in lipid weigh, but neither the lipid nor the wet content was cited, therefore it is not possible to convert the results to compare them to other works. Furthermore, these comparisons must be taken with caution because of the variation on the analytical methods.

In 2014 a study was conducted by Formigaro et al. (2014) to assess the current dietary intake of polycyclic aromatic hydrocarbons in killer whales (*Orcinus orca*) through direct determination in a group of whales in captivity. In this study, sixteen blood samples were taken from four whales. The plasma was obtained through centrifugation and it was stored at −18 °C. Further than the *O. orca* blood samples, 500 g of the fish of each species that was given to feed the whales was taken from each delivery, homogenized in a blender and taken as an individual subsample of that species. Plasma samples were subjected to solid-phase extraction (SPE). The fish tissues were Soxhlet extracted and the organic extracts purified using GPC. PAHs quantitation was performed using GC-TQMS. The total concentration of 16 USEPA-priority PAHs in the plasma of the *O. orca* ranged from 257 to 1910 ng g^{-1} lw. Phenanthrene and pyrene were the compounds detected at the highest concentrations and were present in all the animals. The other detected PAHs in the plasma of the orcas were fluoranthene and fluorene. No other PAH was detected. The author found no differences between the levels of PAHs of individual *O. orca*, and there was no influence of sex, age, length or reproductive status on the levels of PAHs. Also, the authors found no relationship between the PAHs from feeding (fish) with those found in whales' plasma, what occurred because PAHs are efficiently metabolized, and the levels present in a given moment reflect only the recent exposure.

Also, in 2014, Fossi et al. (2014) published a new study where it was investigated feeding habitats and migratory behavior as causes of different toxicological hazard to cetaceans of Gulf of California, Mexico. This study involved, for the first time, both concentration of the PAHs in the cetacean tissues and the protein levels of CYP1A1 and CYP2B and two gene expression biomarkers (AHR and E2F1). Biopsies were obtained from 64 free-ranging cetaceans in the Gulf of California in summer 2008–2009 and in winter 2010: *Balaenoptera physalus* ($n = 8$), *Balaenoptera edeni* ($n = 6$), *Tursiops truncatus* ($n = 13$), *Delphinus capensis* ($n = 12$) *Physeter macrocephalus* ($n = 14$), *Orcinus orca* ($n = 5$), and *Balaenoptera musculus* (n = 6). Samples were immediately stored in liquid nitrogen and the PAHs were analyzed by HPLC-fluorescence as exposed previously by Marsili et al. (2001). The mean concentration of the total PAHs ranged from 4772 to 30,114 ng g^{-1} lw. The lipid and the moisture content were not informed. Highest levels of PAHs were detected in *P. macrocephalus* and *O. orca* in comparison to the other five species. According to the authors, based on CYP1A1 and CYP2B inductions, the high PAHs levels influenced the toxicological responses specially in the migratory species *B. musculus*, that could have bioaccumulated PAHs during summer, where it feeds in the waters off California, USA. The high concentrations of PAHs reported in this work must also be carefully evaluated because of the quantification method, based on fluorescence detection, which results can be biased by other fluorescent compounds than PAHs.

In the Canary Archipelago, García-Álvarez et al. (2014a) evaluated the 16 USEPA-priority PAHs in blubber biopsy samples of sixty-four *Tursiops truncatus*, from 2003 to 2011. After sampling, the tissues were stored at −80 °C. PAHs extraction from 0.03 to 0.31 g of the blubber was performed with dichloromethane in a Soxhlet system with a purification step using GPC. The quantitation was performed using a GC-TQMS. The concentration of the total PAHs ranged from 2526 to 35,864 ng g^{-1} lw. The lipid and the moisture content were not informed. Phenanthrene was detected in 100% of the samples and reached the highest concentrations. Pyrene, naphthalene and chrysene were also found at high frequencies (100%, 95%, and 98%, respectively). All 16 PAHs were detected in some of the samples. However, some of these compounds had low detection frequencies, including anthracene (14%), dibenz[*ah*]

anthracene (3%), and benzo[*ghi*]perylene (3%). The lower molecular weight compounds (di-, tri- and tetra-cyclic) were the most frequent and were present in higher concentrations. The authors stated that PAHs are efficiently metabolized, unlike most organohalogenated compounds, and the levels present in a given moment reflect only the recent exposure, as mentioned by Formigaro et al. (2014).

