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Network analysis to reveal the most commonly detected compounds in predator-prey pairs in freshwater and marine mammals and fish in Europe

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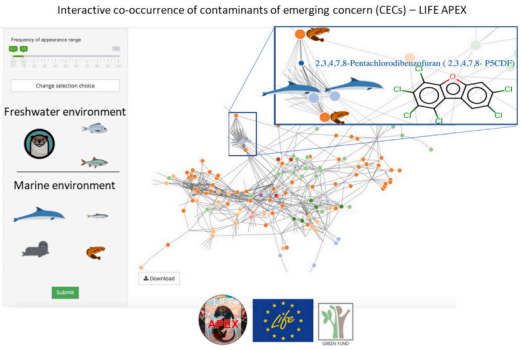
HIGHLIGHTS

- Network analysis visualized occurrence data, revealing top chemical mixtures.
- Web tool was created to explore chemical mixtures in predator-prey pairs.
- Mercury was a predominant heavy metal for both freshwater and marine environments.
- PFAS, BDE, PCB and hexachlorobenzene were predominant mixtures in both environments.
- N-Acetylaminoantipyrine was a predominant pharmaceutical in both environments.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine and freshwater mammalian predators and fish samples, retrieved from environmental specimen banks (ESBs), natural history museum (NHMs) and other scientific collections, were analysed by LIFE APEX partners for a wide range of legacy and emerging contaminants (2545 in total). Network analysis was used to visualize the chemical occurrence data and reveal the predominant chemical mixtures for the freshwater and marine environments. For this purpose, a web tool was created to explore these chemical mixtures in predator-prey pairs. Predominant chemicals, defined as the most prevalent substances detected in prey-predator pairs were identified through this innovative approach. The analysis established the most frequently co-occurring substances in chemical mixtures from AP&P in the marine and freshwater environments. Freshwater and marine environments shared 23 chemicals among their top 25 predominant chemicals. Legacy chemical, including perfluorooctanesulfonic acid (PFOS), brominated diphenyl ethers (BDEs), polychlorinated biphenyls (PCBs), hexachlorobenzene and mercury were dominant chemicals in both environments. Furthermore, *N*-acetylaminoantipyrine was a predominant pharmaceutical in both environments. The LIFE APEX chemical mixture application (https://norman-data.eu/LIFE_APEX_Mixtures) was proven to be useful to establish most prevalent compounds in terms of number of detected counts in prey-predator pairs. Nonetheless, further research is needed to establish food chain associations of the predominant chemicals.

1. Introduction

A prominent and challenging topic in environmental analytical chemistry is the occurrence of legacy and contaminants of emerging concern (CECs) in apex predators and their prey (AP&P). Apex or top predators have characteristics which make them appropriate as sentinel species for monitoring of bioaccumulating contaminants (Gkotsis et al., 2023; Ross, 2000). These characteristics include their high trophic position in food webs (Sanganyado et al., 2020), their long lifespan (Munschy et al., 2020) and diverse nutrition (Fuentes et al., 2023), the possibility of non-invasive sampling (Oliva-Vidal et al., 2022), and the capability to capture temporal and spatial trends (Bignert and Helander, 2015). Moreover, the detection of contaminants with hazardous properties in apex predators is of high interest because they can act as a proxy of contamination for humans (Miccoli et al., 2017; Moriceau et al., 2022). Apex predators are susceptible to the biomagnification of chemicals through the food chain (Androulakakis et al., 2022; Walther et al., 2021) and/or the consumption of contaminated water (Oliva-Vidal et al., 2022). The contamination originates from present and legacy production, use and subsequent release of chemicals through wastewater treatment plants (Du et al., 2014), wide dispersive use, agriculture (Fuentes et al., 2023) and other anthropogenic sources.

Currently, the risk assessment of chemicals for regulatory purposes does only in rare cases take into account the "real life" exposure to multiple chemicals, but mainly relies on the assessment of individual chemicals (Bopp et al., 2018). Key element in (mixture) risk assessment is information on actual exposures in humans, species of interest, or respective media of interest. However, such information is still largely absent, severely limiting mixture risk assessment. The use of environmental and human monitoring data may improve the situation by providing information on intensity, duration, and frequency and/or actual exposure time, and allow assessment of combined exposure patterns to chemicals (Beckers et al., 2023; Finckh et al., 2024).

The majority of published scientific literature focuses on a limited number of compounds within a narrow range of chemical classes, often derived from samples of only a few species and limited to a relatively small geographical area. Some examples include studies on organochlorine pesticides (OCPs) (Munschy et al., 2020), polychlorinated biphenyls (PCBs) (Munschy et al., 2020; Stuart-Smith and Jepson, 2017), perfluoroalkyl substances (PFASs) (Herzke et al., 2023), rodenticides (Cooke et al., 2022; Moriceau et al., 2022; Oliva-Vidal et al., 2022; Parli et al., 2020; Walther et al., 2021), neonicotinoids (Fuentes et al., 2023) and insecticides (Stechert et al., 2014). The application of multi-analyte wide-scope high-resolution mass spectrometry (HRMS) methods has opened new horizons in chemical monitoring and has the potential to support chemical risk assessment and management (Hollender et al., 2017). Advanced analytical instrumentation, mass spectrometric libraries, compound databases and cheminformatic software can support wide-scope screening of thousands of chemicals in environmental samples. The detection of contaminants using these methodologies can provide insights into predominant chemical mixtures in biota (Badry et al., 2022b). These methodologies are becoming more powerful when applied to large sets of environmental samples providing spatial and temporal trends across countries or geographical regions (Marshall and McCluney, 2021).

