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# Occurrence and distribution of PAHs in stranded dolphin tissues from the Northwestern Mediterranean

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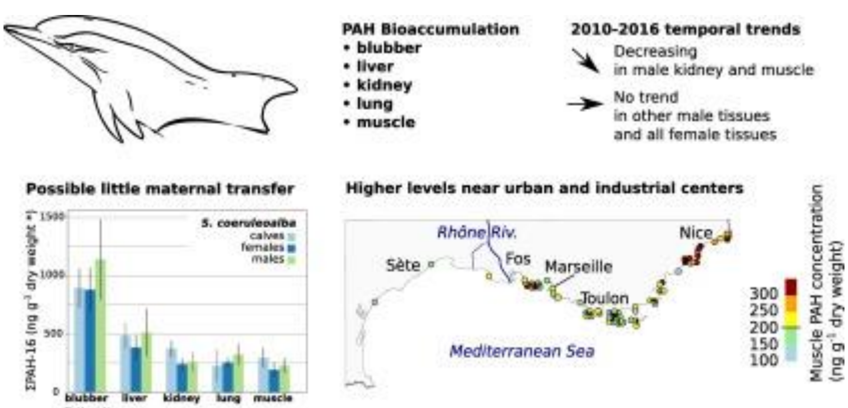
**Abstract :**

There are few cetacean tissue-specific polycyclic aromatic hydrocarbon (PAH) concentration studies in the Mediterranean, despite this region is among the most subjected to chemical contamination. PAH analyses were conducted in different tissues of striped dolphins (Stenella coeruleoalba, N = 64) and bottlenose dolphins (Tursiops truncatus, N = 9) stranded along the French Mediterranean coastline from 2010 to 2016. Comparable levels were measured in S. coeruleoalba and T. trucantus (1020 and 981 ng g−1 lipid weight in blubber, 228 and 238 ng g−1 dry weight in muscle, respectively). The results suggested a slight effect of maternal transfer. The greatest levels were recorded by urban and industrial centers, and decreasing temporal trends were observed in males muscle and kidney, but not in other tissues. As a conclusion, the elevated levels measured could represent a serious threat to dolphins populations in this region, particularly by urban and industrial centers.

**Graphical abstract**



**Highlights**

► PAH were elevated in *Stenella coeruleoalba* and *Tursiops truncatus*. ► Lower levels in *S. coeruleoalba* females suggest little maternal transfer. ► Naphthalene, fluorene, 5- and 6-rings PAHs dominated in all tissues. ► Highest levels were localized by urban and industrial areas. ► Constant time trends in blubber and liver, possibly decreasing in muscle and kidney.

**Keywords** : Striped dolphin, Bottlenose dolphin, PAHs, Mediterranean Sea, Bioaccumulation

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants, which are emitted by human activities implying fossil fuels (*e.g.* petroleum, coal), fossil and non-fossil combustion processes (oil, coal, gas, and biomass burning), but also natural sources (forest fires, volcanoes) (Howsam and Jones, 1998). As a result, PAHs are widespread in all environmental compartments (air, water, soil, biota) and reach the marine environment by atmospheric deposition, remobilization from contaminated soils, and direct release from industrial (*e.g.* petrochemical, refinery, steel production, fossil fuel power plants) and urban centers (road flushing, sewage). In this regard, the North-West Mediterranean Sea is considered as one of the most relevant polluted area (Garcia-Alvarez et al., 2014) with potentially critical PAH levels in sediment (Sarrazin et a., 2006 ; Wafo et al., 2017 ; Bouchoucha et al., 2021), seawater (Guigue et al., 2011 ; Guigue et al., 2014) and marine biota (Sarrazin et al., 2006 ; Dron et al., 2019 ; Bouchoucha et al., 2021).

Bottlenose dolphins (*Tursiops truncatus*) and striped dolphins (*Stenella coeruleoalba*) are the most common cetaceans in the Northwestern Mediterranean (Panigada et al., 2017). According to the several potential sources of PAH in this region (urban and industrial centers, Rhône River inputs), these dolphin populations could be exposed to significant levels of PAHs (Dron et al., 2019 ; Bouchoucha et al., 2021). *T. truncatus* is known to be relatively sedentary and coastal, and the genetic structure of *S. coeruleoalba* populations in the western Mediterranean suggested distinct living areas, with differentiated kinship and inshore/offshore habits (Gaspari et al., 2007 ; Gonzalvo et al. 2016). Dolphins are top predators, and likely mainly exposed to PAHs through feeding, even though no biomagnification of PAH through the marine food chains was evidenced (Takeushi et al., 2009 ; Dron et al., 2019). PAH bioaccumulation in dolphin tissues is also regulated by elimination through metabolic pathways, leading to oxidized compounds such as epoxide and hydroxylated analogues which also contribute to the global

PAH toxicity (Albers and Loughlin, 2003 ; Fair et al., 2010). Also, significant levels of PAHs remaining in dolphins tissues could be an indication of the overload of the mixed-function oxidase system in PAH elimination (Gui et al., 2018). The exposure of dolphins to PAHs can lead to various toxicological effects, such as mutagenicity, carcinogenisity, and developmental issues (Albers and Loughlin, 2003). Obviously, the toxicological consequences of significant exposures to PAHs can be a threat to the dolphins populations, in particular in the North-West Mediterranean where they are also exposed to other pollutants such as PCBs, pesticides and trace metals (Sarrazin et al., 2006 ; Wafo et al, 2014 ; UNEP, 2016 ; Bouchoucha et al., 2021 ; Dron et al., 2022).

In order to evaluate the risks related to PAH exposure in dolphins, and more generally in the marine environment, the determination of PAH levels in dolphins tissues is a prerequisite. However, PAH bioaccumulation data in cetacean remain scarce worldwide (Lourenço et al., 2021), particularly in the Mediterranean. To our knowledge, such measurements in the whole Mediterranean region are limited to 3 reported studies, the latest concerning 2009 samples (Marsili et al., 2001 ; Fossi et al., 2010 ; Marsili et al., 2014). Moreover, these works were mainly concerned by whales. Only a unique study reported PAH bioaccumulation levels in Mediterranean dolphins, with 25 samples collected on free-ranging *S. coeruleoalba* in 1993. Thus, it comes without saying that the data provided in the present paper is essential to assess further PAH contamination of cetaceans in the Mediterranean region. Here, 16 PAH congeners were investigated in 5 tissues (blubber, liver, kidney, lung, and muscle) of 64 *S. coeruleoalba* and 9 *T. truncatus* specimens stranded on the French Mediterranean coastline during the 2010-16 period. The relations between the PAH levels and primary biological variables (sex, life stage) were examined. This dataset represents today a unique feature, contributing to further interpretations of PAH levels in cetaceans according to potential pathways, time evolution, and spatial distributions, which were also discussed here.