García-Álvarez et al. (2014b) also assessed the levels of the 16 USEPA-priority PAHs in *Tursiops truncatus* stranded along the Canary Islands coasts. Twenty-five blubber and twenty-six liver samples were collected from 27 dolphins stranded in different coastal areas of Canary Islands over the period of 1997–2011, and stored at the −80 °C until chemical analysis. The state of decomposition of the animals was classified as very fresh, fresh, moderately autolytic, autolytic, and very autolytic. More than 55% of the samples were included in the first three categories. The analytical method was the same described by García-Álvarez et al. (2014a). The concentration of the total PAHs varied from 10.2 to 162.8 ng g^{-1} lw. Acenaphthylene, anthracene, indene[1,2,3-*cd*] pyrene, and dibenzo[*a*,*h*]anthracene were not detected in any sample. Phenanthrene was the most frequently detected compound of this group, and also the one detected at the highest levels in both tissues.

In 2014, Marsili et al. (2014) produced a new multidisciplinary study of seven male *Physeter macrocephalus* that were found stranded along the Adriatic coast in southern Italy in December 2009. This study involved analyzes of histopathology, virology, bacteriology, parasitology, toxicology, genetics, and analyzes of organic contaminants that were related to the responses of biomarkers, as performed by Fossi et al. (2014). Mazzariol et al., 2011 previously presented these same data of PAHs. The seven organisms found were young males, three of them were still alive and the carcasses of the others were reported as in good condition, however no codes were used to categorize their state of decomposition. PAHs were analyzed in fat, muscle and liver tissues. The samples were freeze-dried and Soxhlet extracted, with subsequent cleanup with Florisil. The analyses of PAHs were performed with HPLC with fluorescence detector. There were differences statistically significant between the concentrations obtained between the tissues, with higher concentration of PAHs found in the muscle. Among the 14 PAHs analyzed, the most abundant were the light ones, with 2 to 3 rings. The concentration values, in lipid weight, ranged from 136.47 to 551.46 ng g^{-1} for fat; 796.16 to 891.84 ng g^{-1} for the liver and 513.41 to 3747.43 ng g^{-1} for the muscle. According to the authors, it is probably related to the prolonged fasting of these animals, which may cause the mobilization of lipophilic contaminants, such as PAHs, from the fat tissue to the muscle and liver. The relatively high levels of contaminants, among them the PAHs, which increased induction of CYP1A1 and CYP2B genes reflected the toxicological stress of these sperm whales. According to the authors, this may have contributed to decrease the immune system's defenses, however PAHs intoxication was not considered the cause of death of these whales.

Weijs et al. (2016) carried out a study in an urbanized region of Australia that evaluated the blubber, skin, liver, kidney and muscle tissue from six Australian humpback dolphins (*Sousa sahulensis*) stranded in Moreton Bay in Queensland, which were collected opportunistically between 2002 and 2014. After sampling, the tissues were stored at −20 °C. Because of the small amount of tissue samples available, the analysis of organic compounds was performed only on the blubber three individuals. The state of decomposition of the carcasses was not described. The PAHs were Soxhlet extracted with n-hexane-acetone (4:1 v:v), and purified through silica column. The 16 USEPA-priority PAHs were quantified using CG-MS. PAHs were detected in only one of the three animals analyzed (ΣPAH = 82 ng g^{-1} lw). In this sample only naphthalene, phenanthrene, acenaphthene, anthracene and fluoranthene were detected, with 65% of the concentration due to naphthalene and 27% to phenanthrene. Despite the relatively high concentration of PAHs in the sediment of Moreton Bay (Kannan and Perrotta, 2008), the low concentration in the dolphins was attributed to the rapid metabolism of PAHs in mammals, as result of the efficiency of the cytochrome P450 enzyme system.