In this study, we applied target analysis for chlorinated, brominated

and fluorinated persistent organic pollutants (POPs) together with wide-scope target screening methods to detect unregulated contaminants which are potentially hazardous to humans and the environment (also known as CECs). Such an effort to apply both established and advanced analytical approaches in big sets of marine and freshwater mammalian predators and selected fish samples in conjunction with new data science tools was conducted in the context of the EU-funded LIFE APEX project (LIFE17 ENV/SK/000355, https://lifeapex.eu/, 2018–2022) (Badry et al., 2022b; Treu et al., 2022). Four analytical laboratories: National and Kapodistrian University of Athens (NKUA; Greece), Environmental Institute (EI; Slovakia), University of Florence (UNIFI; Italy) and Fraunhofer Institute IME (FrIME; Germany) undertook the analyses. NKUA conducted an extensive chemical screening of medium-polarity and high-polarity compounds. Meanwhile, EI and UNIFI focused their efforts on analyzing non-polar compounds, and FrIME specialized in mercury analysis. The occurrence of 2545 CECs was investigated in freshwater and marine top predators in Europe producing in total 503,910 chemical occurrence data points.

The present study comes to introduce the application of a new data science methodology (network analysis) using the LIFE APEX dataset that can aid the evaluation of chemical mixtures in the environment. For this purpose, we developed an interactive web tool able to investigate chemical mixtures in AP&P and used it to identify which chemicals usually co-occur as predominant mixture in the freshwater and marine compartment. Network-analysis visualization and predator-prey pair analysis were used to identify these contaminants. We believe that our type of study can help the chemicals' regulators to design precautionary actions to protect humans and wildlife from CECs by providing insights into the occurrence and distribution of predominant chemicals in the prey and predators.

2. Materials and methods

2.1. Samples

Freshwater (otter) and marine (seal, porpoise and dolphin species) top predators, and freshwater and marine prey species (fish) were collected from 20 European countries: Germany, United Kingdom, Netherlands, Sweden, Italy, France, Austria, Denmark, Czech Republic, Norway, Hungary, Spain, Ukraine, Poland, Belgium, Slovakia, Greece, Portugal, Slovenia, and Romania (Fig. 1). Most samples were pooled samples, the heterogeneous selection of sampling sites for predators and prey prevented the establishment of spatial correlations between predator and prey. These samples were provided by European Environmental Specimen Banks (ESBs), Natural History Museums (NHMs), and research collections (RCs). A total of 145 samples from top predators and prey, collected between 1996 and 2022 in LIFE APEX were used in this study (Table 1). Marine and freshwater fish were sampled by net or rod fishing, where opportunistic sampling was applied for deceased top predators. The seal samples from the Norwegian Arctic were collected from animals harvested by local sport hunters during the open hunting season. The liver was selected as the matrix for analysis in top predator samples due to its role as the primary organ for xenobiotic metabolism and high chemical levels compared to most other organs (Lerapetritou et al., 2009). In contrast, muscle tissue was chosen as the matrix for prey samples since it is frequently chosen in regulatory biota monitoring programmes and therefore allows for better comparison with data collections. The detailed list of samples is provided in Table S1 in the supplementary material and the information is provided in an aggregative manner in Table 1.

The species selection process was conducted meticulously by the members of the LIFE APEX consortium, taking into consideration the availability of samples in NHMs, RCs and ESBs. Based on previous research, Eurasian otters have been identified as indicators for freshwater environment contamination (Kean et al., 2021), and marine mammals have been deemed ideal sentinels for assessing marine ecosystem quality (Sonne et al., 2020).

2.2. Sample pre-treatment

The samples were collected, processed and stored at −80 °C by the ESBs and at −20 °C by NHMs and RCs. Storage of samples at temperature − 20 °C was considered satisfactory given that NHMs and RCs do not have the facilities to reach −80 °C storage temperature. All samples were shipped from the sample suppliers on frozen and delivered to the laboratory at NKUA within two days of shipping. The samples of species listed under CITES Appendices were shipped under respective CITES permits. The samples were lyophilized upon receipt, homogenized using a pestle with mortar or a laboratory blender, and stored at −80 °C until analysis (Badry et al., 2022a). Subsequently, aliquots of the samples were distributed to analytical laboratories at the Environmental Institute, University of Florence and Fraunhofer IME for targeted analyses.

2.3. Wide-scope screening for contaminants of emerging concern

For the application of wide-scope target screening of CECs, the samples were extracted from the lyophilized biota matrices by accelerated solvent extraction (ASE) using a mixture of methanol:acetonitrile (2:1 v/v) as extraction solvent. A two-phase clean-up step of the extracts was conducted by liquid-liquid extraction (LLE) and solid-phase extraction (SPE). In particular, a defatting step, using hexane, was applied before the SPE. Mixed-mode SPE cartridges consisted of Oasis HLB and a mixture of Strata-X-AW (weak anion exchanger), Strata-X-CW (weak cation exchanger) and Isolute ENV+ were used for the extraction of the analytes and the purification of the extract. The final extracts were evaporated to dryness under a gentle nitrogen stream (40 °C), reconstituted to a final volume of 250 μL, using a mixture of methanol:milli-Q (1:1 v/v) and filtered through a 0.22 μm Regenerated Cellulose (RC) membrane filter (Phenomenex, CA, USA) into a 2 mL vial, before the analysis by liquid chromatography coupled with high resolution mass spectrometry (Gkotsis et al., 2022). The screening detection limit (SDL) was estimated at 5 ng/g wet weight (Diamanti et al., 2020).

