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Assessment of contaminants of emerging concern in European apex predators and their prey by LC-QToF MS wide-scope target analysis

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ABSTRACT

Apex predators are good indicators of environmental pollution since they are relatively long-lived and their high trophic position and spatiotemporal exposure to chemicals provides insights into the persistent, bioaccumulative and toxic (PBT) properties of chemicals. Although monitoring data from apex predators can considerably support chemicals' management, there is a lack of pan-European studies, and longer-term monitoring of chemicals in organisms from higher trophic levels. The present study investigated the occurrence of contaminants of emerging concern (CECs) in 67 freshwater, marine and terrestrial apex predators and in freshwater and marine prey, gathered from four European countries. Generic sample preparation protocols for the extraction of CECs with a broad range of physicochemical properties and the purification of the extracts were used. The analysis was performed utilizing liquid (LC) chromatography coupled to high resolution mass spectrometry (HRMS), while the acquired chromatograms were screened for the presence of more than 2,200 CECs through wide-scope target analysis. In total, 145 CECs were determined in the apex predator and their prey samples belonging in different categories, such as pharmaceuticals, plant protection products, per- and polyfluoroalkyl substances, their metabolites and transformation products. Higher concentration levels were measured in predators compared to prey, suggesting that biomagnification of chemicals through the food chain occurs. The compounds were prioritized for further regulatory risk assessment based on their frequency of detection and their concentration

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levels. The majority of the prioritized CECs were lipophilic, although the presence of more polar contaminants should not be neglected. This indicates that holistic analytical approaches are required to fully characterize the chemical universe of biota samples. Therefore, the present survey is an attempt to systematically investigate the presence of thousands of chemicals at a European level, aiming to use these data for better chemicals management and contribute to EU Zero Pollution Ambition.

1. Introduction

Contamination of the environment with a "cocktail" of various and dynamically changing anthropogenic chemicals, including persistent organic pollutants (POPs) and contaminants of emerging concern (CECs), remains a challenging environmental problem due to the excessive production and consumption of chemicals, such as pharmaceuticals, personal care products, plant protection products, food additives, industrial chemicals and flame retardants, amongst others (Ali et al., 2018; Badea et al., 2020; Reichert et al., 2019; Vargas-Berrones et al., 2020). The term CECs has been established for a broad range of non-regulated substances, which are candidates for future regulation due to their consecutive detection in environmental compartments and their potential hazardous properties (Dulio et al., 2018; Gavrilescu et al., 2015; Thomaids et al., 2012). CECs are distributed in aquatic and terrestrial ecosystems through various pathways and, some are biomagnified through the food web to higher trophic levels. Although CECs are present at trace concentrations in the environment, their monitoring, which is not systematic up to date, is highly recommended since they are likely to be of major concern for biodiversity and human health (Badea et al., 2020; Montesdeoca-Esponda et al., 2018).

Biomonitoring studies using wildlife are suitable to assess the spatiotemporal distribution of chemicals, and their possible bioaccumulative properties, if both predator and prey samples have been analyzed (Badry et al., 2020; Crimmins et al., 2018; Espin et al., 2020). Although the establishment of a pan-European biomonitoring survey is of high importance, due to the harmonization of the chemicals management, though European legislation, it requires compliance of sampling, processing, and chemical analysis (García-Fernández et al., 2020; Movalli et al., 2017). Chemical trends in wildlife can expand the knowledge on mixtures of bioaccumulative CECs in organisms and, thus, provide insights into the current ecosystem quality, contribute to the assessment of EU risk mitigation measures of CECs, and ultimately help to underpin more effective regulatory actions and, thereby, protect wildlife and human health from the adverse effects of CECs (Badry et al., 2020; Movalli et al., 2019).

Apex predators have numerous characteristics which make them particularly appropriate as sentinel species for monitoring of CECs, especially for those which have toxic, persistent and bioaccumulative properties. These characteristics include (i) their high trophic position in terrestrial or aquatic food webs, (ii) their long lifespan over which they might bioaccumulate contaminants, (iii) their diverse nutrition, (iv) the availability of non-invasive sampling strategies for gathering samples (for example carcasses, blood and feathers or deserted eggs in raptors), (v) the high lipid content in selected matrices (e.g. liver) which allows the accumulation of lipophilic CECs, (vi) the measurable responses to exposure in chemicals, especially POPs, and (vii) the exposure over time and relatively large spatial areas, enabling surveys of both spatial and temporal distribution of chemicals (Badry et al., 2020; Briels et al., 2019; Cossaboon et al., 2019; Espin et al., 2020; Li et al., 2018; Løseth et al., 2019; Lu et al., 2019; Munschy et al., 2020; Sánchez-Virosta et al., 2020; Sham et al., 2020).

Over the last decade, several studies focused on the occurrence and the potential bioaccumulation of chemicals in organisms through the investigation of specific classes of micropollutants in the upper trophic levels of food webs. The biomonitoring of less polar, semi-volatile and thermally stable micropollutants, such as POPs and Halogenated Organic Chemicals (HOCs), in marine mammals has been widely studied within research projects and monitoring programmes (Aznar-Alemany et al., 2019; Cossaboon et al., 2019; Kajiwara et al., 2008; Law et al., 2013; Weijs et al., 2020). Similarly, widespread contamination with legacy and emerging HOCs has also been reported in raptors (Guerra et al., 2012; Yu et al., 2013) in an effort to assess the chemical contamination in terrestrial food webs. Temporal trends of POPs have been investigated in apex predators from the aquatic ecosystem (Bjurlid et al., 2018; Rotander et al., 2012). The presence of GC-amenable compounds in marine mammals belonging to other classes, such as chlorinated paraffins (CPs), polycyclic aromatic hydrocarbons (PAHs) and plasticizers, has been reported by Guan et al. (2019), Kannan and Perrotta (2008) and Papachlimitzou et al. (2015). Per- and Polyfluoroalkyl Substances (PFAS) constitute a class of CECs, which is at the forefront of scientific interest in recent years due to their wide distribution in aquatic ecosystems and the bioaccumulative properties of certain compounds which make them harmful for apex predators and humans. Recent research has proven that PFAS are present at high concentrations in organisms from both lower and upper trophic levels (Androulakakis et al., 2022; Butt et al., 2010; De Silva et al., 2021; Muir et al., 2019). The presence of PFAS in aquatic organisms of lower trophic levels is well-reported (Catherine et al., 2019; Fliedner et al., 2020), and recently the scientific interest has shifted to the investigation of their occurrence at higher trophic levels of the terrestrial and aquatic food webs (Fujii et al., 2018; Li et al., 2018; Sedlak et al., 2017; Spaan et al., 2020; Wu et al., 2020). For example, through the target analysis of PFAS in 140 liver samples of Eurasian Otters (*Lutra lutra*) gathered from Scandinavia from 1972 till 2011, Roos et al. (2013) observed increasing trends in nine PFAS and extremely high concentrations of Perfluorooctanesulfonic acid (PFOS) which supports the concern of trophic magnification. Moreover, many recent studies focused on other classes of CECs, such as pharmaceuticals, personal care products (Ali et al., 2018; Bean et al., 2018; Montesdeoca-Esponda et al., 2018; Swiacka et al., 2019; Ventura et al., 2002) and plant protection products (Abrantes et al., 2010; Wiens et al., 2019). During the last years, there is a continuously growing interest in the simultaneous investigation of CECs' occurrence in aquatic organisms of the lower trophic levels, mainly fish and mussels, as model organisms of the current legislation. Through the development and application of a multiresidue analytical methodology, Mijangos et al. (2018) reported the occurrence of 41 multiclass organic micropollutants in mussels and fish tissues from Spain. Pico et al. (2019) revealed the presence of 76 compounds, including pharmaceuticals, pesticides, PFAS and endocrine disrupting compounds (EDCs) in fish collected from four Spanish rivers. Moreover, Omar et al. (2019) identified dozens of CECs, classified in four chemical classes, in fish and mussels. Although there are plenty of studies focusing on the determination of CECs in apex predators, their application is mainly restricted to the analysis of specific classes of pollutants, the presence of metabolites and transformation products (M&TPs) is usually overlooked and typically only specimens collected from one selected region or country are investigated. Therefore, there remains a high need for a pan-European biomonitoring study of different classes of CECs and their M&TPs in marine, freshwater and terrestrial apex predators, using high resolution mass spectrometry techniques.

Established low resolution mass spectrometric (LRMS) methodologies have been developed for specific chemical classes and selected analytes, providing sufficient sensitivity and allowing the reliable identification of micropollutants in trace levels (Lee et al., 2019). However, more recently, the evolution of hyphenated analytical

chromatographic coupled with high resolution mass spectrometric (HRMS) techniques has empowered the comprehensive screening of a wide range of CECs, as well as their M&TPs that occur in the environment (Badea et al., 2020; Crimmins et al., 2018; Hollender et al., 2019; Schymanski et al., 2015). For the simultaneous monitoring of thousands chemicals, novel, comprehensive workflows, including wide-scope target analysis, suspect and non-target screening methodologies, have been recently developed (Badea et al., 2020; Crimmins et al., 2018; Gago-Ferrero et al., 2020; Hollender et al., 2017; Krauss et al., 2010).

The EU funded LIFE APEX project (LIFE17 ENV/SK/000355, https://lifeapex.eu/, 2018–2022) aimed to improve the systematic use of chemical monitoring data from apex predators and their prey by regulators towards better chemicals management, reducing, thereby, the exposure to harmful substances and protecting the environment, wildlife and the human health. The implementation of the project included the target analysis of well-known pollutants including dioxins and dioxin-like compounds, chlorinated alkanes, organophosphorus flame retardants (OPFRs), mercury, PCBs, organochlorine pesticides (OCPs), PBDEs and Hexabromocyclododecane, along with wide-scope target and suspect screening for the simultaneous identification of thousands of CECs and M&TPs, in order to demonstrate the chemicals' occurrence at the higher trophic levels.

The primary objectives of the current study were (i) the application of a novel analytical protocol for the simultaneous determination of thousands CECs and their M&TPs in apex predators and prey species at a European level, (ii) the evaluation of different apex predator species as sentinels for biomonitoring studies and (iii) the investigation of potential CECs' occurrence patterns in four countries. For this reason, 67 recent specimens of apex predator and their prey samples were gathered from four countries of Northern Europe (United Kingdom, Germany, the Netherlands, and Sweden) and analyzed for more than 2,200 organic CECs and their M&TPs, classified in different groups of chemicals, using wide-scope target analysis.