Unlike other studies, Binnington et al. (2017) assessed the production of PAHs related to the process of preparation adipose tissue from *Delphinapterus leucas* as a food source for the natives from the northwest Canada in the Arctic region, the same region of the first PAHs reports in cetacean tissues. The analytical procedure involved accelerated solvent extraction (Dionex, ASE), clean up with gel permeation chromatography and final analysis in GC-TQMS. Fat samples from 2 beluga whales (males), aged between 24 and 37 years old, captured during the summer hunting season of 2014 were processed according to native cooking methods. The impacts of the cooking on contaminant levels had little variation, and most cooking processes did not influence the concentrations of PAHs. However, the roasting process increased the concentrations of PAHs, because the burning influenced the deposition of these compounds in the food. The concentrations varied, in lipid weight, between 2.8 and 4.6 ng g^{-1} for the tissues without any treatment and between 181.3 and 265.2 ng g^{-1} for the roasted samples. The fat tissue of the youngest individual presented higher concentration of PAHs than the fat tissue of the oldest one.

Gui et al. (2018) conducted a study with fat samples obtained from 37 *Sousa chinensis* that stranded between 2012 and 2017 in the Pearl River estuary, China. The carcass states of decomposition were indicated and all dolphins used in this study were in good or moderate conditions, carcass code 2 and 3. Fat samples were first wrapped in aluminum foil and then placed in clean plastic bags and frozen at −80 °C. Soxhlet extraction was carried out with n-hexane-dichloromethane (3:1 v:v) and the extracts were purified by gel permeation chromatography (GPC). The PAHs (n = 16) were analyzed using a GC–MS. Concentrations ranged from 17.6 to 6080 ng g^{-1} ww. The relatively high concentrations were associated with the area where these animals live; the Pearl River estuary is a highly urbanized and industrialized region (Gui et al., 2014). Low molecular weight PAHs (2 and 3 rings) were responsible for 99.8% of the total concentrations of the PAHs. Among these, phenanthrene was the most abundant. Among the 4-ring aromatic PAHs, fluoranthene and pyrene were detected in more than 90% of the fat samples, while the other 4–6 aromatic ring PAHs were detected only in some samples. Sex and lipid content had no significant effect on PAHs levels. However, the fat of dolphins stranded in the areas with the highest levels of PAHs in the sediment had significantly higher levels of PAHs than in other areas of the estuary and reflected the contamination of the sampling area. A sharp decline in PAHs levels was observed in the fat tissues sampled over the study period, suggesting that the PAHs load in the area has been gradually reduced. According to the authors, the ratios between phenanthrene and anthracene and fluoranthene and pyrene in sediment indicated both pyrolytic and petrogenic sources of PAHs. Similar conclusion was observed in previous studies on sediments and fish in the same area (Sun et al., 2016; Liu et al., 2017). However, the phenanthrene/anthracene ratios were higher in dolphins than in other matrices, which were associated with the greater water solubility of phenanthrene compared to anthracene, what means that phenanthrene can be absorbed more readily by marine organisms than anthracene (Martinez et al., 2004).