The sampling of 64 *S. coeruleoalba* and 9 *T. truncatus*, found stranded from May 2010 to April 2016, was performed by the National Marine Mammals Stranding Network (RNE), coordinated by the Mediterranean Cetaceans Study Group (MIRACETI), mainly on the eastern part of the French Mediterranean coastline. This area hosts several urban centers (Montpellier, Marseille, Toulon, Nice), intensive agriculture in the west, a major industrial harbor (Fos, 50 km west from Marseille) and a military harbor (Toulon), and the Rhône River delta. Relatively high PAH levels were identified in fish, mussel and sediment in the Fos-Berre and Toulon areas (Zorita et al., 2007 ; Wafo et al., 2017 ; Dron et al., 2019 ; Bouchoucha et al., 2021), and in sediment in many spots along the whole coastline (Bouchoucha et al., 2021).

Body length and sex were determined on site, along with localization and decomposition condition category (DCC). Up to 5 tissues (blubber, liver, kidney, lung and muscle) where sampled when possible, as detailed previously (Dron et al., 2022). Biological data and sampling information are summarized in Supporting Information S1, and full data is provided as a Supplementary Information CSV file. Most *S. coeruleoalba* carcasses were in very fresh state (DCC 1, N = 31) or in intermediate condition DCC 2 and 3 (N = 11 and 18, respectively), while decomposed status DCC 4 and 5 represented only 6.3 % of the carcasses (N = 3 and 1, respectively). The DCC was comparably distributed for *T. truncatus*, with N = 6, 1, 0, 1 and 1 for DCC 1, 2, 3, 4, and 5, respectively.

The tissues mean moisture contents were determined gravimetrically after freeze-drying, and were 25% in the blubber, 70% in the liver, and 75% in the other tissues. In the blubber, lipids represented 71.9 ± 5.8% and 71.7 ± 6.8% for *S. coeruleoalba* and *T. truncatus*, respectively. The newborn and calves were considered separately according to a total length below 120 cm for *S. coeruleoalba* (N= 11), in accordance with previous works (Dron et al., 2022), and no *T. truncatus* calves were sampled. This corresponds to an age of 1.5 years for *S. coeruleoalba* according to Marsili et al. (2004), when calves feeding habits switch from lactation to solid food, affecting their exposure to pollutants (Miyazaki, 1977 ; Calzada et al., 1996).

The 16 PAH congeners defined by the U.S. Environmental Protection Agency (USEPA) priority list, naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (FlA), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), benzo(g,h,i)perylene (Bpe), dibenzo(ah)anthracene (DBA), and indeno(1,2,3-cd)pyrene (IPy), were determined following a long settled and previously described extraction step (Sarrazin, 2006 ; Dron et al., 2019) and analytical procedure (Dron et al. 2021). Briefly, the samples were extracted with acetone in an ultrasonic bath. An aliquot of the supernatant was filtered, and 15 mL of ultrapure water were added. The solution was passed through a 1 g C18 cartridge, and the PAH congeners were eluted with 3 mL acetone followed by 2 mL methanol.

The final extracts were analyzed by gas chromatography (GC, Agilent 6890N), equipped with a mass spectrometry detector (MS, Shimazu QP2010), a deactivated fused-silica guard column (5 m x 0.25 mm) and a fused-silica capillary column (30 m x 0.25 mm x 0.25 µm, Phenomenex ZB-50). Samples were injected in the splitless mode, with the transfer line and injector held at 280 °C. The oven temperature was programmed as follows: 100 °C during 1 min, from 100 °C to 240 °C at 10 °C $min^{-1}$, and from 240 to 280 °C at 1.5 °C $min^{-1}$, hold for 15 min. Quantification was performed in the SIM mode, and detection limits were 1 ng $g^{-1}$ for each congener. Each analysis sequence included at least 3 blank runs and 3 analyses of certified IAEA-451. The relative standard deviation (RSD), as calculated for the certified material IAEA-451 (N = 18), was below 15% for all congeners except Ipy, Acy (both 23.8%) and BbF (28.9%). The concentrations measured in the certified material were consistent with the certified values, confirming the reliability of the method (Supporting Information S2). Duplicate or triplicate runs were realized for each sample, and their mean values were considered.

All the data treatment and analyses were performed with the R software version 3.6 (R Core Team, 2020). The comparisons of means were realized with the Tukey post-hoc test after an analysis of variance (aov and TukeyHSD functions, “stats” package).

The significance of the temporal trends were evaluated by the Kwiatkowski–Phillips–Schmidt–Shin KPSS test (kpss.test function, “tseries” package), which allows for checking trend and level stationarity (Kwiatkowski et al., 1992). In the kpss.test function, the null hypothesis ($p > 0.1$) is stationarity, meaning an homogeneous trend (regular increasing or decreasing trend) when trend stationarity is tested, or no trend when level stationarity is tested. To enhance the visualization of temporal trends, the graphical outputs were supported by moving regression (geom_smooth function, “ggplot2” package) used with LOESS (locally estimated scatterplot smoothing) at a 0.9 span value and a 0.95 confidence interval.

Full data is provided as a Supplementary material CSV file.

The ΣPAH-16 concentrations were comparable in *S. coeruleoalba* (striped dolphins) and *T. truncatus* (bottlenose dolphins), for all tissues (Table 1). These levels fell in the higher end of ranges reported by other studies in cetaceans worldwide, when considering results from GC-MS analyses as recommended by Lourenço et al. (2021). In the Mediterranean region, only a very few works described PAH measurements in cetaceans (Marsili et al., 2001 ; Fossi et al., 2010 ; Marsili et al., 2014), all using HPLC-fluorescence analysis. However, the levels recorded here in dolphins stranded along the French Mediterranean were much lower than those reported for blubber in free-ranging *S. coeruleoalba* (36,200 ng $g^{-1}$ wet weight in average) and *Balaenoptera physalus* (9050 ng $g^{-1}$ wet weight) in the Ligurian and Ionian Seas, in 1993-96 (Marsili et al., 2001). The latter were presumably related to the MT Haven oil spill near Genoa, Italy (1991). Also, a subsequent paper reported lower levels for 2008 samples collected from Ligurian Sea free ranging *B. physalus* blubber, at about 5000 ng $g^{-1}$ dry weight (Fossi et al., 2010). The latest work in the Mediterranean region reported levels down to 329 ng $g^{-1}$ lipid weight in blubber samples of *Physeter macrocephalus* stranded in 2009 along the south Adriatic coastline (Marsili et al., 2014), significantly below what observed in the present study ($p < 0.05$). The contrast observed in this latest study compared to the earlier ones also questioned towards differences due to sampling, between free-ranging biopsies and stranded dolphin tissues. Clearly, further studies and surveys are required to address such primary points. Finally, the ΣPAH-16 concentrations measured here in dolphin tissues were also much higher than in fish of high trophic level in the Mediterranean Sea, *i.e.* 5 - 50 ng $kg^{-1}$ dry weight (Perugini et al., 2007 ; Dron et al., 2019), in accordance with the long lifetime of dolphins, their status of top predators, and high bioaccumulation potential in fat tissues such as blubber and melon.

