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Investigating the potential impact of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) on gene biomarker expression and global DNA methylation in loggerhead sea turtles (*Caretta caretta*) from the Adriatic Sea

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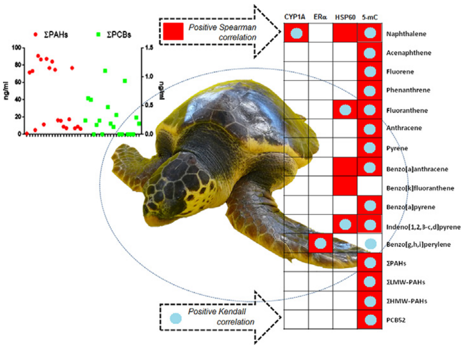
HIGHLIGHTS

- Plasma levels of PCBs and PAHs were detected in Adriatic loggerheads.
- Associations between 3 gene biomarkers assessed and plasma levels of some PAH congeners were found.
- Global DNA methylation was positively correlated with selected PAH congeners and total PAHs.
- Whole blood cell expression of gene biomarkers as strategy for assessing POPs impact on sea turtles.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are priority contaminants that bioaccumulate through the food webs and affect the biology of a variety of resident and migratory species, including sea turtles. Few studies have evaluated toxicological biomarkers of exposure to PAHs and PCBs in these animals. The present paper reports the results of an initial field study to quantify the association between plasma concentrations of PAHs/PCBs and whole blood cell expression of gene biomarkers in juvenile loggerhead sea turtles (*Caretta caretta*) rescued along the Italian coasts of the northern and central areas of the Adriatic Sea. While detectable levels of PAHs were found in all plasma samples examined, only three PCB congeners (PCB52, PCB95, and PCB149) were noted, with detection percentages ranging between 48% and 57%. A significant correlation was found between 3 of the 6 gene biomarkers assessed (HSP60, CYP1A and ERα) and plasma levels of some PAH congeners. In contrast, no significant association between PCB burden and gene expression was observed. The global DNA methylation levels were significantly and positively correlated with the concentrations of most of the PAHs and only one of the PCB congeners (PCB52).

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Biomarkers
Gene transcription

The relation between PAH concentration and gene expression in whole blood cells suggests that these genes may respond to environmental contaminant exposure and are promising candidates for the development of biomarkers for monitoring sea turtle exposure to persistent organic pollutants (POPs).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), two of the most ubiquitous and predominant classes of persistent organic pollutants (POPs), reflect human activities in marine and coastal environments (Field and Sierra-Alvarez, 2008; Perugini et al., 2007). PAHs are released by natural events such as volcano activity and forest fires, as well as by such anthropogenic sources as waste, sewage, and industrial and agricultural outfalls (Zychowski and Godard-Codding, 2017). On the contrary, no known natural sources of PCBs exist and, once released in the environment, they can last for decades because of their persistence and slow degradation (Grossman, 2013).

The North Adriatic basin is particularly vulnerable to the bioaccumulation of both PCBs and PAHs due to the physical-chemical properties and ecological processes that mark this area (Annibaldi et al., 2015; Korlevic et al., 2015; Marini et al., 2008; Pavoni et al., 2003). Previous studies have shown the widespread presence of these contaminants along the eastern coasts of Italy (Manodori et al., 2006; Pavoni et al., 2003). In this regard, we recently monitored the seawater PCB and PAH concentrations in a coastal area of the central Adriatic Sea, finding moderate to high contamination levels of PAHs (Cocci et al., 2017a; Taffi et al., 2014).

PAHs and PCBs are considered priority contaminants that can bioaccumulate and biomagnify across marine ecosystems (Frapiccini et al., 2017; Perugini et al., 2013; Taffi et al., 2014), affecting the biology of a variety of marine organisms (Rodriguez-Hernandez et al., 2017), including loggerhead sea turtles (*Caretta caretta*, Linnaeus 1758) (D'Ilio et al., 2011). PAHs and PCBs move through the food chain in different ways: PAHs biodilute, perhaps because of their potential rapid metabolism and elimination (Amiard-Triquet and Rainbow, 2011; Takeuchi et al., 2009), while PCBs bioaccumulate. The well-known toxic effects of PAHs and PCBs on wildlife range from carcinogenicity to immunosuppression, liver damage, and endocrine disruption (Collier et al., 2014; Olenycz et al., 2015; Zychowski and Godard-Codding, 2017). Their mechanisms of toxicity involve a variety of molecular initiating events and pathways, including modulation in target gene expression (*e.g.* steroid hormone, antioxidant and detoxification genes) and DNA methylation (Cocci et al., 2017c; Dupuy et al., 2014; Mortensen and Arukwe, 2007; Ruiz-Hernandez et al., 2015; Spromberg and Meador, 2005).