2. Materials and methods

2.1. Sampling

Apex predators, which were found dead, were gathered together with freshwater and marine prey species from four European countries - United Kingdom, Germany, the Netherlands and Sweden. Samples were provided by European Environmental Specimen Banks (ESBs), Natural History Museums (NHMs) and other Research Collections (RCs). The majority of the samples were pooled and constituted of 2–5 individuals (in case of apex predators), collected in the same calendar year from closely situated locations to obtain representative samples and gather sufficient sample quantity to perform the required analyses. In total 67 apex predator and prey samples, collected during 2015–2019, were analyzed through wide-scope target analysis. The selected matrix of analysis for apex predator samples was liver since liver is the primary organ of xenobiotic metabolism and a target organ of toxicity for many CECs (Yeh et al., 2017), whereas for prey samples muscle tissue was chosen, as a matrix which is most likely eaten by predator species. The species used in the present study as well as their spatial distribution are depicted in Fig. 1. The prey specimens were freshwater fish; Common Roach (*Rutilus rutilus*, n = 5) and Common Bream (*Abramis brama*, n = 6), as well as marine fish; European Eelpout (*Zoarces viviparous*, n = 3) and Atlantic Herring (*Clupea harengus*, n = 3). Five apex predator species were selected, based on their different habitats and feeding strategies, including one terrestrial raptor; Common Buzzard (*Buteo buteo*, n = 12), one mainly freshwater mammal; Eurasian Otter (*Lutra lutra*, n = 20) and three marine mammals; Harbour Porpoise (*Phocoena phocoena*, n = 5), Harbour Seal (*Phoca vitulina*, n = 10) and Grey Seal (*Halichoerus grypus*, n = 1). Common buzzards have been identified as suitable sentinel species for pan-European biomonitoring (Badry et al., 2020), Eurasian otters as indicators for freshwater environment contamination (Kean et al., 2021) and marine mammals as ideal sentinels for assessing marine ecosystem quality (Fossi and Panti, 2017; Sonne et al., 2020). Multiple marine mammal species were included in the present study, due to their

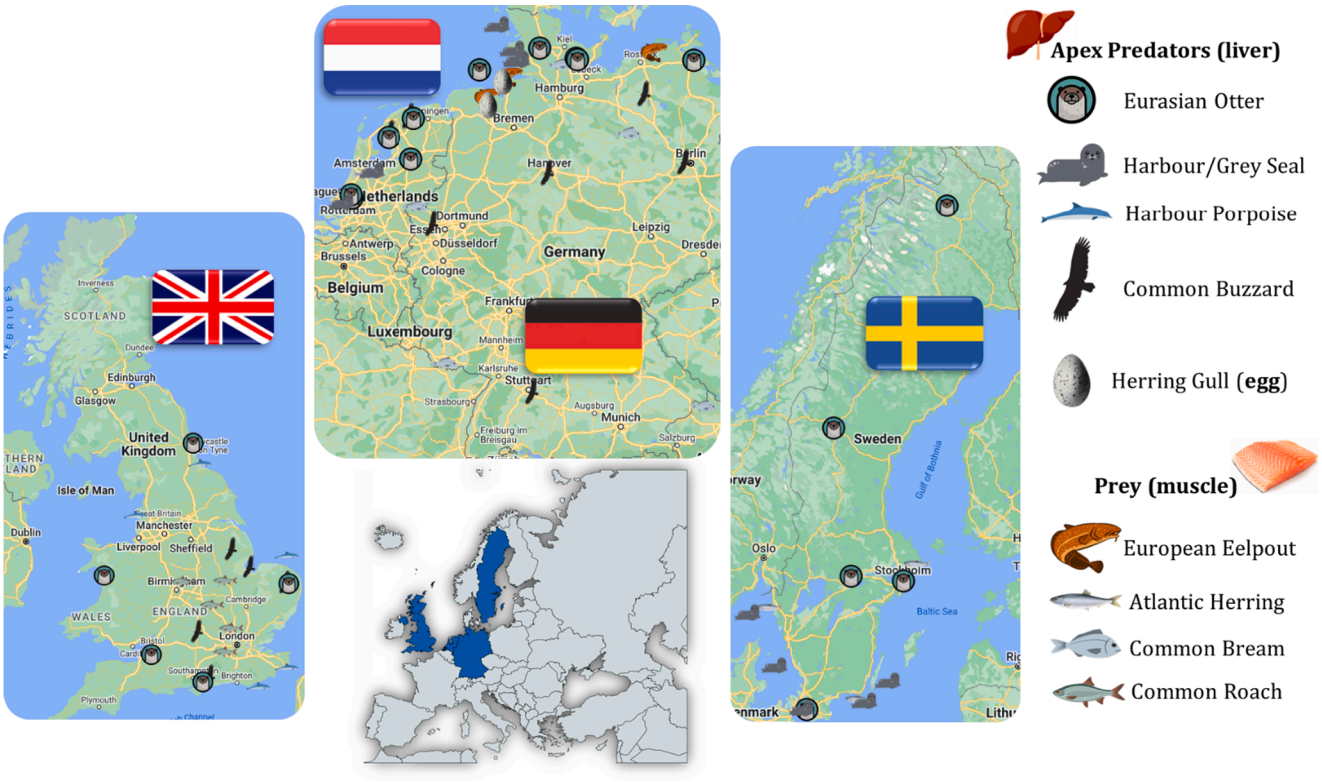

Fig. 1. Spatial distribution of the sampling locations of apex predators and their prey specimens across Europe. An on line version is also available [here].

regional limited distribution across Europe. Furthermore, two eggs from Herring Gull (*Larus argentatus*) were analyzed.

All samples were shipped on dry ice and delivered to the laboratory within two days. Common buzzard, Eurasian Otter and Harbour Porpoise specimens, included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) lists, were shipped under CITES permission and were delivered to the Zoological Museum, National and Kapodistrian University of Athens, Faculty of Sciences, Department of Biology, Panepistimioupolis Athens, which is a registered institution. The samples were lyophilized upon receipt, homogenized using a pestle with mortar or a laboratory blender, and stored at − 80 °C until analysis. Detailed metadata on the samples of the current study was collected by the samples' providers: Center for Ecology & Hydrology, UK Cetacean Strandings Investigation Programme and Cardiff University (United Kingdom), German Environment Agency, Fraunhofer Institute for Molecular Biology and Applied Ecology, University of Veterinary Medicine Hannover, Foundation and Leibniz Institute for Zoo and Wildlife Research (Germany), Naturalis Biodiversity Center and Wageningen University & Research (the Netherlands) and the Swedish Museum of Natural History (Sweden) and listed in the Supplementary Material (SM-2).

2.2. Analytical methodology

A validated generic sample preparation protocol for the simultaneous determination of polar and semi-polar emerging contaminants in liver (Badry et al., 2022) and muscle tissue was used in this study. Briefly, the extraction of the analytes from the lyophilized biota matrices was carried out 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 into a 2 mL vial, before the analysis by liquid chromatography coupled with high resolution mass spectrometry. A detailed description of the analytical methodology, as well as the analytical performance details, are included in Supplementary Material (SM-3).

2.3. Instrumental analysis

The analysis of the final extracts of biota samples was conducted using an ultra-high performance liquid chromatographic system (UHPLC, Dionex UltiMate 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 C_{18} column (2.1 × 100 mm, 2.2 μm) from Thermo Fisher Scientific, connected to an ACQUITY UPLC BEH C_{18} 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. The detailed description of the instrumental analysis is included in Supplementary Material (SM-3).

2.4. Data treatment

Wide-scope target analysis was performed using an in-house developed database of 2,273 contaminants of emerging concern of high environmental significance, including compounds from different chemical classes, having a broad spectrum of applications, use, physicochemical properties and extent of production. The database (https://doi.org/10.5281/zenodo.6012778, last visit: 21/06/2022, version 2019) is available as S21 UATHTARGETS in NORMAN Suspect List Exchange (*https://www.norman-network.com/nds/SLE/*, last visit 21/06/2022). 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 (<2 mDa), 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.

2.5. Quality assurance – Quality control

As described in Badry et al. (2022) and Gago-Ferrero et al. (2020) a smart validation was performed for the tested matrices using a mix of CECs, including representative compounds of the wide-scope target database. The quantification of the detected compounds was performed using the standard addition method and representative structurally related isotope-labeled compounds (Internal Standards, IS). Matrices with the lowest detected compound number and concentration levels used for the quantification experiments, as contaminant-free biota are not commercially available for chemical analysis purposes. Linearity was calculated and evaluated, whereas % Recoveries and % Factors of Matrix Effects were calculated in different concentration levels using standard solutions, spiked and matrix-matched samples (analytes were spiked prior to extraction and prior to instrumental analysis, respectively). Method limits of detection (LODs) were calculated from standard addition curves (using relative peak areas of spiked samples) with the following equation: LOD = 3.3*(Sb/S), where S is the slope of the calibration curve and Sb is the standard deviation of the response. The contaminants that were detected in traces below the limit of quantification (LOQ) were reported as Below Quantification Limit (BQL). For the statistical treatment of the results, the substitution of BQL with LOQ/2 has been performed, as indicated by Directive 2009/90/EC.

Quality Control was conducted by the simultaneous process and monitoring of solvent blanks, procedural blanks, standards, and spiked samples during the analysis of all samples. The detected compounds in procedural blanks, which indicate potential contamination throughout the analytical procedure and instrumental analysis, were subtracted from the samples, but the majority of analytes was not present in the blanks. Two mixes (for positive and negative ionization mode respectively), each containing 18 known substances (Retention Time Indices -RTI- calibrant compounds), were injected in the sequence for the assessment of retention time shift (Aalizadeh et al., 2021) and for additional data treatment of the chromatograms following suspect and non-target screening workflows. The acquired HRMS data have been digitally stored in the NORMAN Digital Sample Freezing Platform (DSFP) for retrospective suspect screening purposes (Alygizakis et al., 2019). A QC sample, including a representative number of CECs included in the target list, was injected every ten injections for the evaluation of good operation and high sensitivity of the instrumentation.

3. Results and discussion

Overall, 145 CECs were detected in the analyzed apex predator and prey samples through wide-scope target analysis, indicating the widespread exposure of wildlife, notably in apex predator species. The determined compounds belonged to different categories, based on their use, application, or chemical class. As depicted in Fig. 2, the majority of the detected compounds were pharmaceuticals (37%), followed by pesticides (plant protection products and biocides) (28%) and PFAS (13%). Detailed occurrence data on the presence of CECs in the apex

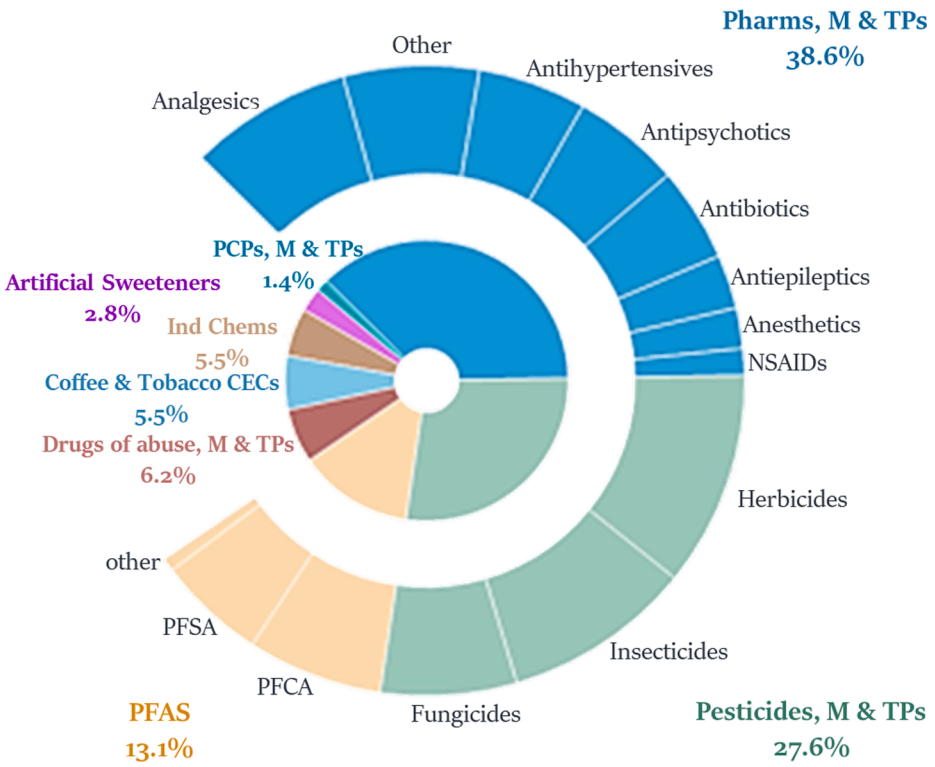

Fig. 2. Chemical classes and sub-classes of the detected contaminants of emerging concern though wide-scope target analysis, based on their use, application, or chemical class. The following abbreviations were used: Pharms: pharmaceuticals; NSAIDs: non-steroidal anti-inflammatory drugs; PFAS: per- and polyfluoroalkyl substances; PFCA: perfluoroalkyl carboxylic acids; PFSA: perfluoroalkyl sulfonic acids; Ind. Chems: industrial chemicals; PCPs: personal care products; M: metabolites; TPs: transformation products.

predators and their prey samples is included in the Supplementary Material (SM-4, Table SM-5).