The ΣPAH-16 levels were also very comparable in kidney, lung and muscles, and slightly higher in liver. In the blubber, on a wet weight basis and considering the tissues water and lipid contents detailed previously, concentration levels were a factor 3 to 7 more elevated than in other tissues, for both species. It is globally admitted that PAH metabolize relatively quickly in marine vertebrates (Lourenço et al., 2021), and the contrasts among tissues were less pronounced than for PCBs in the same *S. coeruleoalba* specimens (Dron et al., 2022). However, the higher PAH levels in blubber, and in a lesser extent in liver, still indicated that the bioaccumulation of PAHs occured in dolphins lipid tissues, as previously observed for *P. macrocephalus* (Marsili et al., 2014). Contrarily to PCBs and organochlorine pesticides, the ΣPAH-16 concentrations were not correlated between tissues, except between muscle and kidney ($R^2 = 0.67$, $p < 0.001$) and in a lesser extent between liver and kidney ($R^2 = 0.30$, $p = 0.037$). The values between muscle and kidney tissues were already strongly correlated for PCBs and organochlorine pesticides (Dron et al., 2022), highlighting the similar characteristics of these two tissues toward organic contaminants bioaccumulation. On the other hand, the lack of correlation of ΣPAH-16 levels in blubber with all other tissues supported the hypothesis of a stronger contribution of metabolic pathways to eliminate PAHs, compared to PCBs (Fair et al., 2010).

**Table 1**
Summary of ΣPAH-16 levels in stranded dolphin tissues (ng $g^{-1}$ lipid weight in blubber, and in ng $g^{-1}$ dry weight in other tissues) from the French NW Mediterranean coastline (N is the number of samples).

| | | blubber | liver | kidney | lung | muscle |
|---|---|---|---|---|---|---|
| *Stenella coeruleoalba* | mean (*N*) | 1020 *(42)* | 464 *(53)* | 269 *(50)* | 280 *(17)* | 228 *(61)* |
| | median | 1000 | 416 | 256 | 270 | 209 |
| | range | 606-1790 | 214-904 | 157-457 | 131-439 | 103-419 |
| *Tursiops truncatus* | mean (*N*) | 981 *(5)* | 457 *(7)* | 303 (7) | 463 *(2)* | 238 *(8)* |
| | median | 893 | 443 | 328 | 463 | 235 |
| | range | 524-1651 | 278-648 | 210-385 | 316-610 | 146-354 |

The state of conservation of the dolphins carcass had no significant incidence on the ΣPAH-16 levels, as deduced from Tukey HSD tests performed for all studied tissues ($p > 0.5$ between all DCC values). This was a noticeable result indicating that PAH levels were not affected by the residence time and the degradation of the bodies. It also presumed that PAH measurements from stranded and free ranging specimens could be compared.

The ΣPAH-16 concentrations in female *S. coeruleoalba* were the lowest compared to males and calves in all tissues, except lung where all had comparable levels (Figure 1). The differences between sexes and calves were less contrasted than for organochlorine contaminants (Dron et al., 2022). Interestingly, males had significantly higher ΣPAH-16 levels in blubber and liver, but calves had the highest levels in muscles and kidney. While significant negative correlations were accordingly observed between body length and ΣPAH-16 in kidney and muscle ($R^2 = 0.22$ and 0.21, respectively, $p < 0.001$), there was no correlation in blubber and liver ($p > 0.1$). Finally a positive correlation was observed between body length and ΣPAH-16 in lung tissues, but covering a limited number of samples compared to the other tissues (Supporting Information S1). These results still corroborated that PAH accumulated preferably in lipid tissues, but that the transfer of PAH through lactation and placental pathways was limited, in particular when compared to PCBs. Surprisingly, female *T. truncatus* presented the highest levels in blubber, liver and kidney, but these results relied on a limited number of specimens (Figure 1).

The most prominent PAH congener was Nap in all tissues of *S. coeruleoalba* as well as of *T. truncatus*, accounting for 17.6% and 20.9% in average, respectively (Figure 2). In the blubber samples of *S. coeruleoalba*, Flu (10.4%) and larger PAHs such as DBA, BaP and Ipy (9.5%, 8.1%, and 12.3%, respectively) had also strong contributions (Supporting Information S3). Comparable contributions were observed in *T. truncatus* blubber, except that Ace (11.6%) was relatively high but not Ipy (5.6%). The higher contribution of Nap to ΣPAH-16 was consistent with previous measurements in cetaceans (Marsili et al., 2014 ; Gui et al., 2018 ; Zhan et al., 2019), and fish (Dron et al., 2019). It was interesting to note that *S. coeruleoalba* calves had higher proportions of low molecular weight PAHs than adults, but lower contributions of high molecular weight PAHs (Figure 2). It could indicate that low molecular weight PAHs are more subjected to maternal transfer (placental and lactation) while high molecular weight PAHs levels are mainly the result of their bioaccumulation with time.

The study of the geographical distribution of the ΣPAH-16 concentrations focused on the tissues of *S. coeruleoalba*, as insufficient observations were available for *T. truncatus*. It should also be noted that studying geographical aspects in stranded dolphins must be taken with caution, due to that the distances covered by drifting carcass or affected individuals as well as the exact perimeter of the living habitat remain relatively unknown (Gui et al., 2018 ; Dron et al., 2022). Nevertheless, most stranding sites were localized within a 300 km coastline including three major urban centers (Marseille, Toulon, Nice), a major industrial area (Fos) and the mouth of the Rhône river (streamflow of 1700 $m^3 s^{-1}$ in average) which brings waters from the anthropized Rhône valley. Interestingly, the ΣPAH-16 highest concentrations in all tissues were found near the urban and industrial centers, as found by Gui et al. (2018) in the Chinese coast around Macau. The highest ΣPAH-16 levels in blubber were measured around Fos and Toulon and were particularly elevated in muscle tissues in the Nice and Fos areas (Figure 3). Similarly, concentrations in kidney were the highest by Fos and Nice, and in liver around all areas subjected to anthropic pressure (Supporting Information S4). This contrasted with the homogeneous PCB levels measured in the same samples on this coastline (Dron et al., 2022). Assuming the hypothesis that metabolic elimination is more efficient towards PAH than PCBs, their bioaccumulation could reflect a shorter integration time, and thus be more affected by local contamination sources.