The analysis of the final extracts of biota samples was conducted using an ultra-high performance liquid chromatographic system (UHPLC, DionexUltiMate 3000 RSLC, Thermo Fisher Scientific) coupled with a hybrid Quadrupole Time-of-Flight (QToF) mass spectrometer (Maxis Impact, Bruker Daltonics). The chromatographic separation was achieved on a reversed-phase (RP) chromatographic system using an Acclaim RSLC C18 column (2.1 × 100 mm, 2.2 μm) from Thermo Fisher Scientific, connected to an ACQUITY UPLC BEH C18 1.7 μm, VanGuard pre-column from Waters, and thermostated at 30 °C. The QToF-MS system was equipped with an electrospray ionization interface (ESI) source, operating in both positive and negative ionization modes. Wide-scope target analysis was performed using an in-house developed database of 2273 contaminants of emerging concern of high environmental significance, including compounds from different chemical classes, having a broad spectrum of applications, use, physico-chemical properties and extent of production. The database is available as a dataset in Zenodo (Thomaidis et al., 2022) and as S21 UATHTARGETS in the NORMAN Suspect List Exchange (Mohammed Taha et al., 2022). The post-acquisition data treatment was conducted using TASQ Client 2.1 and DataAnalysis 5.1 (Bruker Daltonics, Bremen, Germany) software. The HRMS data processing workflow was described in previous publications in detail (Gago-Ferrero et al., 2020; Nikolopoulou et al., 2022). Specifically, the detection of the target compounds was based on strict screening thresholds of mass accuracy (<2mDa), retention time shift (±0.2 min), isotopic fitting (only for the verification of the positive findings) and the presence of qualifier ions (adduct and fragment ions), which confirmed the detection of the analytes.

A more detailed description of the analytical methodology is included in Section 2 of the supplementary material.

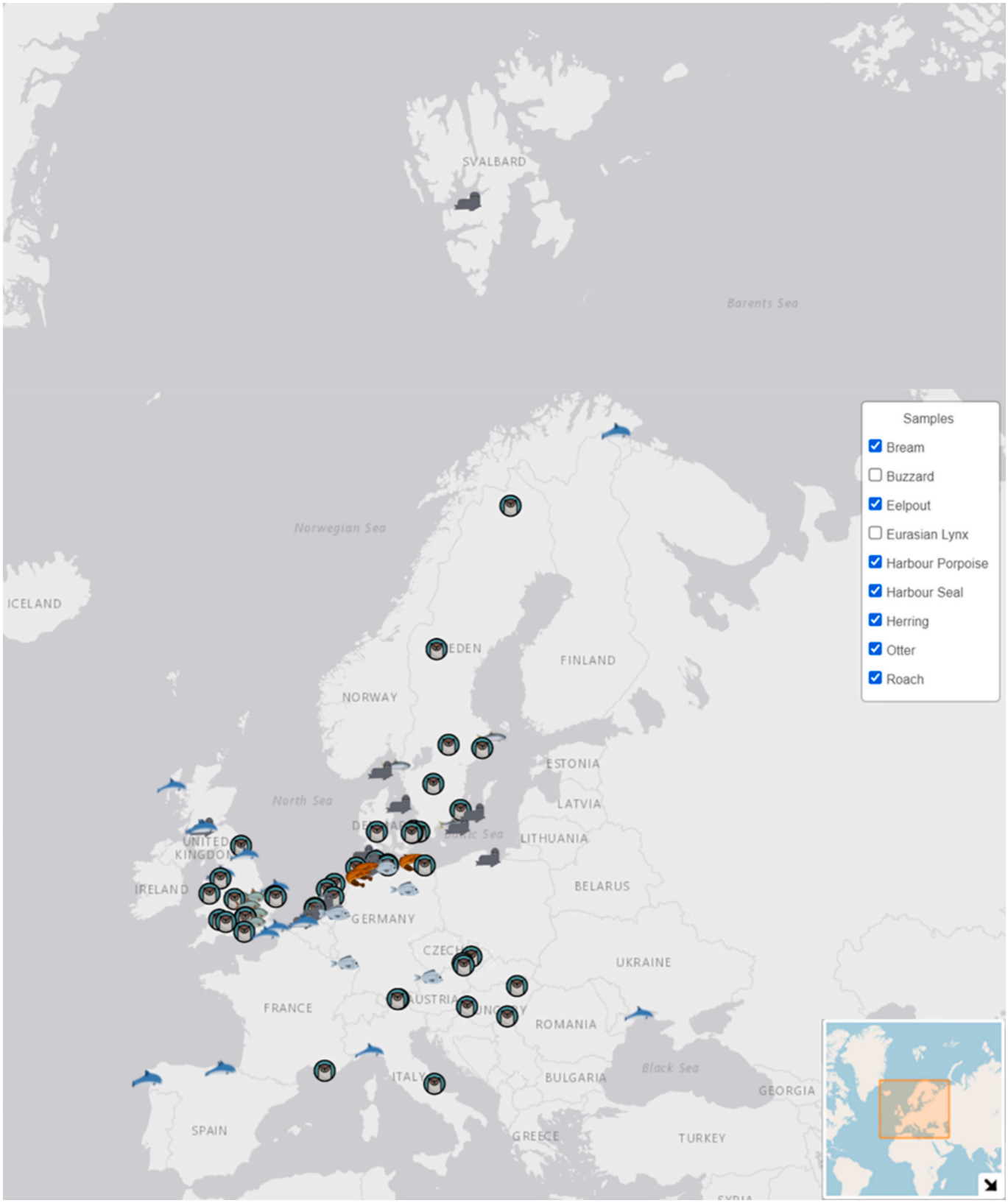

Fig. 1. Sample collection sites and their spatial distribution. An interactive version of the map is available in the following link: https://norman-data.eu/LIFE_APEX_Samples (Accessed on 18th September 2023). For visualization purposes, a uniform icon is used to represent all marine top predator species.

Table 1
Common name, Latin name, number of (pooled) samples, countries and environmental compartment of the LIFE APEX sample collection.