3.1. Pharmaceuticals, personal care products, M&TPs

The increasing use of pharmaceuticals and personal care products has resulted in their widespread presence in the environment. In the framework of the current study 56 pharmaceuticals and personal care products were determined through wide-scope target analysis. The detected compounds belong to different drug classes. The dominant categories were analgesics (21%), antihypertensives (14%), antipsychotics (14%) and antibiotics (13%). A considerable percentage (27%) of the detected compounds were M&TPs, formed through biotic or abiotic processes, indicating their simultaneous presence in the environment alongside to the parent compounds. Therefore, the simultaneous monitoring of CECs, their M&TPs is crucial to conduct a reliable risk assessment, considering the potential harmful effects of both the parent compounds, their M&TPs.

Among personal care products, galaxolide, a synthetic musk and the organic UV-absorbing compound, 2-hydroxy-4-methoxybenzophenone (benzophenone 3) were determined in the tested samples. Galaxolide, used as synthetic musk in washing and personal care products, was present in substantially higher concentration levels in predator samples (mean concentration 491 ng/g w.w.) compared to prey samples (mean concentration 4.97 ng/g w.w.). The occurrence of galaxolide in fish and raptor samples from Germany has been previously reported and it has been characterized as bioaccumulative and toxic but not considered as persistent compound (Badry et al., 2022; Subedi et al., 2012). Benzophenone 3, which is an organic ingredient of many sunscreen lotions (UV filter), was detected in three roach samples from the rivers Lee (Wheathampstead), Thames (Standford-Abington) and Welland (Stamford Meadows) in United Kingdom (prey_UK_2, 4 and 5 respectively), with a mean concentration at 11.5 ng/g w.w. The occurrence of benzophenone 3 in marine organisms from lower trophic levels, mainly fish and crustaceans, has already been reported by Balmer et al. (2005), who revealed the presence of benzophenone 3 in roach from three lakes (Hüttnersee, Greifensee, Zürichsee) in Switzerland at concentrations ranging from 66 to 118 ng/g lipid weight, whereas the concentration levels in freshwater fish collected from Norway (Langford et al., 2015) and Germany (Meinerling and Daniels, 2006) were up to 182 and 21 ng/g d.w., respectively.

Among pharmaceuticals, the highest % Frequency of Appearance (% FoA) and concentration levels were observed for 4-acetamido-antipyrine (4-AAA), a metabolite of the widely used analgesic drug metamizole. The presence of 4-AAA in the various environmental compartments has previously been reported in ground and surface water, as well as wastewater samples (Diamanti et al., 2020; Hernández et al., 2015; Huntscha et al., 2012). The highest cumulative concentration of the two metamizoles' metabolites, 4-AAA and 4-formylamino-antipyrine, was observed in an otter from Bösdorf, Germany (predator_GE_11), whereas the parent compound, metamizole, was not detected, which may be attributed to the rapid metabolism of metamizole in the living organisms (Gómez et al., 2008).

Analgesics represented the 22% of the total number of the detected pharmaceuticals making them the dominant sub-class of pharmaceuticals. Residues of tramadol, which has already been reported in other environmental compartments, such as in wastewater, leachates and fish samples, (Fabunmi et al., 2020; Grabicova et al., 2017; Nika et al., 2020), along with five tramadol's M&TPs (listed in Table SM-4) were present in marine apex predator and prey species. Specifically, the parent compound was determined only in fish samples with a mean concentration of 0.344 ng/g w.w., whereas the concentrations of its M&TPs ranged from 1.20 to 15.6 ng/g w.w. in the apex predators, which may be attributed to the different matrices of analysis. The pattern of higher concentration levels of tramadol's M&TPs in contrast with the parent compound has previously been reported in fish samples from Germany (Boulard et al., 2020).

The occurrence of eight antipsychotic drugs, their M&TPs confirms the extensive consumption of such pharmaceuticals within the European Union (OECD, 2014). The highest total concentrations were observed in the United Kingdom, confirming the high consumption pattern and an incomplete removal during the processes that are applied in wastewater

treatment plants (WWTPs). It is worth highlighting that for a roach sample (prey_UK_2) from the river Lee (Wheathampstead), almost all the detected antipsychotic drugs (total concentration 3.65 ng/g w.w., n = 7) were found. The commonly prescribed selective serotonin re-uptake inhibitor (SSRI) fluoxetine, which has been characterized as a persistent and bioaccumulative compound, due to its stability in hydrolysis and photolysis in the aquatic environment (Fabunmi et al., 2020; Weinberger and Klaper, 2014), was detected only in the aforementioned roach sample at concentration levels comparable to those reported in the literature (Chu and Metcalfe, 2007). Moreover, citalopram and venlafaxine were determined with the highest %FoA, among the other antipsychotic drugs, in the tested samples.

3.2. Pesticides, M&TPs

In total 40 pesticides (plant protection products, biocides, M&TPs) were detected in the biota samples, classified in three main subclasses; herbicides (40%), insecticides (35%) and fungicides (25%), as illustrated in Fig. 2. The total concentration of the detected pesticides was considerably higher in apex predator versus the prey samples, which may be attributed to the different matrices of analysis. The highest concentration levels among predators were observed in otters and harbour porpoises. The fungicide spiroxamine, which has been marketed in the EU since the late 1990s, was detected with the highest %FoA (27% in predators, 21% in prey) among the determined pesticides. Metolachlor-OXA, a metabolite of the herbicide metolachlor, was the most abundant compound among the detected pesticides, determined only in freshwater apex predator liver samples (Eurasian otters) from Germany and Sweden with a mean concentration of 117 ng/g w.w. (range: 15.8–428 ng/g).

Atrazine, a priority substance of the Water Framework Directive, was detected only in the UK specimens, collected between 2014 and 2019 with a detection frequency of 55%, indicating its ubiquitous presence in the UK freshwater, marine and terrestrial ecosystems, despite the ban on its total use in the EU in 2004. The concentration levels in freshwater apex predator specimens (mean concentration 5.78 ng/g w.w.) were 10-fold higher than those detected in roach samples (mean concentration 0.584 ng/g w.w.), indicating a possible biomagnification at the higher trophic levels, although the matrices of analysis were different. It is remarkable that the chloro-s-triazine metabolite of atrazine, desisopropyl-atrazine, was detected (4.74 ng/g w.w.), along with the parent compound, in an otter (predator_UK_8), collected from Hertfordshire, United Kingdom. Furthermore, aldicarb sulfoxide, a metabolite of the carbamate insecticide aldicarb, was detected in a buzzard (predator_NL_2) from the province of Friesland the Netherlands. The high concentration of aldicarb sulfoxide (99.3 ng/g w.w.) depicts a potential biomagnification through the terrestrial food webs, since buzzards mainly feed on small mammals, birds, insects and reptiles (Badry et al., 2020). The herbicide dinoterb was determined with high % FoA (42%) in the fish samples. The presence of dinoterb has been reported in other environmental matrices, such as river water samples from Dniester, Ukraine (Diamanti et al., 2020) and landfill leachates from Greece (Nika et al., 2020).

3.3. Per- and polyfluoroalkyl substances (PFAS)

The presence of 19 PFAS was observed in the analyzed apex predator and prey samples. The majority of the detected PFAS were perfluoroalkyl carboxylic acids (PFCA) (n = 10), whereas eight perfluoroalkyl sulfonic acids (PFSA) and perfluorooctanesulfonamide (PFOSA) were determined through wide-scope target analysis. Despite the higher number of PFCA compared to PFSA, the cumulative concentrations of PFSA ($\sum_8 PFSA_{(prey)}$ = 0.294–111 ng/g w.w. and $\sum_8 PFSA_{(predators)}$ = 4.19 ng/g-17.0 μg/g w.w.) were 10-fold higher than the respective concentrations of PFCA ($\sum_{10} PFCA_{(prey)}$ = 0.469–10.9 ng/g w.w. and $\sum_{10} PFCA_{(predators)}$ = 0.556 ng/g-1.48 μg/g w.w.). However, it should be highlighted that this trend is mainly attributed to PFOS contribution. Furthermore, the cumulative PFSA and PFCA concentrations were higher in freshwater and marine apex predator than the respective prey samples, confirming that PFAS are biomagnified through the food web, as illustrated for freshwater apex predator and prey samples from the United Kingdom in Fig. 3.

PFOS was by far the dominant substance of the present biomonitoring study, with the highest detection frequency (100%) and the highest concentration (16.7 μg/g w.w. in an otter (predator_NL_3) from Overijssel, the Netherlands), although PFOS has been voluntarily phased out by industry in the early 2000 s. Moreover, the mean concentration of the tested fish samples (20.4 ng/g w.w.) exceeded the respective Environmental Quality Standard (EQS, 9.1 ng/g w.w.) established for fish (DIRECTIVE 2013/39/EU), demonstrating the extensive production in former decades and the bioavailability of legacy PFOS in the environment (Wu et al., 2020). These results denote that, although PFOS use is restricted under the REACH regulation and Stockholm Convention, additional mitigation measures should be established for the protection of environment and human health. Accordingly, PFOS was most dominant compound among PFAS in the organisms from the higher trophic levels, which is in accordance with the findings of other studies (Gebbink et al., 2016; Roos et al., 2013; Sedlak et al., 2017; Wu et al., 2020). On the contrary, the detected substitutes for the long-chain Perfluoroalkyl Sulfonic Acids, such as perfluorobutane sulfonic acid (PFBS) and perfluoropentane sulfonic acid (PFPeS), were rarely reported in apex predator and prey samples, indicating their recent production history, their insignificant presence in the aquatic ecosystem and their low bioaccumulation potential in food webs. Linear PFOS and the sum of mono- and di-substituted branched isomers of PFOS were chromatographically separated and quantified in the majority of the samples, whereas the sum of branched isomers of Perfluorohexane sulfonic acid (PFHxS) was also determined in a few samples. PFAS cumulative concentrations observed in the freshwater apex predator species (otter) were considerably higher compared to marine (pinnipeds) and terrestrial (buzzard) apex predator species as depicted in Fig. 4, in which the concentration levels for the different apex predator samples from the Netherlands are illustrated, as well as in the **Figure SM-4**, indicating that organisms in freshwater ecosystems seem to be more exposed to PFAS. PFOSA was not detected in the majority of marine apex predator samples, probably due to its possible biotransformation to PFOS in pinnipeds (Sedlak et al., 2017). Perfluorodecanoic acid (PFDA) was the second most abundant substance among the PFAS and the dominant PFCA with concentrations ranging from 3.59 to 3.73 ng/g w.w. in prey specimens and between below quantification limit (BQL) (<9.78 ng/g) and 930 ng/g w.w. in apex predators. The concentrations of the rest PFCA decreased with increasing chain length from the PFDA to perfluorotetradecanoic acid (PFTeDA), which is in line with PFCA pattern reported by Roos et al. (2013). However this finding is in contrast with the common behavior of PFCA, where concentrations of the odd chain-length PFCA were higher, in most cases, compared to those of their adjacent even chain-length homologues (Sedlak et al., 2017), which may be attributed to the different studied ecosystem and/or year of sampling.