The temporal evolution of the ΣPAH-16 concentrations in *S. coeruleoalba* tissues was also examined, and the results of the KPSS tests for level and trend stationarity are detailed in

Supporting Information S5. Despite the relatively short time frame investigated here (5 years, 2010-2016), significantly decreasing trends were observed for male individuals in muscle (Figure 4) and kidney tissues (Supporting Information S6). Otherwise, no trends were identified, meaning that levels remained stable over the period in blubber and liver tissues (calves, females and males), as well as in calve and female muscle and kidney tissues. Even though the low number of calves restrained the statistical analysis, a slight decrease also appeared graphically in muscle and kidney tissues. The lack of trend for PAHs in female dolphins could be related to the, even though limited, transfer or loss of contaminants during gestation and lactation. The stability of ΣPAH-16 in blubber and liver tissues among time could be attributed to longer integration time and storage capacities of these fat tissues compared to muscle and kidney. PAH temporal trends are particularly scarce in the literature, but clear decreasing trends were reported in the blubber of *Sousa chinensis* (Indo-Pacific humpback dolphins) stranded in the Pearl River estuary, on a comparable time period of 5 years from 2012 to 2017, from 4740 down to 346 $ng^{-1}$ wet weight (Gui et al., 2018). As well, a decreasing trend over 25 years can be presumed from the few separate works on different species in the Mediterranean Sea (Marsili et al., 2001 ; Fossi et al., 2010 ; Marsili et al., 2014).

As the Nice region was a particular hotspot for PAH contamination of stranded *S. coeruleoalba* (Figure 3), and that such hotspots may interfere with temporal trends (Gui et al., 2018), the evolution of the ΣPAH-16 levels with time was additionally investigated excluding this area (*i.e.* excluding longitude > 7° E). The decreasing trend was still significant in male dolphins kidney tissues (Supporting Information S5 and S6), but not in muscle tissues (Supporting Information S5), even though a slight decrease is still visible graphically (Figure 4). Thus, the temporal trends in muscle and kidney could have been affected by a specific event in the Nice region, but could also reflect a diminishing exposure to PAHs, which could eventually echo in the future, to blubber and liver ΣPAH-16 levels.

To conclude, the ΣPAH-16 concentrations measured here in stranded dolphins from the French Mediterranean coastline were not extreme, but still remained at a high level. Correlations were observed between ΣPAH-16 in muscle and kidney tissues, suggesting comparable bioaccumulation characteristics. Female showed lower ΣPAH-16 concentrations than males and calves, indicating a little incidence of maternal transfer during gestation and lactation, in much lower proportions than for organochlorine contaminants such as PCBs. Even though spatial aspects should be taken with care in dolphin bioaccumulation studies, higher levels were found in the most anthropized areas, around urban centers or industrial areas. And finally, the temporal trends were globally steady, but decreasing trends were still observed in males kidney and muscle tissues.

These results highlighted the necessity of sampling at least two different tissues for PAH bioaccumulation monitoring surveys in cetaceans, for instance blubber and muscle, as bioaccumulation characteristics may differ among tissues and consisted in a valuable source of information for further interpretations. They also confirmed that PAH pollution remains a strong matter of concern in the Mediterranean marine environment, and that further monitoring in cetaceans will be essential to evaluate and better understand contamination trends and pathways.

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Figure Captions

***Figure 1. (2-columns width)***

*Mean ΣPAH-16 concentrations and standard deviations (error bars) in a) Stenella coeruleoalba and b) Tursiops truncatus tissues among sex (and young S. coeruleoalba length < 120 cm). Letters above bars indicate significant differences between sexes and youngs (Tukey HSD test, $p < 0.05$) and numbers at the bottom of the bars indicate the corresponding number of samples. * Blubber concentrations in ng $g^{-1}$ lipid weight.*

***Figure 2. (2-columns width)***

*Mean relative contributions (%) and standard deviations of congeners to ΣPAH-16 in a) S. coeruleoalba blubber, b) S. coeruleoalba muscle, and c) in T. truncatus blubber.*

***Figure 3. (2-columns width)***

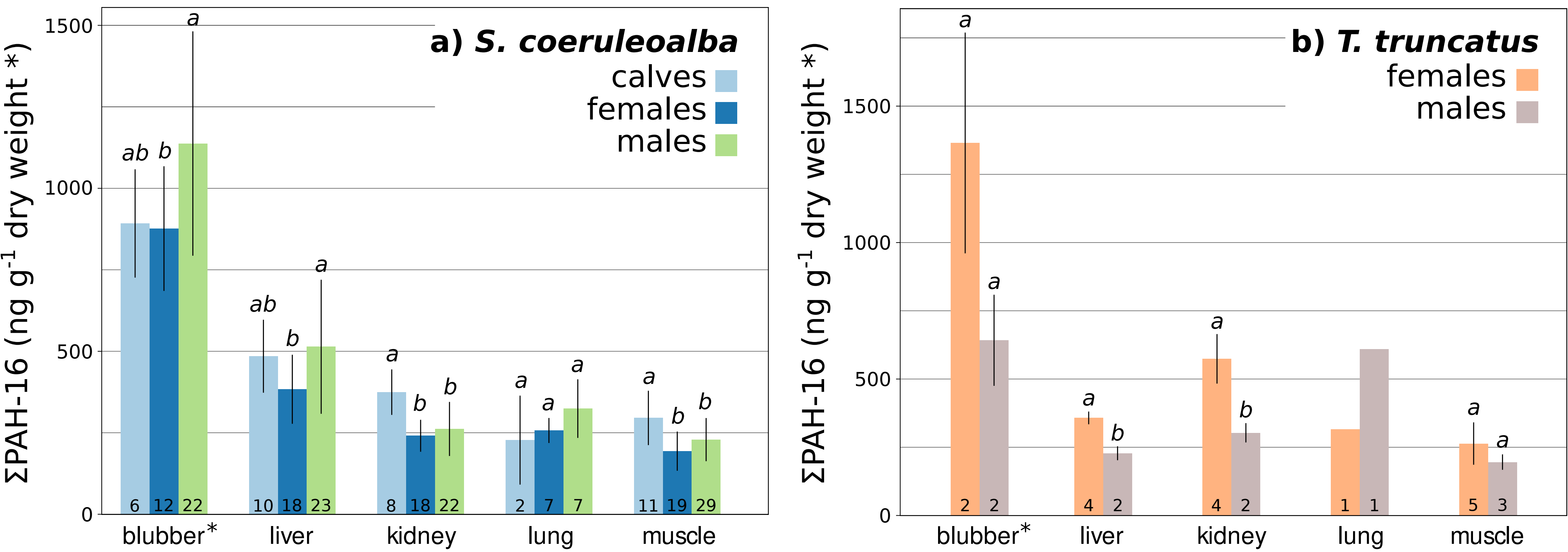
*Geographical distribution of the ΣPAH-16 levels measured in blubber and muscle tissues of S. coeruleoalba stranded on the French Mediterranean coast (solid bars in the legends indicate medians).*