Changes in gene expression of Estrogen Receptors (ERs), Cytochrome P4501A (CYP1A) and heat-shock proteins (HSPs) have been found to be sensitive biomarkers of environmental exposure to PCBs and PAHs in different fish species (Cocci et al., 2017a; Huang et al., 2016; Mahmood et al., 2014). Moreover, exposure to selected PAHs was found to affect both gene-specific and global DNA methylation in zebrafish (Fang et al., 2013). Ecotoxicological studies on marine wildlife have shown the usefulness of biomarkers, which can serve as indicators of PAH/PCB exposure and elucidate potential underlying mechanisms (Cocci et al., 2017a,c; Sarkar et al., 2006; Viarengo et al., 2007).

Concerning sea turtles, alarming levels of POPs, including PAHs, PCBs and organochlorine pesticides (OCPs), have recently been found in live loggerheads, suggesting that these pollutants are a real threat to sea turtle conservation (Bucchia et al., 2015; Camacho et al., 2012, 2013, 2014; Hamann et al., 2010; Keller et al., 2004a; Novillo et al., 2017). However, there is evidence that PAHs and PCBs are consistently the predominant POPs (in concentration) in sea turtles from distinct geographic areas. In this regard, it has been demonstrated that regional geographic differences in pollution correlate to differing PAH/PCB concentrations detected in sea turtles, with higher levels of exposure found in individuals rescued in the northern Adriatic Sea, which is a semi-closed and highly anthropized basin (Bucchia et al., 2015; Lazar and Gracan, 2011; Lazar et al., 2011). Interestingly, the northern and middle Adriatic Sea, with its shallow water and rich benthic communities, is a major feeding habitat for turtles in the demersal stage (Lucchetti et al., 2016). These turtles, especially juvenile specimens, show strong fidelity to these specific foraging areas (Casale et al., 2012). In fact, tag returns (Casale et al., 2007) and satellite tracking data (Casale et al., 2012) have demonstrated the long-term permanence of juveniles in the neritic area. Overall, the North Adriatic Sea can be considered an important developmental area for juvenile loggerheads which then move southward along the Italian coast by taking advantage of favorable currents (Casale and Margaritoulis, 2010).

Although the number of studies monitoring the effects of pollution in sea turtles continues to increase, contributing to the definition of the baseline levels of these contaminants, only limited information is available on the potential mechanisms by which PAHs and PCBs affect these species. It has been shown that bioaccumulation of PAHs results in hematological changes in both juvenile and nesting population of loggerhead sea turtles (Camacho et al., 2013; Casal and Oros, 2009; Lutcavage et al., 1997). Similarly, high PCB concentrations were found to correlate with certain biochemical parameters of blood (Camacho et al., 2013), and possibly to contribute to causing anemia in sea turtles (Keller et al., 2004b). However, few studies have evaluated the toxicological biomarkers of exposure to PAHs and PCBs in sea turtles.

Thus, the present study aimed to determine PAH and PCB concentrations in the blood (collected in a non-lethal manner) of loggerhead turtles rescued along the Italian coasts of the northern and middle Adriatic Sea, and to investigate the relationship between pollutant burden levels and whole blood mRNA abundance profiles of gene biomarkers involved in physiological mechanisms such as chemical detoxification (CYP1A), the immune and endocrine systems (Interleukin 1β (IL-1β) and ER α), respiratory pathways (cytochrome *c* oxidase subunit 1 (COI)) and stress response (HSP60, 90). Gene expression data provide quantitative information at the molecular level that can be used for early detection of biological disturbance and serious health risk associated with environmental contamination. There may be many benefits to using blood as a target tissue for evaluating molecular biomarkers that indicate potential pathological changes in other organs of the body due to systemic exposure (Morey et al., 2016), especially in endangered species. Significant positive correlations between gene biomarker expression in blood cells and contaminant exposure have recently been found in different turtle species, in both *in vivo* and *in vitro* studies (Cocci et al., 2017b; Drake et al., 2017). We also investigated whether loggerhead exposure to PAHs/PCBs is associated with altered levels of global DNA methylation, in order to examine the potential correlation of a single epigenetic endpoint with marine pollutants in this reptilian species.

2. Material and methods

2.1. Sampling

In the present study, we selected 20 live immature loggerhead sea turtles found entangled in fishing nets or cold-stunned along the Italian coasts of the northern and middle Adriatic Sea (Fig. 1) between 2014