3.4. Industrial chemicals

Among Industrial Chemicals, eight compounds (benzenesulfonamide, bisphenol A, 2,4-dinitrophenol, nonylphenol, benzotriazole, tolytriazole; the sum of 4- and 5-methyl-benzotriazole, 2-aminobenzothiazole, and 2-hydroxy-benzothiazole) were detected in at least one of the samples. 2-hydroxy-benzothiazole (2-OH-BTH) and benzotriazole were determined with the highest %FoA (31% and 16%, respectively) among Industrial Chemicals. Although a “cocktail” of six industrial chemicals was observed with high detection frequency in freshwater prey samples gathered from the United Kingdom, this trend was not followed in the respective predator (otter), as none of the screened industrial chemicals was detected (**Figure SM-6**).

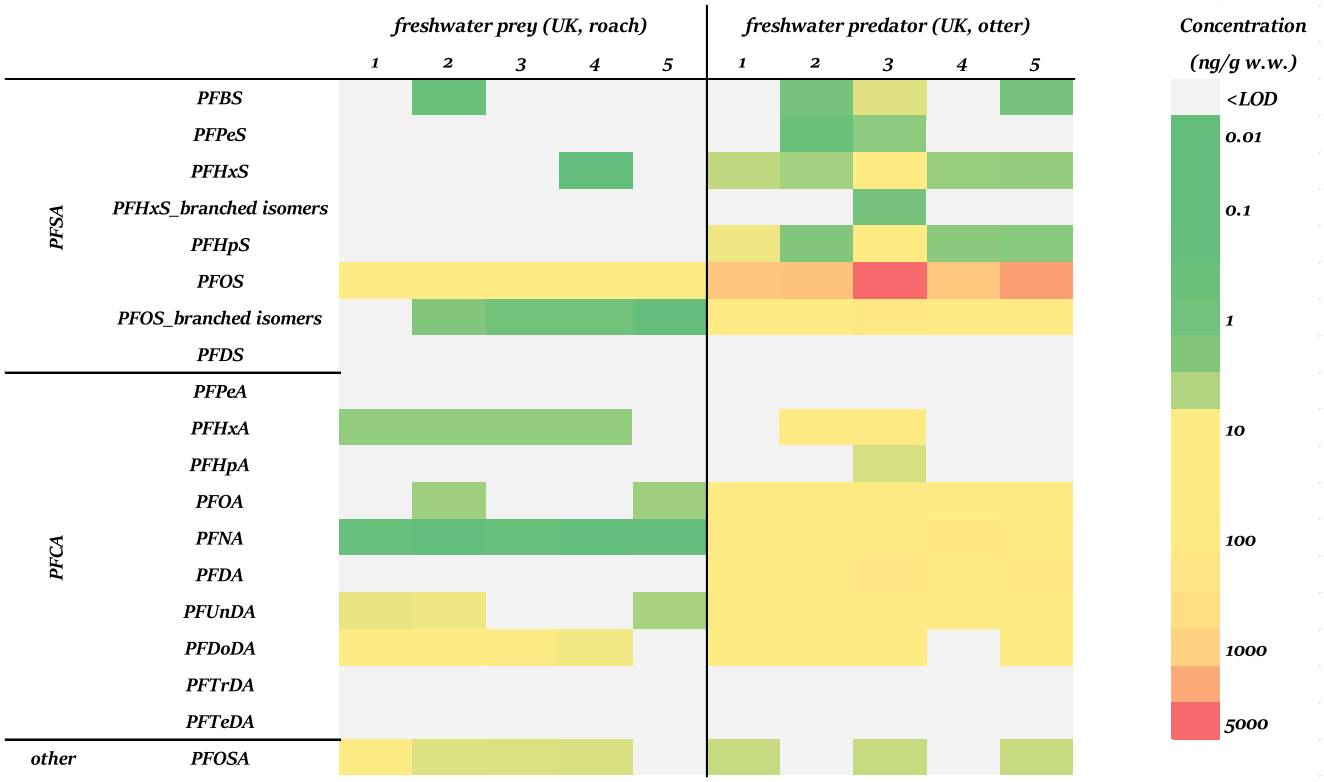

Fig. 3. Concentration levels (ng/g w.w.) of PFAS in freshwater apex predators and their prey samples, gathered from the United Kingdom. Grey tiles in the heatmap refer to concentrations below the limit of detection (LOD) for muscle tissue and liver respectively.

Fig. 4. Comparison of the PFAS concentrations in apex predators from different trophic webs, collected across the Netherlands. Concentration levels (ng/g ww.) of PFAS are provided as a heatmap, where grey tiles refer to concentrations below the limit of detection (LOD).

Furthermore, it is worth highlighting that 2-OH-BTH and 2-amino-benzothiazole (2-NH_2-BTH) were ubiquitous in Swedish freshwater and marine predator species (mean concentration 84.8 ng/g w.w. and BQL (<6.73 ng/g w.w.), 90% and 40% FoA, respectively), indicating that Swedish freshwater and marine (Baltic Sea) ecosystems are heavily impacted by anthropogenic activities. The chemical 2-OH-BTH was determined in 21 of the samples at concentrations ranging from BQL (6.95 ng/g w.w.) to 206 ng/g w.w., observed in freshwater fish and predator species respectively. The presence of 2-OH-BTH and 2-NH_2-BTH has been reported in aquatic organisms from the lower trophic

levels. Trabalón et al. (2017) reported the presence of benzothiazole and four of its derivatives in different aquatic biota samples from Tarragona, Spain; 2-NH_2-BTH was detected in 80% of the tested samples at concentrations up to 70 ng/g d.w., whereas 2-OH-BTH was determined at concentrations below the quantification limit (50 ng/g d.w.). Chen et al. (2020) reported the presence of 2-OH-BTH at concentrations ranging from 15.4 to 26.1 ng/g d.w. in fish from Taiwan, while Jia et al. (2019) calculated concentrations for 2-OH-BTH (FoA 89%) and 2-NH_2-BTH up to 512 and 15.7 ng/g d.w., respectively, in molluscs from the Bohai Sea, China. The endocrine disrupting compound Bisphenol A was detected only in three roach samples from the United Kingdom in concentrations below the quantification limit (<3.25 ng/g w.w.). These low levels are in accordance with the literature where up to 5.79 ng/g (average 2.54 ng/g) have been reported in fish samples from Klang River estuary, Malaysia (Omar et al., 2019). However, high levels of bisphenol A up to 200 ng/g d.w. have been detected in molluscs from the Bohai Sea, China (Liao and Kannan, 2019).

3.5. CECs of other chemical classes

The wide-scope target analysis revealed the presence of eight coffee and tobacco related CECs in apex predator and their prey samples. Residues of nicotine were detected in a quarter of the organisms from higher (25% FoA) and lower (26% FoA) trophic levels, but the concentrations in predators (mean concentration 26.4 ng/g w.w.) were considerably higher than those in prey (mean concentration 3.03 ng/g w.w.). Furthermore, as illustrated in **Figure SM-7**, three M&TPs of nicotine (cotinine, hydroxy-cotinine, nor-nicotine) were determined with higher detection frequency and concentration levels in freshwater apex predator samples than in the respective fish samples from Germany, which may be attributed to the different matrices of analysis (liver *vs* muscle). Cotinine, the predominant metabolite of nicotine, was omnipresent in half of the analyzed apex predator samples in concentrations ranging from 5.03 to 309 ng/g w.w. In general, the detection rate and the reported concentrations of the M&TPs were higher compared to the parent compounds in biota from the upper trophic levels, a trend which has been previously reported in other studies (e.g. Badry et al., 2022). Caffeine and its M&TPs had a lower detection frequency than tobacco related CECs in the samples. Caffeine concentrations ranged from BQL (<3.07 ng/g w.w.) to 3.83 μg/g w.w. It should be emphasized that in a buzzard sample (predator_GE_3) collected in Baden-Württemberg, Germany, the highest concentration of caffeine was observed, as well as of theobromine and theophylline, two M&TPs of caffeine (concentrations 32.4 and 38.7 ng/g w.w. respectively), indicating the importance of simultaneous monitoring of both parent compounds, M&TPs for a comprehensive risk assessment.

Residues of nine Drugs of Abuse, M&TPs (amphetamine, codeine, dihydro-codeine, nor-fentanyl, 3-methylnor-fentanyl, ketamine, norketamine, methamphetamine, and sulpiride) were determined in the tested samples. Ketamine, a drug with anesthetic effects, and its main TP, nor-ketamine, were detected only in one buzzard sample (predator_GE_2) from Lower Saxony, Germany. This, along with the presence of a mixture of medicinal products in elevated concentrations (e.g., the nonsteroidal anti-inflammatory drug meloxicam, concentration 104 ng/g w.w.) in the same sample may be the result of drug administration as one individual in the pooled sample received veterinary treatment prior to death. Similarly, the opioid analgesic codeine along with dihydrocodeine were detected in a roach (prey_UK_2) from river Lee (Wheathampstead, United Kingdom). The highest drugs of abuse detection frequency was observed in specimens collected across the United Kingdom, reflecting a high human consumption rate. The detection of numerous drugs of abuse in lower trophic level organisms from the United Kingdom has been reported in the literature (Miller et al., 2021, 2019), whereas the knowledge on for the presence of such compounds in apex predators from the United Kingdom is limited. Moreover, the TP of the opioid analgesic fentanyl, 3-methylnor-fentanyl, pointed out the highest concentration (177 ng/g w.w.) among the other drugs of abuse in the same samples. The detection of amphetamine and methamphetamine in apex predator and prey specimens was expected, since traces of such compounds have previously been reported in wastewater samples from Sweden (Östman et al., 2014), the Netherlands (Bijlsma et al., 2012) and the United Kingdom (Kasprzyk-Hordern et al., 2009; Kasprzyk-Hordern and Baker, 2012; Mwenesongole et al., 2013), as well as in white-tailed eagles (*Haliaeetus albicilla*) from Northern Germany (Badry et al., 2022). The presence of drugs of abuse, such as amphetamine, methamphetamine and codeine, in organisms from the lower trophic levels has been included in research findings of previously published monitoring studies (Malev et al., 2020; Yin et al., 2019). Yin et al., 2019 determined amphetamine and codeine in fish collected from Beijing urban rivers, with a high detection frequency at concentrations up to 6.1 and 5.3 ng/g, respectively.

Residues of four artificial sweeteners (acesulfame, cyclamic acid, saccharine, and aspartame) were reported in the tested biota samples, indicating a potential incomplete removal by WWTPs' technologies and, thus, their continuous accumulation in the aquatic ecosystem (Scheurer et al., 2009). The high concentration levels of saccharine and cyclamic acid may be attributed to wastewater discharges, since these artificial sweeteners have been characterized as ideal chemical tracers or indicators, due to their stability during the WWTPs' processes. The highest frequency of appearance was observed in apex predators, in particular in terrestrial, marine and freshwater predators from the Netherlands, which points out a possible biomagnification at higher trophic levels. All the aforementioned artificial sweeteners were detected at extremely high cumulative concentration (2.84 μg/g w.w.) in an Eurasian otter sample (predator_UK_7), gathered from North-East England.