Name	Latin name	No, of pooled samples	Countries	Years of sample collection	Environmental compartment
Eelpout	*Zoarces viviparus*	3	1 (Germany)	2015	Marine (prey)
Bearded Seal	*Erignathus barbatus*	1	1 (Norway)	2019	Marine (predator)
Grey Seal	*Halichoerus grypus*	4	4 (Sweden, Germany, UK, Poland)	2015–2020	Marine (predator)
Harbour Seal	*Phoca vitulina*	30	4 (Netherlands, Sweden, Germany, Belgium)	2000–2021	Marine (predator)
Ringed Seal	*Pusa hispida*	1	1 (Norway)	2020	Marine (predator)
Herring	*Clupea harengus*	3	1 (Sweden)	2018	Marine (prey)
Harbour Porpoise	*Phocoena phocoena*	11	4 (UK, Norway, Ukraine, Belgium)	2017–2020	Marine (predator)
Bottlenose dolphin	*Tursiops truncatus*	4	3 (Italy, UK, Spain)	2015–2020	Marine (predator)
Short-beaked common dolphin	*Delphinus delphis*	3	2 (UK, Spain)	2015–2019	Marine (predator)
Striped dolphin	*Stenella coeruleoalba*	2	1 (Spain)	2017–2019	Marine (predator)
White-beaked dolphin	*Lagenorhynchus albirostris*	1	1 (UK)	2016–2017	Marine (predator)
Eurasian Otter	*Lutra lutra*	58	10 (UK, Netherlands, Sweden, Germany, Italy, France, Austria, Denmark, Czechia, Hungary)	2003–2018	Freshwater (predator)
Bream	*Abramis brama*	18	2 (Germany, Netherlands)	1996–2019	Freshwater (predator)
Roach	*Rutilus rutilus*	5	1 (UK)	2014–2017	Freshwater (predator)

2.4. Target analysis for PCBs, HCB and PBDEs

For the determination of polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and polybrominated diphenyl ethers (PBDEs) at trace and ultra-trace levels, careful cleaning of analytical glassware is crucial. Limit of detection (LOD) for PCBs ranged from 0.006 to 0.5 ng/g ww, LOD for HCB was 0.001 ng/g ww, LOD for PBDEs ranged from 0.003 to 0.013 ng/g ww. The extraction of samples involved blending the lyophilized sample with anhydrous sodium sulfate and placing it in an extraction cellulose thimble. The thimble was then placed in a Soxhlet extractor, filled with a mixture of dichloromethane and hexane. The sample was extracted for 12 h using Soxhlet, and the extract was reduced, cleaned up using a multilayer silica gel column, and washed with hexane. The procedure was repeated in case lipid removal was incomplete. The lipid content was determined gravimetrically by evaporating the sample, recording the weight, and calculating the lipid content as a percentage.

For PCBs and HCB analysis, a low bleed DB-5MS column (nominal length: 30 m, nominal diameter: 250 μm, nominal film thickness: 0.25 μm) was used. For PBDEs analysis, a short 15 m DB5-MS column with a 5 % phenyl, 95 % dimethylarylenesiloxane stationary phase was used to meet EPA Method 1614 resolution requirements. Gas chromatography electrospray mass spectrometry (GC-EI-MS) was used for the determination of PCBs and HCB, whereas Gas chromatography negative chemical ionization mass spectrometry (GC-NCI-MS) was used for PBDEs.

Detailed method and its quality assurance are provided in section 3 of the supplementary material.

2.5. Target screening for dioxins and dioxin-like compounds, chlorinated alkanes, novel organophosphorus flame retardants and decloran plus

ASE was used for the extraction of dioxin, dioxin-like compounds and chlorinated alkanes. The cell test DR CALUX® was used for screening of dioxin-like activities, GC-HRMS was used for the determination of 29 regulated dioxin-like compounds (and other persistent organic pollutants with dioxin-like potencies, such as mixed halogenated dioxins/biphenyls), GC-NCI-MS for the determination of C10-C13 and C14-C17 polychlorinated alkanes based on ISO/DIS 12010.

Detailed method and its quality assurance are provided in Section 4 of the supplementary material.

2.6. Mercury

Determination of total mercury (Hg) was conducted by Fraunhofer IME. Solid mercury analyser permits interference-free analysis of solid and liquid samples for total mercury content. Automatic sample combustion was carried out at approx. 1000 °C in a current of oxygen. Following combustion of the sample and catalytic conversion of the combustion gases, elemental mercury is selectively concentrated by amalgam formation and then measured by means of atomic-absorption spectrometry (AAS). LOD for mercury was 0.082 ng/g ww and limit of quantification (LOQ) 0.25 ng/g ww.

When a compound was detected at concentration levels above LOD but below the LOQ, LOQ/2 was used for the statistical treatment of the results (European Commission, 2009). When a compound was <LOD, it was considered non-detected and replaced by zero.

Detailed method and its quality assurance are provided in Section 5 of the supplementary material.

2.7. Network analysis

Graphs were selected because they represent entities and their relationships. In this case, the occurrence of chemicals in studied AP&P samples, and the predominant chemicals as in the ones co-occurring in AP&P pairs. Graph analytics are increasingly used for interconnected data and are powerful for gaining insights from the relationships between data. They help to uncover the workings of intricate systems and networks at massive scales. They consist of the following elements; nodes, which are the objects that make up the graph and represent entities, edges which are the links between the nodes and represent connections of entities and properties which represent attributes/metadata of nodes and edges. In our analysis, The nodes represent the fish muscle and top predator liver samples from LIFE APEX. The connections/edges represent legacy contaminants and CECs that were present in the samples. Two samples are connected through a compound node in case a substance is commonly detected in these two samples. The thickness of the connection/edge represents the logarithm of the concentration level. The length of the connection/edges is determined by 3D coordinates (xyz) of the two points, resulting in nodes of samples with similar chemical profiles clustering more closely to each other, thereby enabling the visual characterisation of chemical mixtures. On top of the visualization of chemical occurrence in AP&P, the system also calculates the co-occurrence in prey-predator pair for each chemical. This pairing method displays all potential pairs of AP&P where the same chemical or a similar chemical mixture is present. Prioritisation is based on the number of prey-predator pairs, as a higher pair count suggests a potential association of chemical occurrences across different levels of the food web. Frequency of appearance was calculated for informative reasons only. The efforts resulted in the R-based shiny web tool with open access for visualization of chemical occurrence in the LIFE APEX samples. The application is available at https://github.com/nalygizakis/ChemicalMixtures

3. Results and discussion

3.1. Interactive tool to investigate chemical mixtures in AP&P

Network analysis visualization was used to demonstrate how data obtained from national and Europe-wide screening campaigns can be used to identify chemicals typically co-occurring in apex predators across Europe and thus might be considered as predominant mixtures of concern. The present study does not assess any mixture risks but reports on combined exposure. The chemical mixture graph database is available at https://norman-data.eu/LIFE_APEX_Mixtures/. A screenshot of the tool is presented in Fig. 2. The tool allows filtering of the chemicals by their total frequency of appearance (frequency of appearance; FoA, 0.5–100 %) in species and matrix. The results are presented in an interactive network analysis map. The results which were used to generate the interactive graph map are downloadable in CSV format.