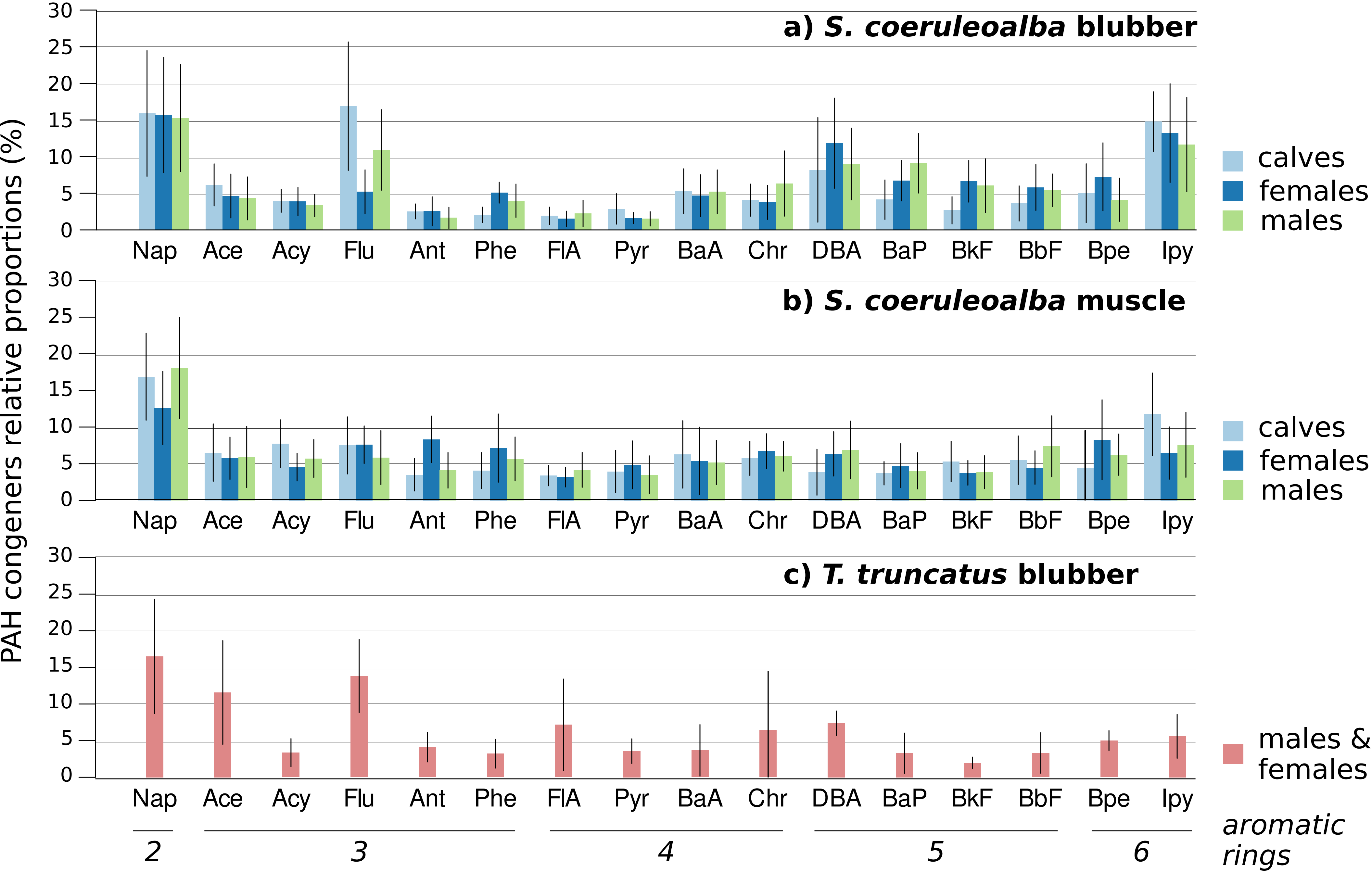
***Figure 4. (2-columns width)***

*Evolution of PAH concentrations from 2010 to 2016 in muscle tissues of all studied Stenella coeruleoalba a) calves, b) females and c) males, and S. coeruleoalba individuals restricted to longitude < 7° d) calves, e) females and f) males.*

Figure(s) 1

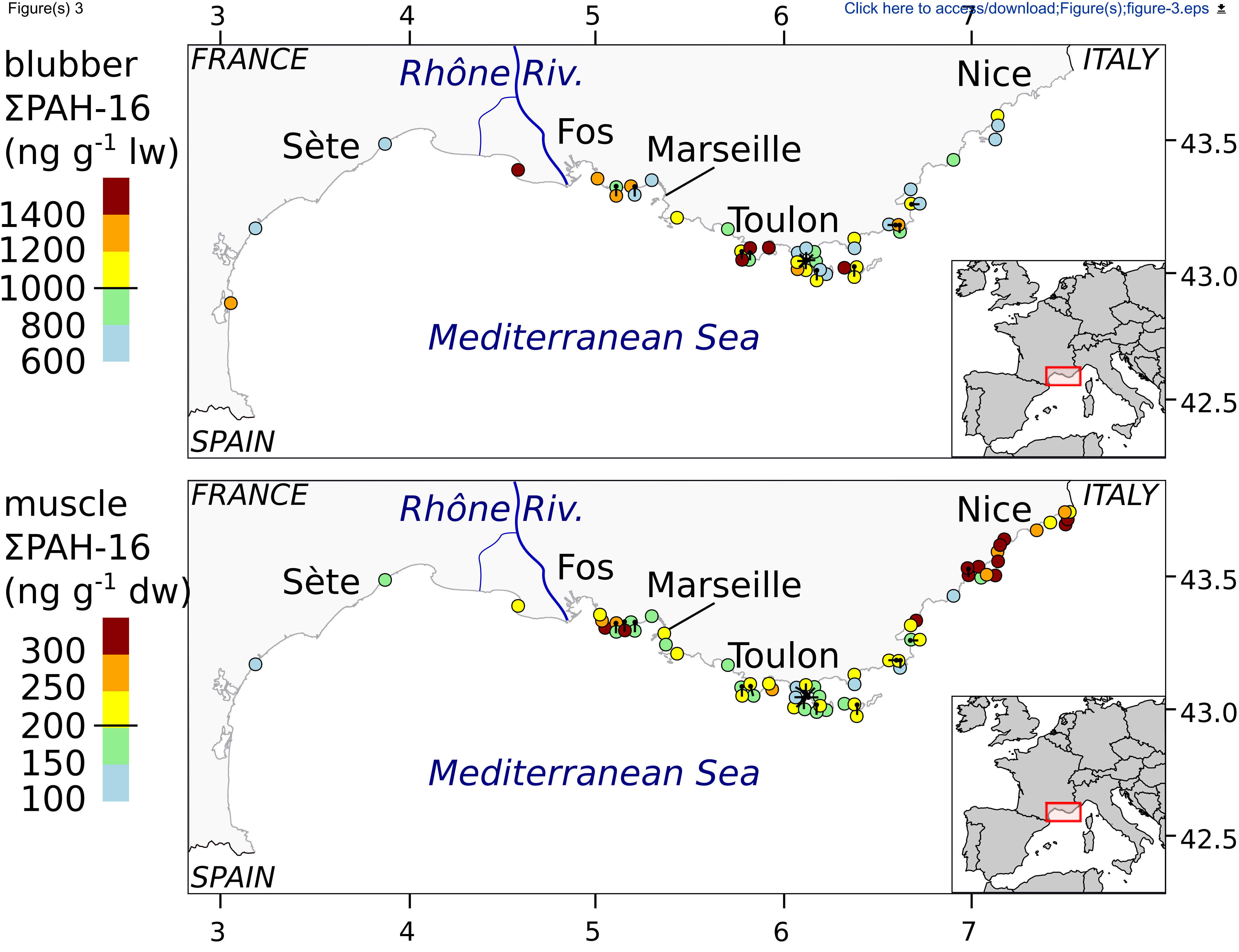
Click here to access/download;Figure(s);figure-1.eps