3.6. Prioritization of the detected CECs

The prioritization of detected CECs is of significant value for regulatory authorities in identifying the substances for which mitigation measures may be required to protect environment, wildlife and human health in a One Health approach (Dulio et al., 2018). In an effort to prioritize the substances of the greatest concern in the apex predator and prey samples, a scoring system was designed using risk-relevant parameters. A score of 0 to 100 was assigned to each of two occurrence data values (maximum detected maximum concentration, frequency of appearance for apex predator and prey specimens) producing a cumulative score of 0 to 200 for predators, and a score of 0 to 200 for prey, and a total predator–prey score of 0 to 400 (**Table SM-6**). The 50 top prioritized CECs, based on their %FoA and their maximum detected concentration, are summarized in **Table SM-7**. To exclude from the list compounds with extremely high concentrations in selected samples, a threshold of 30% in FoA (in one of the tested subsets; prey and predator samples) was applied, resulting in the 33 top CECs that are listed in **Table SM-8**. Prey and predator samples were considered separately when applying FoA threshold, due to the different matrix of analysis and different profile of detected chemicals. Fig. 5 illustrates the presence of these compounds in the apex predator and prey specimens, analyzed in the current biomonitoring study.

One-third of the prioritized compounds were PFAS, indicating their widespread presence in the environment, while another one-third were pharmaceuticals and their M&TPs. While almost all PFAS detected in the samples included in the EU Directive 2020/2184 on the quality of water intended for human consumption (DIRECTIVE (EU) 2020/2184), 11 PFAS were on the list with the compounds of high priority, indicating the importance of implementing mitigation measures for PFAS at a European level. Moreover, five of the prioritized compounds (benzotriazole, PFOS, PFOA, PFNA, PFDA) were also reported among the 13 priority compounds in a study investigating the occurrence of emerging contaminants in South Korean effluent wastewaters through suspect and non-target screening methodologies (Choi et al., 2021). Additionally, the simultaneous occurrence of parent compounds and M&TPs

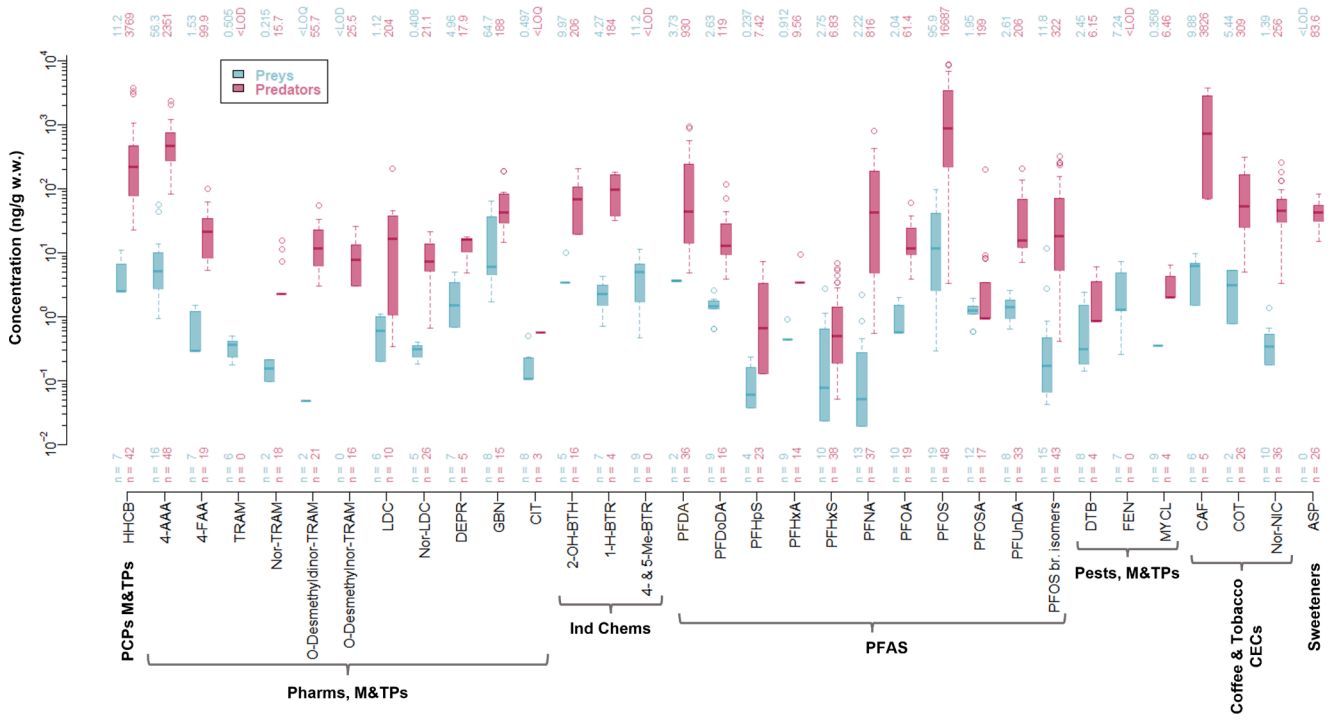

Fig. 5. Box-whisker plots of the observed concentration levels of the 33 top prioritized CECs, determined through wide-scope target analysis, in the apex predator (n = 48) and prey samples (n = 19). The maximum concentrations are indicated in the upper part of the figure, whereas below each box the number (n) of samples with concentrations above LOQ is provided. The abbreviated compound names are listed in **Table SM-8**.

(tramadol, lidocaine and their M&TPs) in the list of the prioritized compounds, as well as the elevated concentration levels in apex predators (liver) compared with the prey (muscle tissue) samples, supported the aforementioned statement that parent compounds and M&TPs should be monitored for a comprehensive risk assessment.

In an effort to understand the physicochemical properties of the top prioritized compounds, their lipophilicity was investigated, as illustrated in Fig. 6. The majority of the prioritized substances in the biota samples had a strong lipophilic character (logP > 2), reinforcing the necessity for continuous monitoring of such substances in apex

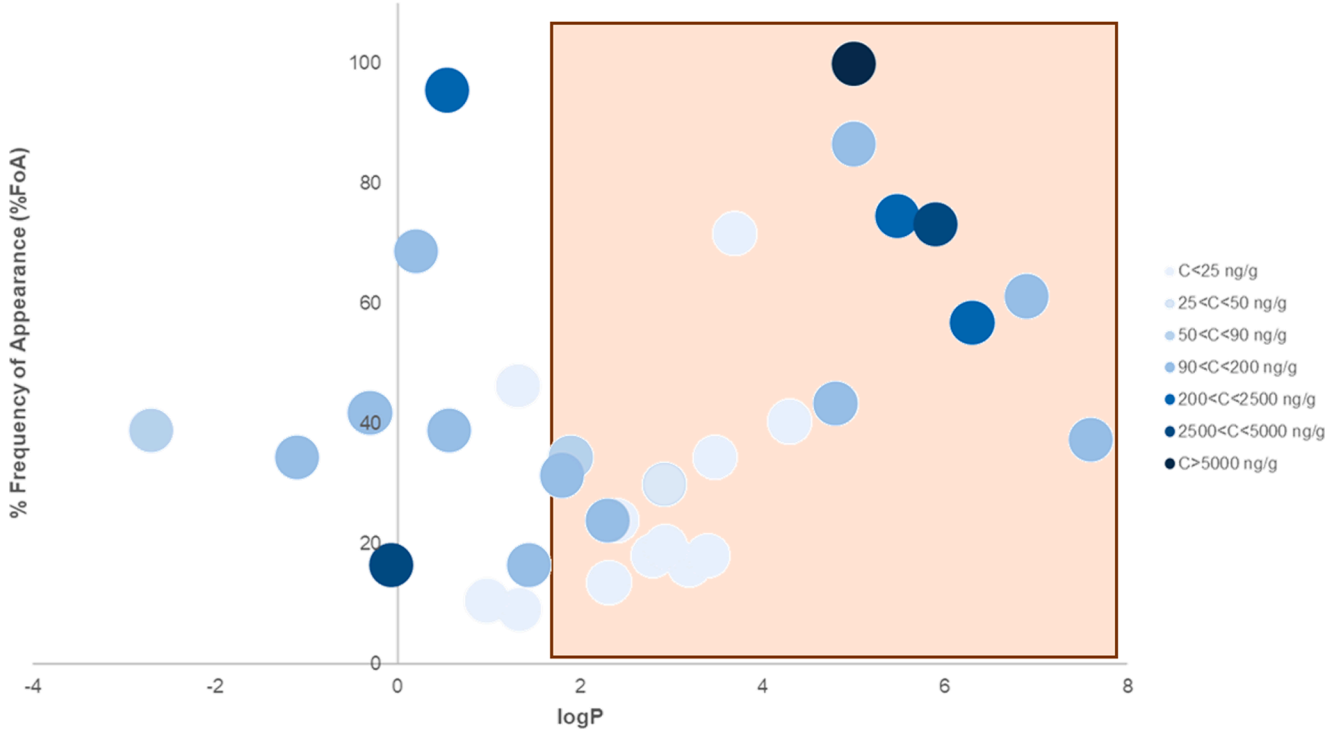

Fig. 6. Lipophilicity (logP) of 33 prioritized CECs. The most frequently detected substances and highest concentrations were observed for CECs with high lipophilic character (log P > 2, marked square in the figure). The logP values of the chemicals used for this figure are provided in the **Table SM-8**.

predators for the assessment of the potential harmful effects in the environment and human health. However, based on the outcome of the current study, the presence of (semi)polar substances should not be neglected, since, for example, some PFAS are binding to proteins or amino acids (Maso et al., 2021), instead of lipids, and, consequently, low Kow values may result in high exposure concentrations in organisms. A holistic analytical approach for the simultaneous determination of CECs with a broad range of physicochemical properties is, therefore, crucial to reveal the overall chemical fingerprint of CECs in the various environmental compartments. Moreover, additional orthogonal analysis by gas chromatography (GC) coupled to HRMS is required to capture non-polar contaminants as well, while the presence of lipophilic compounds that can be determined by both LC and GC analysis will be verified.

Prioritization scores and rankings may vary significantly based on prioritization parameters. Although uncertainties may exist in the present prioritization scheme (e.g. fish from riverine systems are constantly exposed to persistent and pseudo-persistent compounds due to effluent wastewater emissions), it does highlight compounds with high environmental significance based on their occurrence and/or high concentrations in organisms from various trophic levels. In the context of the LIFE APEX project, all CECs determined through wide-scope target analysis were examined for potential (v)PBT properties and prioritized under REACH by the Janus tool (https://www.vegahub.eu/portfolio-item/janus/), an *in silico* (Q)SAR model designed by the German Environment Agency (UBA) (Benfenati et al., 2016).

4. Conclusions

In the current biomonitoring study, which was part of the EU-funded LIFE APEX project, 67 freshwater, marine and terrestrial apex predator and prey samples from four European countries were analyzed by LC-ESI-QToF MS. The acquired HRMS data were screened for the presence of more than 2,200 contaminants of emerging concern through wide-scope target analysis. Therefore, the present survey is an attempt to systematically investigate the presence of thousands of chemicals at a European level, aiming to use these data for better chemicals management and contribute to EU Zero Pollution Ambition. The results indicate that apex predators and their prey are exposed to a diverse "cocktail" of contaminants of emerging concern. Specifically, wide-scope target analysis revealed the presence of 145 chemicals belonging to different categories, based on their main use, application or chemical class. The concentration levels for the majority of the detected CECs through wide-scope target analysis were lower compared to the concentrations of legacy pollutants, monitored in the context of LIFE APEX project, which are part of another publication. The majority of the determined compounds were pharmaceuticals, while pesticides and per- and polyfluoroalkyl substances were also omnipresent in the samples. The results of the current study also highlight the presence of considerably higher concentrations of chemicals in apex predators compared to their prey, although these samples were not co-located in time and space. In future biomonitoring studies in order to receive more reliable results on the bioaccumulative properties of CECs, organisms from different trophic levels, gathered from the same ecosystem and region, should be obtained. Furthermore, a large variety of metabolites and transformation products were determined, demonstrating the capabilities of HRMS in revealing the current state of environmental pollution. The simultaneous monitoring of CECs, along with their metabolites and transformation products is crucial since many compounds may be metabolized in high extend after their uptake from the organisms. The experience of human biomonitoring can support the screening of metabolites since it may reveal main metabolic pathways that can be used to predict additional metabolites of frequently detected CECs in apex predators to be investigated in future studies through suspect screening. Compounds included in the Stockholm Convention, such as PFOS and PFOA, were widespread in the samples, despite the established EU-wide mitigation measures, indicating that additional measures for the restriction of their use should be implemented. Moreover, all detected compounds observed in both apex predator and prey samples were prioritized based on their detection frequency and maximum concentration. Among the top prioritized compounds, the most ubiquitous chemicals in the samples had a strong lipophilic character, whereas the presence of more polar substances, underlines the power of HRMS techniques for revealing the "fingerprint" of the chemical pollution in the organisms. Additional orthogonal analysis by GC is required to capture non-polar contaminants, whereas the application of suspect and non-target screening workflows in the already acquired chromatograms will allow a comprehensive chemical characterization of the samples.