A chapter of the book "Oil Spill Environmental Forensics Case Studies" (Stout and Wang, 2018) was dedicated to the study of the "fingerprint" of oil spilled during the accident in the Gulf of Mexico on the Deepwater Horizon Platform, Texas, USA. This study involved analysis of several matrices (water, sediment, soil and biological samples). Among the biological samples, analysis of petroleum hydrocarbons, PAHs and petroleum biomarkers were performed on a lung tissue sample from a *Tursiops truncatus* carcass found dead during the spill event (Stout et al., 2018). The lung tissue sample was extracted with dichloromethane using a tissue homogenizer (Turrax), cleaned up through GPC, and the analysis of 50 PAHs was performed by GC–MS. The results were expressed in wet weight. The total PAHs concentration was 162 ng g^{-1}. This work also reported that petroleum biomarkers such as hopanes and steranes were not detected and the majority of the

compounds observed were alkyl-naphthalenes, which was considered consistent with the oil vapor phase from the spill, suggesting inhalation and that there was no aspiration of oil in the liquid or aerosol phase.

Recently Sanganyado et al. (2018) published a review of current knowledge and future perspectives regarding bioaccumulation of organic pollutants in *Sousa chinensis* in South Asia (Hong Kong and Xiamen, China). The trends of bioaccumulation of organic pollutants were discussed, focusing on sources, physicochemical properties and usage patterns. In addition, factors that influence bioaccumulation were examined, such as sex, age, food intake and specific distribution in the different studies were evaluated. A total of 12 studies carried out in the region were evaluated and only two contemplated the PAHs (Cagnazzi et al., 2013; Leung et al., 2005). In both studies, high concentrations of PAHs were observed for *S. chinensis*, ranging from 6680 (Leung et al., 2005) to 86,711 ng g^{-1} lw (Cagnazzi et al., 2013), and the remarkable predominance of naphthalene. According to Leung et al. (2005), these high concentrations of PAHs were attributed to oil storage tanks and oil refineries in the region. In this study, the sources were evaluated through indexes normally used in sediment and the metabolization of PAHs was not considered, which may bias the interpretation of the indices. According to the authors, the sources were mixed from petrogenic introductions, from burning coal and biomass. According to Sanganyado et al. (2018), sampling is probably the most critical step in monitoring and characterizing organic pollutants in marine mammals. Taking into account that some parameters such as size, sex, age and frequency are limited when samples are obtained only from stranded animals, non-invasive collection such as biopsy could improve the assessment, however, there is still concern about the effect of this procedure on animal health. Jefferson and Hung (2008) described *S. chinensis* reacting to the dart by causing a wound. A major challenge with biopsy sampling is the heterogeneous distribution of lipids and contaminants in fat. Therefore, there is a need for validation of biopsy sampling to establish stratification levels. However, the authors indicated this sampling technique as a viable alternative to sampling stranded carcasses for the *S. chinensis*.

Zhan et al. (2019) described 16 PAHs in stranded pregnant *Physeter microcephalus*, found alive, from the Huizhou coast of the South China Sea that was sampled immediately during necropsies. Tissue samples were packed in clean plastic storage bags and frozen at −20 °C until further analysis. The analytical procedures were the same described in previous studies (Gui et al., 2014; Gui et al., 2018). Several tissues were analyzed, blood, muscle, liver, lung, placenta, heart, mammary gland, ovary, umbilical cord, epidermis, and blubber. The PAHs were concentrated at higher levels in the liver than those measured in the blubber tissue, suggesting that liver is the main organ for metabolizing PAHs (Takeuchi et al., 2009) and that the tissue used for analysis of PAHs is an important factor when comparing data between studies. Mazzariol et al. (2011) also demonstrated that PAHs were significantly lower in the blubber of sperm whales than in the liver.

Sun et al. (2020) reported concentration of 16 PAHs in the brain and blubber from eight *Sousa chinensis* stranded coast of the Pearl River Estuary, China, from 2016 to 2017. The samples were stored at −20 °C prior extraction. The samples (0.5 g) were freeze-dried and Soxhlet extracted using hexane and dichloromethane. GPC was used to clean up the organic extracts. PAHs were quantified by GC–MS. The quality assurance and the quality control of the analytical method were detailed described, blanks of the methods, spiked blanks, matrix spikes, and 20% of duplicated samples were included in the tests. The lipid contents were provided but not the moisture contents. The mean concentrations of total PAHs in the tissues of *S. chinensis* ranged from 367 ± 261 ng g^{-1} ww in brain to 4513 ± 3366 ng g^{-1} ww in blubber.