PAH congeners relative proportions (%)
a) S. coeruleoalba blubber
b) S. coeruleoalba muscle
c) T. truncatus blubber
30
25
20
15
10
5
0
Nap Ace Acy Flu Ant Phe FlA Pyr BaA Chr DBA BaP BkF BbF Bpe Ipy
calves
females
males
males & females
2
3
4
5
6
aromatic rings

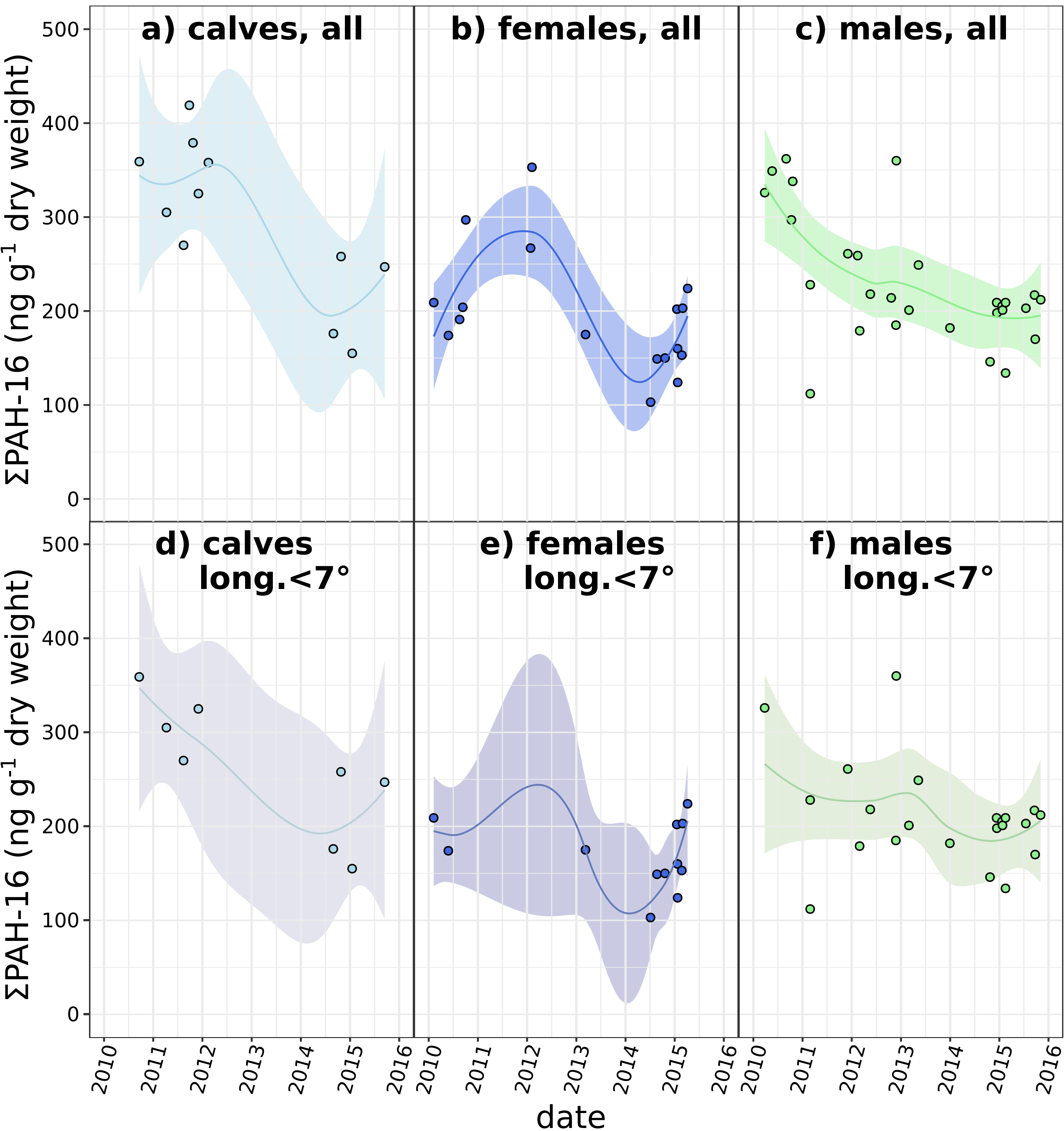
Figure(s) 3




blubber
ΣPAH-16
(ng g-1 lw)
1400
1200
1000
800
600
muscle
ΣPAH-16
(ng g-1 dw)
300
250
200
150
100
FRANCE
ITALY
SPAIN
Rhône Riv.
Sète
Fos
Marseille
Toulon
Nice
Mediterranean Sea
3
4
5
6
7
43.5
43.0
42.5

Figure(s) 4





a) calves, all
b) females, all
c) males, all
d) calves long.<7°
e) females long.<7°
f) males long.<7°
ΣPAH-16 (ng g-1 dry weight)
0
100
200
300
400
500
2010
2011
2012
2013
2014
2015
2016
date

# Occurrence and distribution of PAHs in stranded dolphin tissues from the Northwestern Mediterranean

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## SUPPORTING INFORMATION

Supporting Information S1 – Analytical quality results from certified material IAEA 451.

Supporting Information S2 – PAH congeners relative contributions in *S. coeruleoalba* and *T. truncatus* stranded on the French Mediterranean coastline.

Supporting Information S3 – Geographical distribution of the ΣPAH-16 levels measured in liver and kidney tissues of S. coeruleoalba stranded on the French Mediterranean coastline.

Supporting Information S4 – Detailed p-values obtained in KPSS tests among hypothetical temporal variations in the studied period (2010 – 2015) and realized on *Stenella coeruleoalba* data in all studied tissues, for the whole dataset and excluding the Nice area (longitude < 7°E).