For example, when zooming in the top left area of the graph (Fig. 2a), one can visualize the connection between two otter (LIFE APEX 25 and LIFE APEX 49) and two bream samples (LIFE APEX 40 and LIFE APEX 3).

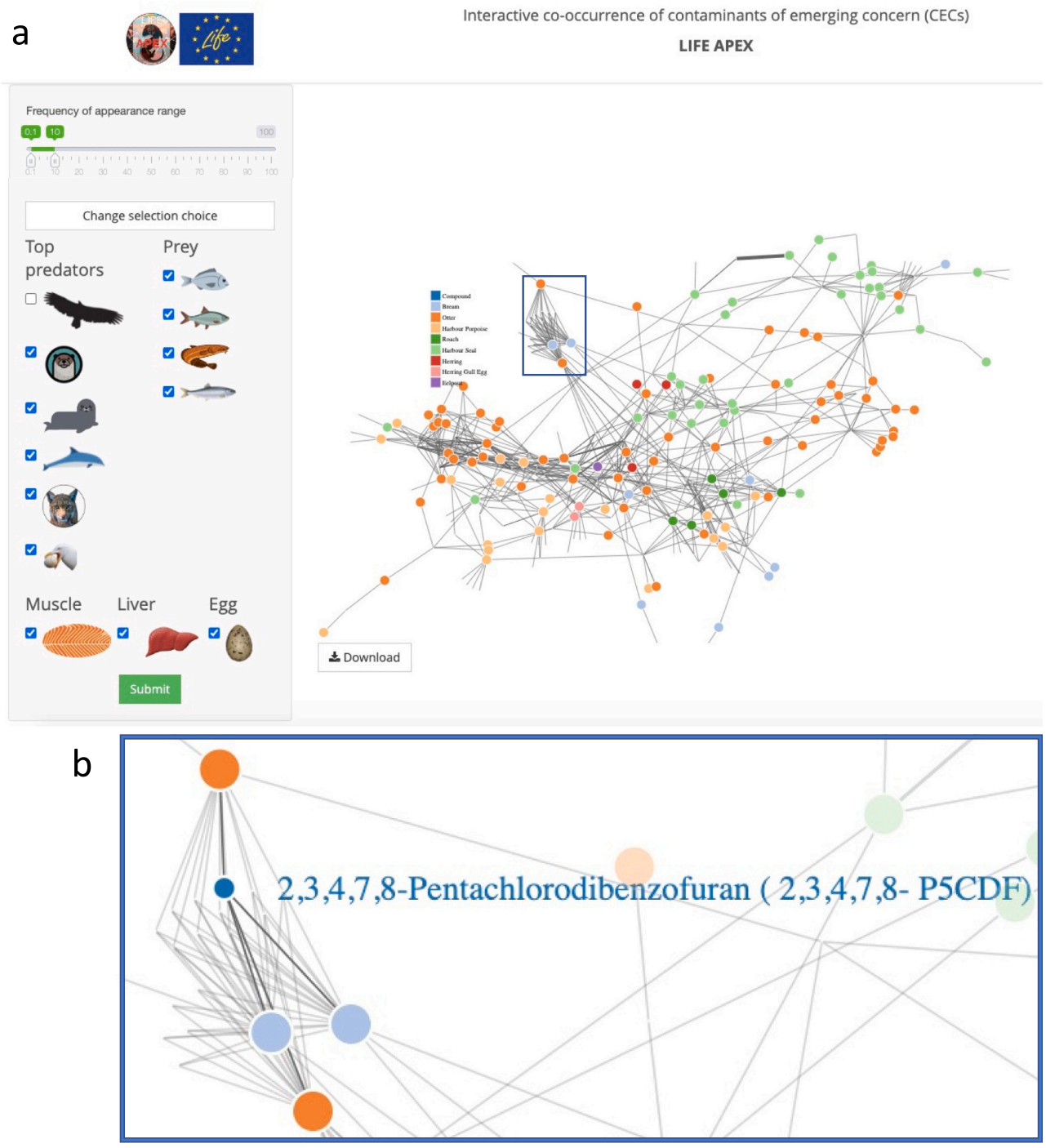

Fig. 2. a) Screenshot from the tool developed to reveal the chemical mixtures (https://norman-data.eu/LIFE_APEX_Mixtures/, accessed on 23 Apr 2024). The tool is accessible via the LIFE APEX Chemical Occurrence Data. b) Chemical mixture identified in both otter livers (LIFE APEX 25 from UK and LIFE APEX 49 from SE) and bream filets (LIFE APEX 40 from NL and LIFE APEX 3 from DE). Each connecting point represents a commonly detected chemical, with 2,3,4,7,8-P5CDF highlighted as an example. The commonly detected compounds in four samples are shown in figure (b).

As shown in Fig. 2b, the substance 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-P5CDF) was detected in these four samples together with 73 other substances forming a unique chemical cocktail (could be retrieved by applying the appropriate filters on the graph analytics). Therefore, the graph can reveal associations between samples given the detected chemical mixtures and identify compartment-specific contaminants such as 2,3,4,7,8-P5CDF for freshwater. The data can be downloaded from the "download" button as shown in Fig. 2a.