5. PAHs concentrations in pinnipeds and sirenians

Studies reporting PAHs in seals and sea lions, collectively called pinnipeds, and sea cows or sirenians are even scarcer than in cetaceans.

Concentrations of PAHs in free-ranging pinnipeds tissues were first reported by Hellou et al. (1990). In this study, 16 PAHs were analyzed in the muscle tissue of four seals, hooded seal (*Cystophora cristata*), ringed seal (*Pusa hispida*), harbor seal (*Phoca vitulina*) and harp seal (*Pagophilus groenlandicus*) captured in the Newfoundland-Labrador waters, Canada. Once caught, the whole animal was frozen immediately and sampled later in the laboratory, or dissected on site and a portion of the important organs frozen (−20 °C). The analytical method was the same presented by the authors for whales and dolphin's tissues, previously mentioned. The total PAHs concentrations, expressed on dw, in Venezuelan oil equivalents, ranged from 0.28 to 3.34 μg g^{-1}, and from 0.10 to 0.80 μg g^{-1} in chrysene equivalents. Fluoranthene and pyrene were the major hydrocarbons presents, while phenanthrene and anthracene were the second major PAHs detected.

Only after 18 years a new study involving PAHs in pinnipeds was published. Stimmelmayr et al. (2018), conducted a study on PAHs levels in oiled seals stranded in the Bering Strait, Alaska. In this study, two spotted seals (*Phoca largha*) and a ringed seal (*Pusa hispida*) with visible signs of oil encrustation were evaluated. Tissue samples (skin, lung, liver, fat, kidney, muscle), tracheal contents, stomach contents, feces, and bile were collected for PAHs analysis. For comparison, tissues from 13 non-oiled seals stranded between 2012 and 2014 were also analyzed. The samples were stored at −20 °C. The carcasses status was classified as codes 2 or 3. The organic contaminants were extracted with dichloromethane using accelerated solvent extraction. The cleanup was performed with gel permeation and analysis in GC-TQMS (45 PAHs). In the bile, PAHs metabolites were analyzed using HPLC coupled to a fluorescence detector. The analyzed tissues of the non-oiled seals showed concentrations below the limit of quantification, except for the muscle (ΣPAHs: 6.4 ± 8.8 ng g^{-1} ww) and stomach contents (ΣPAHs: 4.4 ± 5.2 ng g^{-1} ww). For oiled seals, all samples had a total PAHs concentration, in wet weight, below 44 ng g^{-1}, but the skin sample (280 ng g^{-1}). According to the authors, the concentration of PAHs were low in the inner tissues of the oiled-seals because the effective metabolism and excretion of PAHs by vertebrates. Relatively higher levels of total PAHs were measured only on skin and fat from samples collected from visibly oiled sites. Considering the same animal and same tissue, the higher concentrations of PAHs were found in visible oiled parts of animals. 25 ng g^{-1} ww of PAHs were found in fat sampled from the part with visible oil, meanwhile in the fat removed from a non-oiled part of the same animal the concentration of PAHs was 12 ng g^{-1} ww. Similar conclusion was reported for skin samples. Regardless the presence of oil, the main PAHs found were those with 2–3 aromatic rings. Analyzes of PAHs-metabolites in bile from two oiled individuals indicated exposure to PAHs, but the levels of some metabolites were similar to or lower than the levels of PAHs in non-oiled seals.

6. Conclusions

In general, there are few studies on the analysis of PAHs in marine mammals and the majority of the works is focused on the persistent organic pollutants such as PCBs, organochlorine pesticides, PBDEs and perfluorinated compounds and the PAHs were treated as accessory data.