The obtained HRMS data are stored in a well-organized database system (NORMAN network DSFP), in order to be used by EU regulators and, thus, support the "zero-pollution" policy by restricting, where necessary, the production, use, or import of chemicals endangering Europe's environment. Furthermore, the chemicals detected in this study will be prioritized, based on their (v)PBT properties, using the Janus *in-silico* tool to inform better chemicals' management through the implementation of effective risk mitigation measures. The data reported here will be supported by additional studies providing time series data (1996–2018) to reveal time trend patterns of chemicals' emission in the environment and their uptake by biota. Finally, the current state of chemical contamination in apex predators, in Europe will be expanded by the analysis of samples from additional 19 countries across Europe, to identify which chemicals should be systematically monitored and/or further restricted. Overall, an integrated approach for monitoring and evaluation of chemicals was applied through the LIFE APEX project to link the environment, wildlife and humans together.

CRediT authorship contribution statement

Georgios Gkotsis: Methodology , Validation , Formal analysis , Investigation , Data curation , Writing – original draft , Writing – review & editing , Visualization. **Maria-Christina Nika:** Methodology , Investigation , Data curation , Writing – review & editing. **Varvara Nikolopoulou:**Investigation , Data curation , Writing – review & editing. **Nikiforos Alygizakis:** Writing – review & editing. **Erasmia Bizani:** Project administration, Funding acquisition , Writing - review & editing. **Reza Aalizadeh:** Writing – review & editing. **Alexander Badry:** Resources , Writing – review & editing. **Elizabeth Chadwick:** Resources , Writing – review & editing. **Alessandra Cincinelli:** Writing – review & editing. **Daniela Claßen:** Writing – review & editing. **Sara Danielsson:** Resources , Writing – review & editing. **René Dekker:** Resources , Writing – review & editing. **Guy Duke:** Funding acquisition , Writing – review & editing. **Wiebke Drost:** Writing – review & editing. **Natalia Glowacka:** Project administration , Writing – review & editing. **Bernd Göckener:** Writing – review & editing. **Hugh A.H. Jansman:** Resources , Writing – review & editing. **Monika Juergens:** Resources , Writing – review & editing. **Burkhard Knopf:** Writing – review & editing. **Jan Koschorreck:** Resources , Writing – review & editing. **Oliver Krone:** Resources , Writing – review & editing. **Tania Martellini:** Writing – review & editing. **Paola Movalli:** Resources , Writing – review & editing. **Sara Persson:** Resources , Writing – review & editing. **Elaine D. Potter:** Resources , Writing – review & editing. **Simon Rohner:** Resources , Writing – review & editing. **Anna Roos:** Resources , Writing – review & editing. **Emily O' Rourke:** Resources , Writing – review & editing. **Ursula Siebert:** Resources , Writing – review & editing. **Gabriele Treu:** Funding acquisition , Writing - review & editing. **Nico W. van den Brink:** Resources , Writing – review & editing. **Lee A. Walker:** Resources , Writing – review & editing. Rosie Williams: Resources , Writing – review & editing. Jaroslav Slobodnik: Project administration , Funding acquisition , Writing – review & editing. Nikolaos **S. Thomaidis:** Supervision , Resources , Funding acquisition , Project administration , Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Aalizadeh, R., Alygizakis, N.A., Schymanski, E.L., Krauss, M., Schulze, T., Ibáñez, M., McEachran, A.D., Chao, A., Williams, A.J., Gago-Ferrero, P., Covaci, A., Moschet, C., Young, T.M., Hollender, J., Slobodnik, J., Thomaidis, N.S., 2021. Development and Application of Liquid Chromatographic Retention Time Indices in HRMS-Based Suspect and Nontarget Screening. Anal. Chem. 93, 11601–11611. https://doi.org/10.1021/ACS.ANALCHEM.1C02348/SUPPL_FILE/AC1C02348_SI_002.XLSX.

Abrantes, N., Pereira, R., Gonçalves, F., 2010. Occurrence of Pesticides in Water, Sediments, and Fish Tissues in a Lake Surrounded by Agricultural Lands: Concerning Risks to Humans and Ecological Receptors. Water, Air Soil Pollut. 212, 77–88. https://doi.org/10.1007/s11270-010-0323-2.

Ali, A.M., Thorsen Rønning, H., Sydnes, L.K., Alarif, W.M., Kallenborn, R., Al-Lihaibi, S. S., 2018. Detection of PPCPs in marine organisms from contaminated coastal waters of the Saudi Red Sea. Sci. Total Environ. 621, 654–662. https://doi.org/10.1016/j.scitotenv.2017.11.298.

Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., Oswaldova, M., Slobodnik, J., 2019. NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in "digitally frozen" environmental samples. TrAC Trends Anal. Chem. 115, 129–137. https://doi.org/10.1016/J.TRAC.2019.04.008.

Androulakakis, A., Alygizakis, N., Gkotsis, G., Nika, M.C., Nikolopoulou, V., Bizani, E., Chadwick, E., Cincinelli, A., Claßen, D., Danielsson, S., Dekker, R.W.R.J., Duke, G., Glowacka, N., Jansman, H.A.H., Krone, O., Martellini, T., Movalli, P., Persson, S., Roos, A., O'Rourke, E., Siebert, U., Treu, G., van den Brink, N.W., Walker, L.A., Deaville, R., Slobodnik, J., Thomaidis, N.S., 2022. Determination of 56 per- and polyfluoroalkyl substances in top predators and their prey from Northern Europe by LC-MS/MS. Chemosphere 287. https://doi.org/10.1016/j.chemosphere.2021.131775.

Aznar-Alemany, Ò., Yang, X., Alonso, M.B., Schneider Costa, E., Torres, J.P.M., Malm, O., Barceló, D., Eljarrat, E., 2019. Preliminary study of long-range transport of halogenated flame retardants using Antarctic marine mammals. Sci. Total Environ. 650, 1889–1897. https://doi.org/10.1016/j.scitotenv.2018.09.287.

Badea, S., Geana, E., Niculescu, V., Ionete, R., 2020. Recent progresses in analytical GC and LC-MS based-methods for the detection of emerging chlorinated and brominated contaminants and their transformation products in aquatic environment. Sci. Total Environ. 772, 137914 https://doi.org/10.1016/j.scitotenv.2020.137914.

Badry, A., Krone, O., Jaspers, V.L.B., Mateo, R., García-Fernández, A., Leivits, M., Shore, R.F., 2020. Towards harmonisation of chemical monitoring using avian apex predators: Identification of key species for pan-European biomonitoring. Sci. Total Environ. 731, 139198 https://doi.org/10.1016/j.scitotenv.2020.139198.

Badry, A., Treu, G., Gkotsis, G., Nika, M.C., Alygizakis, N., Thomaidis, N.S., Voigt, C.C., Krone, O., 2022. Ecological and spatial variations of legacy and emerging contaminants in white-tailed sea eagles from Germany: Implications for prioritisation and future risk management. Environ. Int. 158, 106934 https://doi.org/10.1016/j.envint.2021.106934.

Balmer, M.E., Buser, H.R., Müller, M.D., Poiger, T., 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. Environ. Sci. Technol. 39, 953–962. https://doi.org/10.1021/es040055r.

Bean, T.G., Rattner, B.A., Lazarus, R.S., Day, D.D., Burket, S.R., Brooks, B.W., Haddad, S. P., Bowerman, W.W., 2018. Pharmaceuticals in water, fish and osprey nestlings in Delaware River. Environ. Pollut. 232, 533–545. https://doi.org/10.1016/j.envpol.2017.09.083.

Benfenati, E., Cappelli, C.I., Petoumenou, M.I., Pizzo, F., Lomardo, A., Albanese, F., Roncaglioni, A., Manganaro, A., Lemke, F., 2016. PROMETHEUS – Prioritization Of chemicals: a METHodology Embracing PBT parameters into a Unified Strategy. Umweltbundesamt Texte | 28, 1–69.

Bijlsma, L., Emke, E., Hernández, F., De Voogt, P., 2012. Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry. Chemosphere 89, 1399–1406. https://doi.org/10.1016/j.chemosphere.2012.05.110.

Bjurlid, F., Roos, A., Ericson Jogsten, I., Hagberg, J., 2018. Temporal trends of PBDD/Fs, PCDD/Fs, PBDEs and PCBs in ringed seals from the Baltic Sea (Pusa hispida botnica) between 1974 and 2015. Sci. Total Environ. 616–617, 1374–1383. https://doi.org/10.1016/j.scitotenv.2017.10.178.

Boulard, L., Parrhysius, P., Jacobs, B., Dierkes, G., Wick, A., Buchmeier, G., Koschorreck, J., Ternes, T.A., 2020. Development of an analytical method to quantify pharmaceuticals in fish tissues by liquid chromatography-tandem mass spectrometry detection and application to environmental samples. J. Chromatogr. A 1633, 461612. https://doi.org/10.1016/J.CHROMA.2020.461612.

Briels, N., Norstrand Torgersen, L., Castaño-Ortiz, J.M., Engvig Loseth, M., Herzke, D., Nygård, T., Bustnes, J.O., Ciesielski, T.M., Poma, G., Malarvannan, G., Covaci, A., Jaspers, V.L.B., 2019. Integrated exposure assessment of northern goshawk (Accipiter gentilis) nestlings to legacy and emerging organic pollutants using non-destructive samples. Environ. Res. 178, 108678 https://doi.org/10.1016/j.envres.2019.108678.

Butt, C.M., Berger, U., Bossi, R., Tomy, G.T., 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci. Total Environ. 408, 2936–2965. https://doi.org/10.1016/j.scitotenv.2010.03.015.

Catherine, M., Nadege, B., Charles, P., Yann, A., 2019. Per Fluoroalkyl substances (PFASs) in the marine environment: Spatial distribution and temporal profile shifts in shellfish from French coasts. Chemosphere 228, 640–648. https://doi.org/10.1016/j.chemosphere.2019.04.205.

Chen, C.H., Chung, W.H., Ding, W.H., 2020. Determination of benzotriazole and benzothiazole derivatives in marketed fish by double-vortex-ultrasonic assisted matrix solid-phase dispersion and ultrahigh-performance liquid chromatography-high resolution mass spectrometry. Food Chem. 333, 127516 https://doi.org/10.1016/j.foodchem.2020.127516.

Choi, Y., Lee, J.H., Kim, K., Mun, H., Park, N., Jeon, J., 2021. Identification, quantification, and prioritization of new emerging pollutants in domestic and industrial effluents, Korea: Application of LC-HRMS based suspect and non-target screening. J. Hazard. Mater. 402, 123706 https://doi.org/10.1016/J.JHAZMAT.2020.123706.