The network analysis can be conducted for any possible combination of species that were collected and analysed within LIFE APEX. Nonetheless, owing to the pooling of samples, the findings portray an average exposure estimation for the evaluated species. The individual concentrations alongside corresponding effect thresholds (minimum effect concentrations, toxicity thresholds, etc.) are required for the comprehensive assessment of mixture risk. However, two combinations were identified to reveal the chemical mixtures in freshwater and marine predators and their prey (Fig. S12 in supplementary material);

- the top predator otter (58 samples) and the prey species bream and roach (23 samples) representing the chemical contamination of the freshwater ecosystem
- the top predator seal (36 samples), dolphin (10 samples) and harbour porpoise (11 samples) and the prey species herring (3) and eelpout (3) representing the chemical contamination of the marine ecosystem

Moreover, it has to be noted that sample collection aimed to screen chemicals across a wide-spatial European distribution and not to characterize food chain accumulation. Therefore, the presented links need to be interpreted with caution and must be verified given that the samples were not taken exactly in the same spatio-temporal context.

3.2. Predominant chemical mixtures in freshwater ecosystems

Predominant chemical mixtures contain most frequently co-occurring substances in the investigated samples. In order to establish the predominant chemical mixtures, present in the AP&P of the freshwater ecosystems, a search on the chemical mixture graph database for

Table 2
The 25 most frequently co-occurring substances in chemical mixtures from AP&P of the freshwater environment.

Compound	CAS RN	Chemical Group	Limit of Quantification (μg/kg ww)	Median concentration in predator (otters – liver) μg/kg ww	Median concentration in prey (fish – muscle) μg/kg ww	FoA
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	PFAS	0.25	3813	40.97	100
Mercury	7439-97-6	Heavy metal	0.245	1970	60.25	98.8
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl (PCB 180)	35065-29-3	IC	0.006	54.32	68.26	97.6
2,2′,3,4,4′,5′-Hexachlorobiphenyl (PCB 138)	35065-28-2	IC	0.003	51.2	109.07	97.6
2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB 153)	35065-27-1	IC	0.003	2.95	0.47	97.6
2,2′,4,4′,6-Pentabromodiphenyl ether (BDE 100)	89084-64-8	IC	0.013	2.89	3.23	97.6
2,2′4,4′-Tetrabromodiphenyl ether (BDE 47)	5436-43-1	IC	0.013	18.53	10.24	97.6
2,3′,4,4′,5-Pentachlorobiphenyl (PCB 118)	31508-00-6	IC	0.003	15.33	40.37	97.6
2,4,4′-Tribromodiphenyl ether (BDE 28)	41318-75-6	IC	0.013	1.33	0.13	97.6
2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB 156)	38380-08-4	IC	0.006	8.48	0.03	96.3
2,3,3′,4,4′-Pentachlorobiphenyl (PCB 105)	32598-14-4	IC	0.003	3.61	7.22	96.3
Hexachlorobenzene	38380-08-4	IC	0.001	8.2	2.43	96.3
2,3,3′,4,4′,5′-Hexachlorobiphenyl (PCB 157)	69782-90-7	IC	0.003	1.45	0.97	95.1
2,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 167)	52663-72-6	IC	0.003	2.43	4.48	95.1
2,2′,4,4′,5,6′-Hexabromodiphenyl ether (BDE 154)	207122-15-4	IC	0.025	1.24	0.77	93.9
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl (PCB 189)	39635-31-9	IC	0.003	1.27	0.78	93.9
2,2,4,5,5′-Pentochlorobiphenyl (PCB 101)	37680-73-2	IC	0.003	0.85	89.9	91.5
2,2′,4,4′,5,5′-Hexabromodiphenyl ether (BDE 153)	68631-49-2	IC	0.025	2.95	0.47	92.7
2,2′,4,4′,5-Pentabromodiphenyl ether (BDE 99)	60348-60-9	IC	0.013	1.41	0.09	91.5
2,3,4,4′,5-Pentachlorobiphenyl (PCB 114)	74472-37-0	IC	0.003	0.24	0.75	80.5
2′,3,4,4′,5-Pentachlorobiphenyl (PCB 123)	65510-44-3	IC	0.003	0.29	7.15	75.6
3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126)	57465-28-8	IC	0.003	1.34	0.05	85.4
3,3′,4,4′-Tetrachlorobiphenyl (PCB 77)	32598-13-3	IC	0.003	0.27	0.66	69.5
N-Acetylaminoantipyrine	83-15-8	Ph&TPs	0.85	502.56	2.28	76.8
2,2′,4,5′-Tetrabromodiphenyl Ether (BDE 49)	243,982-82-3	IC	0.013	0.61	0.9	68.3

the co-occurring compounds in the collected samples of 58 otter, 18 bream and 5 roach over the full frequency of appearance range (0.5–100 %) was performed. The top 25 compounds in the number of predator-prey pairs are listed in Table 2.

These include one PFAS, one heavy metal, one representative of pharmaceuticals and transformation products (Ph&TPs) and 22 industrial chemicals (ICs). Being the most predominant detected chemical, perfluorooctanesulfonic acid (PFOS) showed ubiquitous presence in all predator and prey samples at a median concentration of 3813 and 41.0 μg/kg ww, respectively. Similar concentration levels for PFOS have been reported in otters from USA (Kannan et al., 2002). The predominant ICs include 14 PCBs, 7 BDEs, and hexachlorobenzene. PCBs, BDEs and hexachlorobenzene are known persistent substances that have been reported in mammals across the world (Basu et al., 2007). Furthermore, mercury was frequently detected with 98.8 % FoA. Mercury has been reported in otter livers in many places in the world (Dibbern et al., 2021; Yates et al., 2005). However, it has been proven that mercury exhibits significant regional variations in livers of mammals (Yates et al., 2005). The source identification of the emerging compounds could reveal the major point sources of the release to the environment. It was demonstrated that the graphic analytics of the LIFE APEX website could be utilized to establish the distribution and major components of the chemical mixtures in each sample type. Nonetheless, further analysis is required to obtain the food chain associations between animals in the freshwater ecosystems, and the contamination sources of the predominant chemicals.