There is a great divergence between the analytical methods for the determination of PAHs in biological tissues. Many of the published studies used the fluorescence detector technique to quantify the PAHs. Remarkably, in these works in which fluorescence detector was applied, the highest concentrations of PAHs were found in the tissues. It must be considered that fluorescence detectors are not compound-specific and can be biased by other fluorescent compounds than PAHs; it means that unlike the mass spectrometer, fluorescence detectors cannot distinguish coeluted peaks what can result in overestimated concentrations or false positives. Further, it must be considered the lack of internal standardization when fluorescence analysis is applied reducing the precision and accuracy of the method.

The use of different solvents for extraction of the PAHs may have

influenced the results, especially when the concentrations were presented on a lipid weight basis, because different solvents have different efficiencies for lipid extraction and also differ in the classes of lipids that can be extracted, which in turn affect the final reported result. The differences in the normalization of concentrations by wet weight, dry weight, lipid weight, chrysene or oil equivalent or normalization by the percentage of extractable organic matter or by some oil, makes it difficult to compare the results of the different works since the moisture and lipid contents were not reported in most of them. Important information such as amount of extracted tissue (which influences quantification limits), details about the quality control of the analytical method such as the recovery of the surrogate compounds, quality of the analytical blanks, use of certified standards, repeatability and reproducibility were also not cited in most studies.

The older studies were carried out with carcasses of animals found dead, it can be assumed that the tissue was chosen according to its availability, regardless of the state of decomposition of the carcass, which can compromise the results because the tissues could be exposed and subject to external contamination and because the PAHs could have been biodegraded by microorganisms. More recent works have evaluated living organisms, with non-destructive sampling, where researchers choose to collect blood and biopsy samples. These methods avoid problems with the reduced number of samples, which collection is often random in the case of carcasses, and with sex or age of the animal assessment, which might be difficult to unveil due in advanced states of decomposition. With more focused non-destructive sampling, it is also possible to choose animals with compatible health status and body mass to analyze and compare. On the other hand, comparisons with results in carcasses are impaired.

In general, despite efforts to compare individuals taking into account sex or age, published studies did not show any correlation between these parameters and PAHs concentrations. The relatively high concentrations of PAHs in the tissues were usually associated with the influence of urban and industrialized areas in which the organisms lived, or eventually with an oil spill or oil products.

The highest concentrations of PAHs presented in all published works involving marine mammals occurred when the results were normalized by the lipid weight, what may be result not only of the high concentration of PAHs but also of the low lipid content in the tissue. As the lipid content in the analyzed tissue most likely do not correspond to the average levels in the animal, it is not possible to extrapolate these concentrations of PAHs to the body weight of the animal. It is also necessary to carefully evaluate the results of the studies in which the PAHs quantification was performed using a fluorescence detector, because, as discussed, it is not a selective detector and therefore there is a tendency to increase the results when this method is used.

Naphthalene was the predominant PAH found in the tissues of marine mammals in almost all published works. However, naphthalene is one of the most common contaminants in PAHs analyses. Therefore, there is also the question whether this compound is more bioavailable to animals, which would justify its predominance, or whether the concentrations found derive from analytical contamination. This is a difficult question because most of the works do not describe the quality control procedures and there is no available or commercial standard reference material for PAHs in tissues of marine mammals.

CRediT authorship contribution statement

Rafael André Lourenço: Conceptualization, Investigation, Writing - Original Draft and Review & Editing. Josilene da Silva: Investigation, Writing - Original Draft and Review. Satie Taniguchi: Investigation, Writing - Review, Fabiana Dias da Costa Gallotta - Writing Review. Marcia Caruso Bícego: Conceptualization, Investigation, Writing - Original Draft and Review.

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Consent to publish

Not applicable.

Declaration of competing interest

The authors declare that they have no competing interests.

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