Chu, S., Metcalfe, C.D., 2007. Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1163, 112–118. https://doi.org/10.1016/j.chroma.2007.06.014.

Cossaboon, J.M., Hoh, E., Chivers, S.J., Weller, D.W., Danil, K., Maruya, K.A., Dodder, N. G., 2019. Apex marine predators and ocean health: Proactive screening of halogenated organic contaminants reveals ecosystem indicator species. Chemosphere 221, 656–664. https://doi.org/10.1016/j.chemosphere.2019.01.050.

Crimmins, B.S., Mccarty, H.B., Fernando, S., Milligan, M.S., Pagano, J.J., Holsen, T.M., Hopke, P.K., 2018. Integrating non-targeted and targeted chemical screening in Great Lakes fish monitoring programs. J. Great Lakes Res. 44, 1127–1135. https://doi.org/10.1016/j.jglr.2018.07.011.

De Silva, A.O., Armitage, J.M., Bruton, T.A., Dassuncao, C., Heiger-Bernays, W., Hu, X.C., Kärrman, A., Kelly, B., Ng, C., Robuck, A., Sun, M., Webster, T.F., Sunderland, E.M., 2021. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. Environ. Toxicol. Chem. 40, 631–657. https://doi.org/10.1002/ETC.4935.

Diamanti, K.S., Alygizakis, N.A., Nika, M.C., Oswaldova, M., Oswald, P., Thomaidis, N.S., Slobodnik, J., 2020. Assessment of the chemical pollution status of the Dniester River Basin by wide-scope target and suspect screening using mass spectrometric techniques. Anal. Bioanal. Chem. 412, 4893–4907. https://doi.org/10.1007/S00216-020-02648-Y.

DIRECTIVE (EU) 2020/2184 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2020 on the quality of water intended for human consumption, 2020.. Off. J. Eur. Union L 435, 1–62.

DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, 2013.. Off. J. Eur. Union.

Dulio, V., Bavel, B. Van, Lundén, E.B., Harmsen, J., Hollender, J., Schlabach, M., Slobodnik, J., Thomas, K., Koschorreck, J., 2018. Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations. Environ. Sci. Eur. https://doi.org/10.1186/s12302-018-0135-3.

Espin, S., Andevski, J., Duke, G., Eulaers, I., Gomez-Ramirez, P., Hallgrimsson, G.T., Bjorn, H., Dorte, H., Jaspers, V.L.B., Krone, O., Lourenco, R., Maria-Mojica, P., Martinez-Lopez, E., Mateo, R., Movalli, P., Sanchez-Virosta, P., Shore, R.F., Sonne, C., van den Brink, N.W., van Hattum, B., Vrezec, A., Wernham, C., Garcia-Fernandez, A.J., 2020. A schematic sampling protocol for contaminant monitoring in raptors. Ambio. https://doi.org/10.1007/s13280-020-01341-9.

Fabunmi, I., Sims, N., Proctor, K., Oyeyiola, A., Oluseyi, T., Olayinka, K., Kasprzyk-Hordern, B., 2020. Multi-residue determination of micropollutants in Nigerian fish from Lagos lagoon using ultrasound assisted extraction, solid phase extraction and ultra-high-performance liquid chromatography tandem mass spectrometry. Anal. Methods 12, 2114–2122. https://doi.org/10.1039/d0ay00411a.

Fliedner, A., Rüdel, H., Dreyer, A., Pirntke, U., Koschorreck, J., 2020. Chemicals of emerging concern in marine specimens of the German Environmental Specimen Bank. Environ. Sci. Eur. 32 https://doi.org/10.1186/s12302-020-00312-x.

Fossi, M.C., Panti, C., 2017. Sentinel Species of Marine Ecosystems. Oxford Res. Encycl. Environ. Sci. https://doi.org/10.1093/ACREFORE/9780199389414.013.110.

Fujii, Y., Kato, Y., Sakamoto, K., Matsuishi, T., Harada, K.H., Koizumi, A., Kimura, O., Endo, T., Haraguchi, K., 2018. Tissue-specific bioaccumulation of long-chain perfluorinated carboxylic acids and halogenated methylbipyrroles in Dall' s porpoises (Phocoenoides dalli) and harbor porpoises (Phocoena phocoena) stranded in northern Japan. Sci. Total Environ. 616–617, 554–563. https://doi.org/10.1016/j.scitotenv.2017.10.033.

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H. P., Hollender, J., Thomaidis, N.S., 2020. Wide-scope target screening of > 2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. J. Hazard. Mater. 387, 121712 https://doi.org/10.1016/j.jhazmat.2019.121712.

García-Fernández, A.J., Espín, S., Gómez-Ramírez, P., Martínez-López, E., Navas, I., 2020. Wildlife Sentinels for Human and Environmental Health Hazards in Ecotoxicological Risk Assessment. Roy K. Ecotoxicological QSARs. Methods Pharmacol. Toxicol. Humana, New York, NY 77–94.

Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., Fava, F., 2015. Emerging pollutants in the environment: Present and future challenges in biomonitoring, ecological risks and bioremediation. N. Biotechnol. 32, 147–156. https://doi.org/10.1016/j.nbt.2014.01.001.

Gebbink, W.A., Bossi, R., Rigét, F.F., Rosing-Asvid, A., Sonne, C., Dietz, R., 2016. Observation of emerging per- and polyfluoroalkyl substances (PFASs) in Greenland marine mammals. Chemosphere 144, 2384–2391. https://doi.org/10.1016/j.chemosphere.2015.10.116.

Gómez, M.J., Sirtori, C., Mezcua, M., Fernández-Alba, A.R., Agüera, A., 2008. Photodegradation study of three dipyrone metabolites in various water systems: Identification and toxicity of their photodegradation products. Water Res. 42, 2698–2706. https://doi.org/10.1016/j.watres.2008.01.022.

Grabicova, K., Grabic, R., Fedorova, G., Fick, J., Cerveny, D., Kolarova, J., Turek, J., Zlabek, V., Randak, T., 2017. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Res. 124, 654–662. https://doi.org/10.1016/j.watres.2017.08.018.

Guan, K.-L., Liu, Y., Luo, X.-J., Zeng, Y.-H., Mai, B.-X., 2019. Short- and medium-chain chlorinated paraffins in aquatic organisms from an e-waste site: Biomagnification and maternal. Sci. Total Environ. 708, 134840 https://doi.org/10.1016/j.scitotenv.2019.134840.

Guerra, P., Alaee, M., Jiménez, B., Pacepavicius, G., Marvin, C., MacInnis, G., Eljarrat, E., Barceló, D., Champoux, L., Fernie, K., 2012. Emerging and historical brominated flame retardants in peregrine falcon (Falco peregrinus) eggs from Canada and Spain. Environ. Int. 40, 179–186. https://doi.org/10.1016/j.envint.2011.07.014.

Hernández, F., Ibáñez, M., Botero-Coy, A.-M., Bade, R., Bustos-López, M.C., Rincón, J., Moncayo, A., Bijlsma, L., 2015. LC-QTOF MS screening of more than 1,000 licit and illicit drugs and their metabolites in wastewater and surface waters from the area of Bogotá. Colombia. Anal. Bioanal. Chem. 407, 6405–6416. https://doi.org/10.1007/s00216-015-8796-x.

Hollender, J., Schymanski, E.L., Singer, H.P., Ferguson, P.L., 2017. Non-target Screening with High Resolution Mass Spectrometry in the Environment: Ready to Go? Environ. Sci. Technol. 51, 11505–11512. https://doi.org/10.1021/acs.est.7b02184.

Hollender, J., Bavel, B.V., Dulio, V., Farmen, E., Furtmann, K., Koschorreck, J., Kunkel, U., Krauss, M., Munthe, J., Schlabach, M., Slobodnik, J., Stroomberg, G., Ternes, T., Thomaidis, N.S., Togola, A., Tornero, V., 2019. High resolution mass spectrometry- based non-target screening can support regulatory environmental monitoring and chemicals management. Environ. Sci. Eur. 31, 1–11. https://doi.org/10.1186/s12302-019-0225-x.

Huntscha, S., Singer, H.P., McArdell, C.S., Frank, C.E., Hollender, J., 2012. Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1268, 74–83. https://doi.org/10.1016/j.chroma.2012.10.032.

Jia, J., Zhu, Q., Liu, N., Liao, C., Jiang, G., 2019. Occurrence of and human exposure to benzothiazoles and benzotriazoles in mollusks in the Bohai Sea. China. Environ. Int. 130, 104925 https://doi.org/10.1016/j.envint.2019.104925.

Kajiwara, N., Watanabe, M., Wilson, S., Eybatov, T., Mitrofanov, I.V., Aubrey, D.G., Khuraskin, L.S., Miyazaki, N., Tanabe, S., 2008. Persistent organic pollutants (POPs) in Caspian seals of unusual mortality event during 2000 and 2001. Environ. Pollut. 152, 431–442. https://doi.org/10.1016/j.envpol.2007.06.075.

Kannan, K., Perrotta, E., 2008. Polycyclic aromatic hydrocarbons (PAHs) in livers of California sea otters. Chemosphere 71, 649–655. https://doi.org/10.1016/j.chemosphere.2007.11.043.

Kasprzyk-Hordern, B., Baker, D.R., 2012. Estimation of community-wide drugs use via stereoselective profiling of sewage. Sci. Total Environ. 423, 142–150. https://doi.org/10.1016/j.scitotenv.2012.02.019.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. Illicit drugs and pharmaceuticals in the environment - Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. Environ. Pollut. 157, 1773–1777. https://doi.org/10.1016/j.envpol.2009.03.017.

Kean, E.F., Shore, R.F., Scholey, G., Strachan, R., Chadwick, E.A., 2021. Persistent pollutants exceed toxic thresholds in a freshwater top predator decades after legislative control. Environ. Pollut. 272, 116415 https://doi.org/10.1016/J.ENVPOL.2020.116415.

Krauss, M., Singer, H., Hollender, J., 2010. LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns. Anal. Bioanal. Chem. 397, 943–951. https://doi.org/10.1007/s00216-010-3608-9.

Langford, K.H., Reid, M.J., Fjeld, E., Øxnevad, S., Thomas, K.V., 2015. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. Environ. Int. 80, 1–7. https://doi.org/10.1016/j.envint.2015.03.012.

Law, R.J., Losada, S., Barber, J.L., Bersuder, P., Deaville, R., Brownlow, A., Penrose, R., Jepson, P.D., 2013. Alternative flame retardants, Dechlorane Plus and BDEs in the blubber of harbour porpoises (Phocoena phocoena) stranded or by caught in the UK during 2008. Environ. Int. 60, 81–88. https://doi.org/10.1016/j.envint.2013.08.009.

Lee, S., Kim, K., Jeon, J., Moon, H., 2019. Ecotoxicology and Environmental Safety Optimization of suspect and non-target analytical methods using GC / TOF for prioritization of emerging contaminants in the Arctic environment. Ecotoxicol. Environ. Saf. 181, 11–17. https://doi.org/10.1016/j.ecoenv.2019.05.070.

Li, Y., Gao, K., Duo, B., Zhang, G., Cong, Z., Gao, Y., Fu, J., Zhang, A., Jiang, G., 2018. Analysis of a broad range of perfluoroalkyl acids in accipiter feathers: method optimization and their occurrence in Nam Co Basin. Tibetan Plateau. Environ. Geochem. Health 40, 1877–1886. https://doi.org/10.1007/s10653-017-9948-z.

Liao, C., Kannan, K., 2019. Species-specific accumulation and temporal trends of bisphenols and benzophenones in mollusks from the Chinese Bohai Sea during 2006–2015. Sci. Total Environ. 653, 168–175. https://doi.org/10.1016/j.scitotenv.2018.10.271.