From quantitative perspective, PFAS was the chemical class with the highest concentration of individual analytes in livers from otters (0.3–9962 μg/kg), followed by Ph&TPs (15–3200 μg/kg) and ICs (0.02–489 μg/kg), with the lowest concentration found for the stimulants and transformation products (S&TPs; 16–256 μg/kg). The high upper ranges of some chemical classes were high due to the high concentration in individual sample (e.g. over 8000 μg/kg PFOS or over 2300 μg/kg of *N*-acetylaminoantipyrine in some samples).

Nonetheless, the cumulative concentration of chemical classes is associated with the number of compounds analysed. A more wide-scope chemical analysis, such as the inclusion of suspect screening, could reveal the more comprehensive occurrence profile of these chemical classes. On the contrary, as far as freshwater prey species (muscle) were concerned, the predominant chemical class was Ph&TPs (0.99–65 μg/kg) followed by ICs (0.02–11 μg/kg), PFAS (0.11–3.7 μg/kg) and finally S&TPs with concentration ranges 0.18–1.4 μg/kg.

Detection of PFAS was mainly observed in freshwater otter samples from the Netherlands and the United Kingdom. In a study about the occurrence of PFAS in otters in England and Wales, a negative association between the concentrations of some PFAS (including PFOA) in the liver of otters and the distance the polytetrafluoroethylene manufacturing facility was found (O'Rourke et al., 2022). PFAS level in otters was also found to be associated with arable land, which could be due to sewage sludge application, and waste water treatment works (O'Rourke et al., 2022). In general, higher concentration levels were measured in freshwater predator samples compared to prey ones. Specifically, concerning the concentration range was higher in freshwater predators (otter liver) than in the freshwater prey (bream and roach muscle).

ICs were mainly detected in UK liver predators (otter) ($n = 7$ ICs detected) and German prey species (bream muscle) ($n = 5$ ICs detected). Total dioxin content was observed at the highest concentration in predator samples (200–430 μg/kg) among the other ICs. The second most important IC was PCB 169 (96 μg/kg). Such a finding may be of concern as it has been suggested already 20 years ago that PCBs are among the major drivers for the decline of otters in Europe (Smit et al., 1998). Moreover, due to the persistent nature of some PCBs, they could remain in top predators in freshwater (including otters) at threatening levels even decades after legislative restrictions on PCBs in the region (Kean et al., 2021). The most frequent ICs for the freshwater prey were total dioxin content (BEQ: 2.3–11 μg/kg) and PCB 28 (0.21–14 μg/kg) both detected in bream muscle.

3.3. Predominant chemical mixtures in marine ecosystems

In order to establish the predominant chemical mixtures, present in the AP&P of the marine ecosystems, a search on the chemical mixture graph database for the co-occurring compounds in the collected samples of eelpouts, herring, dolphin, harbour porpoise and seal over the full frequency of appearance range was performed. The top 25 compounds in the number of predator-prey pairs are listed in Table 3. The top 25 significant constituents of chemical mixtures found in marine AP&P include PFOS, mercury, 14 PCBs, 7 BDEs, *n*-acetylaminoantipyrine. 23 of these chemicals were also among the top 25 predominant chemicals found in freshwater AP&P. Mercury and PFOS were the most commonly detected compounds which showed ubiquitous presence in marine AP&P, with median concentration of 14,676 and 310.60 μg/kg ww in predator samples; 100.87 and 5.96 μg/kg ww in prey samples, respectively. Globally, these predominant chemicals have been produced and used in high quantities and were regulated in the 1990s and 2000s.

The top predominant chemical class was PFAS (10.2–17,000 μg/kg) in top predators in the marine environment (Table 3). The next most important chemical class was Ph&TPs (15–3200 μg/kg), followed by ICs (0.01–489 μg/kg) and finally S&TPs (16–256 μg/kg). The predominant chemical class for marine prey species was Ph&TPs (0.99–65 μg/kg) followed by PFAS (0.94–63 μg/kg), ICs (0.02–11 μg/kg) and S&TPs (0.18–1.4 μg/kg). The predominant chemical mixtures were detected in lower concentrations in prey samples for all chemical classes.

Higher concentration levels of PFAS were measured in marine predator samples (98.4–17,000 μg/kg) compared to fish samples (0.94–63 μg/kg). In both marine predators and fish species, PFOS is the predominant PFAS detected followed by PFDA. Similar distribution patterns of PFAS have been observed in marine mammals in China (Lam et al., 2016), Greenland (Greaves et al., 2012), Iceland and the US (Spaan et al., 2020). PFOS remained as the dominant PFAS in marine mammals for around two decades following the phasing out of PFOS production (Villanger et al., 2020). The high abundance of PFOS in marine species is of concern due to its toxic properties marine organisms (Sant et al., 2021).

The presence of ICs was detected in the livers of marine predators and prey species from Germany, the UK and The Netherlands. Outside of PCDD/F/dlPCB (BEQ), the most frequently detected ICs in marine top predator samples were PCBs (congeners 180, 156, 153, 138, 118, 105). In marine prey species, the most important ICs were PCB 28 (14 μg/kg) and total dioxin content (BEQ- 11 μg/kg). Nonetheless, PCBs account for 14 of the 25 most frequently co-occurring compounds in marine species. These compounds could bioaccumulate through the complex food web of the marine ecosystem which make them one of the top predominant chemical groups in the marine top predators (Khairy et al., 2021).

4. Conclusions

LIFE APEX partners collected and analysed 145 marine mammal and fish samples for persistent pollutants and a wide range of emerging contaminants. The data was visualized using co-occurrence analysis via graph analytics and analysed using mammal-fish count pairs. Mercury and long-term regulated PFOS, PBDEs, PCBs and HCB were still the dominant compounds in the samples from both environments. The data indicate that the environment is responding to chemical management as these substances are internationally and regionally regulated in environmental, emissions and chemical legislations. The findings of this study offer supporting evidence for chemical regulators to effectively manage potentially hazardous substances. Our analysis provided evidence for ubiquitous chemical occurrence of the compound n-Acetylaminoantipyrine. The application of new data science techniques to tackle challenging issues can be beneficial for researchers and chemical

Table 3
The 25 most frequently co-occurring substances in AP&P in the marine environment.