Supporting Information S5 – Temporal evolution of PAH concentrations from 2010 to 2016 for calves, females and males Stenella coeruleoalba in blubber, liver and kidney tissues.

**Supporting Information S1. Summary of biological data and sampling information (N = number of individuals, sd = standard deviation, DCC = decomposition condition category).**

| | *S. coeruleoalba (N)* | *T. truncatus (N)* |
|---|---|---|
| **Total individuals** | **64** | **9** |
| female adults | 31 | 5 |
| male adults | 20 | 3 |
| calves | 11 | 0 |
| unidentified adults | 2 | 1 |
| **Average body length ± sd (min - max)** | 167 ± 39 cm (90 - 210 cm) | 228 ± 57 cm (150 - 310 cm) |
| **DCC** | | |
| 1 | 31 | 6 |
| 2 | 11 | 1 |
| 3 | 18 | 0 |
| 4 | 3 | 1 |
| 5 | 1 | 1 |
| **Tissues sampled** | | |
| blubber | 42 | 5 |
| liver | 53 | 7 |
| kidney | 50 | 7 |
| lung | 17 | 2 |
| muscle | 61 | 8 |

**Supporting Information S2. Analytical quality results from certified material IAEA 451.**

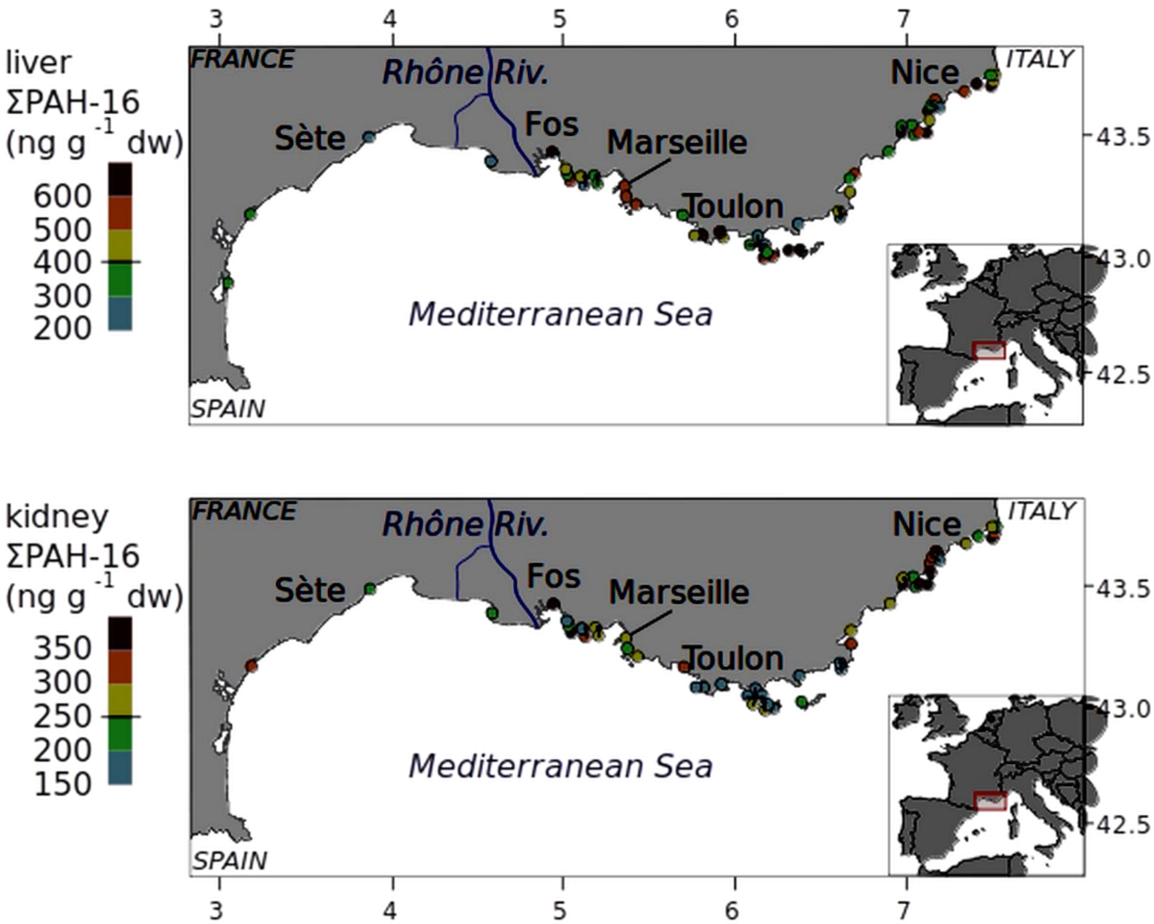
| PAH congener | Certified value ($ng\ g^{-1}$) | Measured value ($ng\ g^{-1}$) | N | Relative standard deviation (RSD, %) |
|---|---|---|---|---|
| Naphthalene* | 14.80±1.20 | 13.50±2.04 | 18 | 15.1 |
| Acenaphthene*** | 2.18 | 1.41±0.10 | 18 | 7.1 |
| Acenaphthylene** | 2.01±0.40 | 2.52±0.60 | 18 | 23.8 |
| Fluorene*** | 2.62 | 2.10±0.010 | 18 | 0.5 |
| Anthracene** | 5.07±1.10 | 5.64±0.65 | 18 | 11.5 |
| Phenanthrene* | 15.80±5.60 | 17.63±2.42 | 18 | 13.7 |
| Fluoranthene* | 49.30±3.20 | 47.52±4.12 | 18 | 8.7 |
| Pyrene* | 40.00±4.60 | 44.63±4.22 | 18 | 9.5 |
| Benzo(a)anthracene* | 19.20±1.30 | 18.77±2.46 | 18 | 13.1 |
| Chrysene* | 26.90±2.00 | 26.07±3.32 | 18 | 12.7 |
| Dibenzo(ah)anthracene* | 5.32±1.36 | 5.67±0.16 | 18 | 2.8 |
| Benzo(a)pyrene* | 18.20±2.40 | 17.51±0.13 | 18 | 0.7 |
| Benzo(k)fluoranthene* | 14.70±3.20 | 16.25±1.38 | 18 | 8.5 |
| Benzo(b)fluoranthene* | 35.80±6.20 | 38.18±11.05 | 18 | 28.9 |
| Benzo(ghi)perylene* | 19.50±2.40 | 17.93±1.40 | 18 | 7.8 |
| Indeno(123-cd)pyrene** | 23.80±1.20 | 19.24±4.57 | 18 | 23.8 |