Compound	CAS RN	Chemical Group	Limit of Quantification (μg/kg ww	Median concentration in marine predators liver μg/kg ww	Median concentration in fish muscle μg/kg ww	Frequency of appearance (FoA)
Mercury	7439-97-6	Heavy metal	0.245	14,676	100.87	100
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	PFAS	0.25	310.6	5.96	100
2,2′,3,4,4′,5′-Hexachlorobiphenyl (PCB 138)	35065-28-2	IC	0.003	46.94	4.49	98.4
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl (PCB 180)	35065-29-3	IC	0.006	24.31	1.92	98.4
2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB 153)	35065-27-1	IC	0.003	70.09	6.88	98.4
2,2′4,4′-Tetrabromodiphenyl ether (BDE 47)	5436-43-1	IC	0.013	2.98	0.55	98.4
2,3′,4,4′,5-Pentachlorobiphenyl (PCB 118)	31508-00-6	IC	0.003	2.89	1.36	98.4
2,3,3′,4,4′-Pentachlorobiphenyl (PCB 105)	32598-14-4	IC	0.003	2.89	1.36	98.4
2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB 156)	38380-08-4	IC	0.006	1.09	0.27	98.4
Hexachlorobenzene	118-74-1	IC	0.001	1.26	1.16	98.4
2,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 167)	52663-72-6	IC	0.003	0.31	0.18	96.8
2,2′,4,4′,6-Pentabromodiphenyl ether (BDE 100)	89084-64-8	IC	0.013	1.21	0.18	95.2
2,2,4,5,5′-Pentochlorobiphenyl (PCB 101)	37680-73-2	IC	0.003	4.54	0.8	91.9
2,3,3′,4,4′,5′-Hexachlorobiphenyl (PCB 157)	69782-90-7	IC	0.003	0.71	0.08	88.7
2,4,4′-Tribromodiphenyl ether (BDE 28)	41318-75-6	IC	0.013	0.75	0.18	95.2
2,2′,5,5′-Tetrachlorobiphenyl (PCB 52)	35693-99-3	IC	0.25	5.24	2.5	82.3
2,3,4,4′,5-Pentachlorobiphenyl (PCB 114)	74472-37-0	IC	0.003	0.31	0.06	85.5
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl (PCB 189)	39635-31-9	IC	0.003	0.49	0.2	95.2
2,2′,4,4′,5-Pentabromodiphenyl ether (BDE 99)	60348-60-9	IC	0.013	0.9	0.08	82.3
N-Acetylaminoantipyrine	83-15-8	Ph&TPs	0.85	344.02	3.65	67.7
2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether (BDE 183)	207122-16-5	IC	0.025	0.61	0.08	85.5
2′,3,4,4′,5-Pentachlorobiphenyl (PCB 123)	65510-44-3	IC	0.003	0.36	0.12	74.2
3,3′,4,4′-Tetrachlorobiphenyl (PCB 77)	32598-13-3	IC	0.003	0.25	0.05	53.2
2,2′,4,5′-Tetrabromodiphenyl Ether (BDE 49)	243982-82-3	IC	0.013	0.19	0.07	50
2,2′,4,4′,5,6′-Hexabromodiphenyl ether (BDE 154)	207122-15-4	IC	0.025	1.11	0.19	74.2

regulators. In our study, we highlight the use of graph analytics to address chemical mixtures in the freshwater and marine environments. However, it should be noted that sample collection and the graphic analytics of the LIFE APEX website aimed to achieve a wide-spatial distribution and not to establish food chain associations. Therefore, the presented links need to be taken with caution and must be verified given that the samples were not taken in the same spatial context.

CRediT authorship contribution statement

Nikiforos Alygizakis: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Niki Kostopoulou:** Writing – review & editing, Data curation. **Georgios Gkotsis:** Writing – review & editing, Investigation, Data curation. **Maria-Christina Nika:** Writing – review & editing, Investigation, Data curation. **Anastasia Orfanioti:** Writing – review & editing, Data curation. **Kelsey Ng:** Writing – original draft, Methodology, Investigation, Data curation. **Erasmia Bizani:** Writing – review & editing, Investigation. **Varvara Nikolopoulou:** Writing – review & editing, Investigation, Data curation. **Alexander Badry:** Writing – review & editing, Resources, Investigation. **Andrew Brownlow:** Writing – review & editing, Resources. **Cinzia Centelleghe:** Writing – review & editing, Resources. **Elizabeth A. Chadwick:** Writing – review & editing, Resources. **Tomasz M. Ciesielski:** Writing – review & editing, Resources. **Alessandra Cincinelli:** Writing – review & editing, Resources, Data curation. **Sara Danielsson:** Writing – review & editing, Data curation. **Rene W.R.J. Dekker:** Writing – review & editing, Project administration, Funding acquisition. **Guy Duke:** Writing – review & editing, Investigation. **Natalia Glowacka:** Writing – review & editing, Project administration. **Pavel Gol'din:** Writing – review & editing, Resources. **Hugh A.H. Jansman:** Writing – review & editing, Resources. **Thierry Jauniaux:** Writing – review & editing, Resources. **Burkhard Knopf:** Writing – review & editing, Data curation. **Jan Koschorreck:** Writing – review & editing, Resources, Data curation. **Oliver Krone:** Writing – review & editing, Resources. **Xabier Lekube:** Writing – review & editing, Resources. **Tania Martellini:** Writing – review & editing, Data curation. **Paola Movalli:** Writing – review & editing, Funding acquisition, Data curation. **Emily O'Rourke:** Writing – review & editing,

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Declaration of competing interest

All the authors declare no conflict of interest.

Data availability

All data is available in the publication and the supplementary material.

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Sample distribution

All samples were shipped following the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) lists.

Disclaimer

The content of this article reflects only the authors' views and the Research Executive Agency is not responsible for any use that may be made of the information it contains.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.175303.

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