* certified values
** recommended values
*** information values

**Supporting Information S3. PAH congeners relative contributions (%) to ΣPAH-16 in the different tissues of *Stenella coeruleoalba* and *Tursiops truncatus* stranded on the French Mediterranean coastline.**

| ***S.coeruleoalba*** | **blubber** | **liver** | **kidney** | **lung** | **muscle** | **average** |
|---|---|---|---|---|---|---|
| Nap | 15.4 | 20.1 | 17.9 | 17.9 | 16.4 | **17.6** |
| Ace | 4.6 | 7.3 | 6.0 | 4.1 | 5.9 | **5.6** |
| Acy | 3.5 | 6.4 | 6.2 | 7.3 | 5.8 | **5.9** |
| Flu | 10.4 | 8.3 | 6.7 | 5.9 | 6.6 | **7.6** |
| Ant | 2.0 | 4.9 | 5.2 | 5.0 | 5.3 | **4.5** |
| Phe | 4.2 | 6.1 | 5.7 | 6.0 | 5.9 | **5.6** |
| FlA | 2.1 | 4.0 | 4.4 | 4.1 | 3.7 | **3.7** |
| Pyr | 1.7 | 3.3 | 4.3 | 4.9 | 4.0 | **3.6** |
| BaA | 4.9 | 6.4 | 5.8 | 5.8 | 5.3 | **5.6** |
| Chr | 5.1 | 4.1 | 5.1 | 4.9 | 6.2 | **5.1** |
| DBA | 9.5 | 4.9 | 5.5 | 6.3 | 6.2 | **6.5** |
| BaP | 8.1 | 3.4 | 3.6 | 4.0 | 4.2 | **4.7** |
| BkF | 5.8 | 3.8 | 4.4 | 4.0 | 4.0 | **4.4** |
| BbF | 5.3 | 4.7 | 5.3 | 5.6 | 6.0 | **5.4** |
| Bpe | 5.2 | 5.0 | 5.6 | 6.1 | 6.4 | **5.7** |
| Ipy | 12.3 | 7.1 | 8.3 | 8.0 | 7.9 | **8.7** |
| ***T. truncatus*** | **blubber** | **liver** | **kidney** | **lung** | **muscle** | **average** |
| Nap | 16.5 | 22.2 | 18.7 | 31.1 | 16.2 | **20.9** |
| Ace | 11.6 | 7.5 | 8.4 | 1.9 | 12.6 | **8.4** |
| Acy | 3.4 | 9.1 | 8.6 | 2.7 | 10.1 | **6.8** |
| Flu | 13.8 | 8.1 | 9.8 | 1.0 | 6.5 | **7.9** |
| Ant | 4.1 | 5.9 | 6.2 | 5.2 | 7.7 | **5.8** |
| Phe | 3.2 | 6.0 | 5.4 | 3.7 | 6.8 | **5.0** |
| FlA | 7.2 | 12.4 | 6.7 | 1.8 | 4.3 | **6.5** |
| Pyr | 3.6 | 3.4 | 2.8 | 2.0 | 3.1 | **3.0** |
| BaA | 3.7 | 3.5 | 4.3 | 3.1 | 4.8 | **3.9** |
| Chr | 6.5 | 2.6 | 3.6 | 2.1 | 4.9 | **3.9** |
| DBA | 7.4 | 2.8 | 3.9 | 17.1 | 3.8 | **7.0** |
| BaP | 3.3 | 2.3 | 2.9 | 6.1 | 3.7 | **3.7** |
| BkF | 2.0 | 3.4 | 4.3 | 3.3 | 3.0 | **3.2** |
| BbF | 3.3 | 3.2 | 3.3 | 1.8 | 3.5 | **3.0** |
| Bpe | 5.0 | 4.7 | 6.9 | 9.9 | 5.8 | **6.5** |
| Ipy | 5.6 | 3.0 | 4.1 | 7.2 | 3.2 | **4.6** |

**Supporting Information S4. Geographical distribution of the ΣPAH-16 levels measured in liver and kidney tissues of *Stenella coeruleoalba* stranded on the French Mediterranean coastline.**



**Supporting Information S5. Detailed p-values obtained in Kwiatkowski–Phillips–Schmidt–Shin (KPSS) tests for level and trend stationarity among hypothetical temporal variations in the studied period (2010 – 2015) and realized on *Stenella coeruleoalba* data in all studied tissues, for the whole dataset and excluding the Nice area (longitude < 7°E).**

| **All individuals** | Gender/age | N | Level stationary | Trend stationary | Conclusion |
|---|---|---|---|---|---|
| blubber | males | 22 | >0.1 | 0.04 | variable |
| | females | 12 | >0.1 | 0.08 | stationary |
| | calves | 6 | >0.1 | >0.1 | stationary |
| liver | males | 23 | >0.1 | >0.1 | stationary |
| | females | 18 | >0.1 | 0.04 | variable |
| | calves | 10 | >0.1 | 0.08 | stationary |
| kidney | males | 22 | **0.02** | >0.1 | **trend** |
| | females | 18 | >0.1 | >0.1 | stationary |
| | calves | 8 | 0.08 | 0.04 | variable |
| lung | males | 7 | >0.1 | 0.06 | stationary |
| | females | 7 | >0.1 | 0.03 | variable |
| | calves | 2 | NA | NA | NA |
| muscle | males | 29 | **0.02** | 0.09 | **trend** |
| | females | 19 | >0.1 | >0.1 | stationary |
| | calves | 11 | >0.1 | >0.1 | stationary |
| **Longitude<7° E** | Gender/age | N | Level stationary | Trend stationary | Conclusion |
| blubber | males | 21 | >0.1 | 0.03 | variable |
| | females | 12 | >0.1 | 0.08 | stationary |
| | calves | 4 | >0.1 | 0.03 | variable |
| liver | males | 19 | >0.1 | >0.1 | stationary |
| | females | 12 | >0.1 | 0.08 | stationary |
| | calves | 7 | >0.1 | 0.01 | variable |
| kidney | males | 17 | **0.04** | >0.1 | **trend** |
| | females | 12 | >0.1 | 0.05 | variable |
| | calves | 5 | 0.09 | 0.03 | variable |
| lung | males | 7 | >0.1 | 0.06 | stationary |
| | females | 8 | >0.1 | 0.03 | variable |
| | calves | 2 | NA | NA | NA |
| muscle | males | 23 | >0.1 | >0.1 | stationary |
| | females | 14 | >0.1 | 0.09 | stationary |
| | calves | 8 | 0.10 | 0.04 | variable |

KPSS level test : Null hypothesis is level stationary (globally stable)
KPSS trend test : Null hypothesis is trend stationary (regular trend)

**Supporting Information S6. Temporal evolution of PAH concentrations from 2010 to 2016 for *Stenella coeruleoalba* calves, females and males in blubber (a), b) and c)), liver (d), e) and f)) and kidney (g), h) and i)) tissues.**

supplementary data

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**Supplementary Interactive Plot Data (CSV)**

data_dolphinPAH_FR-Medit.csv