Løseth, M.E., Briels, N., Flo, J., Malarvannan, G., Poma, G., Covaci, A., Herzke, D., Nygård, T., Bustnes, J.O., Jenssen, B.M., Jaspers, V.L.B., 2019. White-tailed eagle (Haliaeetus albicilla) feathers from Norway are suitable for monitoring of legacy, but not emerging contaminants. Sci. Total Environ. 647, 525–533. https://doi.org/10.1016/j.scitotenv.2018.07.333.

Lu, Z., De Silva, A.O., Provencher, J.F., Mallory, M.L., Kirk, J.L., Houde, M., Stewart, C., Braune, B.M., Avery-Gomm, S., Muir, D.C.G., 2019. Occurrence of substituted diphenylamine antioxidants and benzotriazole UV stabilizers in Arctic seabirds and seals. Sci. Total Environ. 663, 950–957. https://doi.org/10.1016/j.scitotenv.2019.01.354.

Malev, O., Lovrić, M., Stipaničev, D., Repec, S., Martinović-Weigelt, D., Zanella, D., Ivanković, T., Sindičić Đuretec, V., Barišić, J., Li, M., Klobučar, G., 2020. Toxicity prediction and effect characterization of 90 pharmaceuticals and illicit drugs measured in plasma of fish from a major European river (Sava, Croatia). Environ. Pollut. 266 https://doi.org/10.1016/j.envpol.2020.115162.

Maso, L., Trande, M., Liberi, S., Moro, G., Daems, E., Linciano, S., Sobott, F., Covaceuszach, S., Cassetta, A., Fasolato, S., Moretto, L.M., De Wael, K., Cendron, L., Angelini, A., 2021. Unveiling the binding mode of perfluorooctanoic acid to human serum albumin. Protein Sci. 30, 830. https://doi.org/10.1002/PRO.4036.

Meinerling, M., Daniels, M., 2006. A validated method for the determination of traces of UV filters in fish using LC-MS/MS. Anal. Bioanal. Chem. 386, 1465–1473. https://doi.org/10.1007/s00216-006-0706-9.

Mijangos, L., Ziarrusta, H., Zabaleta, I., Usobiaga, A., Olivares, M., Zuloaga, O., Etxebarria, N., Prieto, A., 2018. Multiresidue analytical method for the determination of 41 multiclass organic pollutants in mussel and fish tissues and biofluids by liquid chromatography coupled to tandem mass spectrometry. Anal. Bioanal. Chem. 411, 493–506. https://doi.org/10.1007/s00216-018-1474-z.

Miller, T.H., Ng, K.T., Bury, S.T., Bury, S.E., Bury, N.R., Barron, L.P., 2019. Biomonitoring of pesticides, pharmaceuticals and illicit drugs in a freshwater invertebrate to estimate toxic or effect pressure. Environ. Int. 129, 595–606. https://doi.org/10.1016/J.ENVINT.2019.04.038.

Miller, T.H., Ng, K.T., Lamphiere, A., Cameron, T.C., Bury, N.R., Barron, L.P., 2021. Multicompartment and cross-species monitoring of contaminants of emerging

concern in an estuarine habitat. Environ. Pollut. 270, 116300 https://doi.org/10.1016/J.ENVPOL.2020.116300.
Montesdeoca-Esponda, S., Checchini, L., Del Bubba, M., Sosa-Ferrera, Z., Santana-Rodriguez, J.J., 2018. Analytical approaches for the determination of personal care products and evaluation of their occurrence in marine organisms. Sci. Total Environ. 633, 405–425. https://doi.org/10.1016/j.scitotenv.2018.03.182.
Movalli, P., Dekker, R., Koschorreck, J., Treu, G., 2017. Bringing together raptor collections in Europe for contaminant research and monitoring in relation to chemicals regulations. Environ. Sci. Pollut. Res. 24, 24057–24060. https://doi.org/10.1007/s11356-017-0096-x.
Movalli, P., Duke, G., Ramello, G., Dekker, R., Vrezec, A., Shore, R.F., García-Fernández, A., Wernham, C., Krone, O., Alygizakis, N., Badry, A., Barbagli, F., Biesmeijen, K., Boano, G., Bond, A.L., Choresh, Y., Bolding Christensen, J., Cincinelli, A., Danielsson, S., Dias, A., Dietz, R., Eens, M., Espín, S., Eulaers, I., Frahnert, S., Fuiz, T.I., Gkotsis, G., Glowacka, N., Gomez-Ramirez, P., Grotti, M., Guiraud, M., Hosner, P., Johansson, U., Jaspers, V.L.B., Kamminga, P., Koschorreck, J., Knopf, B., Kubin, E., LoBrutto, S., Lourenco, R., Martellini, T., Martinez-Lopez, E., Mateo, R., Nika, M.-C., Nikolopoulou, V., Osborn, D., Pauwels, O., Pavia, M., Pereira, M.G., Ruedel, H., Sanchez-Virosta, P., Slobodnik, J., Sonne, C., Thomaidis, N.S., Topfer, T., Treu, G., Vainola, R., Valkama, J., Van der Mije, S., Vangeluwe, D., Warren, B.H., Woog, F., 2019. Progress on bringing together raptor collections in Europe for contaminant research and monitoring in relation to chemicals regulation. Environ. Sci. Pollut. Res. 26, 20132–20136.
Muir, D., Bossi, R., Carlsson, P., Evans, M., De Silva, A., Halsall, C., Rauert, C., Herzke, D., Hung, H., Letcher, R., Riget, F., Roos, A., 2019. Levels and trends of poly- and perfluoroalkyl substances in the Arctic environment - An update. Emerg. Contam. 5, 240–271. https://doi.org/10.1016/j.emcon.2019.06.002.
Munschy, C., Vigneau, E., Bely, N., Héas-Moisan, K., Olivier, N., Pollono, C., Hollanda, S., Bodin, N., 2020. Legacy and emerging organic contaminants: Levels and profiles in top predator fish from the western Indian Ocean in relation to their trophic ecology. Environ. Res. 188, 109761 https://doi.org/10.1016/j.envres.2020.109761.
Mwenesongole, E.M., Gautam, L., Hall, S.W., Waterhouse, J.W., Cole, M.D., 2013. Simultaneous detection of controlled substances in wastewater. Anal. Methods 5, 3248–3254. https://doi.org/10.1039/c3ay40655e.
Nika, M.C., Ntaiou, K., Elytis, K., Thomaidi, V.S., Gatidou, G., Kalantzi, O.I., Thomaidis, N.S., Stasinakis, A.S., 2020. Wide-scope target analysis of emerging contaminants in landfill leachates and risk assessment using Risk Quotient methodology. J. Hazard. Mater. 394, 122493 https://doi.org/10.1016/J.JHAZMAT.2020.122493.
Nikolopoulou, V., Aalizadeh, R., Nika, M.C., Thomaidis, N.S., 2022. TrendProbe: Time profile analysis of emerging contaminants by LC-HRMS non-target screening and deep learning convolutional neural network. J. Hazard. Mater. 428, 128194 https://doi.org/10.1016/J.JHAZMAT.2021.128194.
OECD, 2014. "Pharmaceutical consumption", in Health at a Glance: Europe 2014. OECD Publishing, Paris. https://doi.org/https://doi.org/10.1787/health_glance_eur-2014-35-en.
Omar, T.F.T., Aris, A.Z., Yusoff, F.M., Mustafa, S., 2019. Occurrence and level of emerging organic contaminant in fish and mollusk from Klang River estuary, Malaysia and assessment on human health risk. Environ. Pollut. 248, 763–773. https://doi.org/10.1016/j.envpol.2019.02.060.
Östman, M., Fick, J., Näsström, E., Lindberg, R.H., 2014. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci. Total Environ. 472, 862–871. https://doi.org/10.1016/j.scitotenv.2013.11.081.
Papachlimitzou, A., Barber, J.L., Losada, S., Bersuder, P., Deaville, R., Brownlow, A., Penrose, R., Jepson, P.D., Law, R.J., 2015. Organophosphorus flame retardants (PFRs) and plasticisers in harbour porpoises (Phocoena phocoena) stranded or bycaught in the UK during 2012. Mar. Pollut. Bull. 98, 328–334. https://doi.org/10.1016/j.marpolbul.2015.06.034.
Pico, Y., Belenguer, V., Corcellas, C., Diaz-cruz, M.S., Eljarrat, E., Farré, M., Gago-ferrero, P., Huerta, B., Navarro-Ortega, A., Petrovic, M., Rodríguez-Mozaz, S., Sabater, L., Santín, G., Barcelo, D., 2019. Contaminants of emerging concern in freshwater fish from four Spanish Rivers. Sci. Total Environ. 659, 1186–1198. https://doi.org/10.1016/j.scitotenv.2018.12.366.
Reichert, G., Hilgert, S., Fuchs, S., Azevedo, C.R., J.,, 2019. Emerging contaminants and antibiotic resistance in the different environmental matrices of Latin America. Environ. Pollut. 255, 113140 https://doi.org/10.1016/j.envpol.2019.113140.
Roos, A., Berger, U., Jarnberg, U., van Dijk, J., Bignert, A., 2013. Increasing Concentrations of Perfluoroalkyl Acids in Scandinavian Otters (Lutra lutra) between 1972 and 2011: A New Threat to the Otter Population? Environ. Sci. Technol. 47, 11757–11765.
Rotander, A., van Bavel, B., Polder, A., Rigét, F., Atli Auðunsson, G., Wing Gabrielsen, G., Víkingsson, G., Bloch, D., Dam, M., 2012. Polybrominated diphenyl ethers (PBDEs) in marine mammals from Arctic and North Atlantic regions, 1986–2009. Environ. Int. 40, 102–109. https://doi.org/10.1016/j.envint.2011.07.001.
Sánchez-Virosta, P., León-Ortega, M., Calvo, J.F., Camarero, P.R., Mateo, R., Zumbado, M., Luzardo, O.P., Eeva, T., García-Fernandez, A.J., Espín, S., 2020. Blood concentrations of 50 elements in Eagle owl (Bubo bubo) at different contamination scenarios and related effects on plasma vitamin levels. Environ. Pollut. 265, 115012 https://doi.org/10.1016/j.envpol.2020.115012.
Scheurer, M., Brauch, H.J., Lange, F.T., 2009. Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). Anal. Bioanal. Chem. 394, 1585–1594. https://doi.org/10.1007/s00216-009-2881-y.
Schymanski, E.L., Singer, H.P., Slobodnik, J., Ipolyi, I.M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., Thomaidis, N.S., Bletsou, A., Zwiener, C., Ibáñez, M., Portolés, T., Boer, R. De, Reid, M.J., Onghena, M., 2015. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis 6237–6255. https://doi.org/10.1007/s00216-015-8681-7.
Sedlak, M.D., Benskin, J.P., Wong, A., Grace, R., Greig, D.J., 2017. Per- and polyfluoroalkyl substances (PFASs) in San Francisco Bay wildlife: Temporal trends, exposure pathways, and notable presence of precursor compounds. Chemosphere 185, 1217–1226. https://doi.org/10.1016/j.chemosphere.2017.04.096.
Sham, R.C., Shi Ru Tao, L., King Yan Mak, Y., Kin Chung Yau, J., Cheung Wai, T., King Yan Ho, K., Zhou, G.-J., Li, Y., Wang, X., Mei Yee Leung, K., 2020. Occurrence and trophic magnification profile of triphenyltin compounds in marine mammals and their corresponding food webs. Environ. Int. 137, 105567. https://doi.org/10.1016/j.envint.2020.105567.
Sonne, C., Siebert, U., Gonnsen, K., Desforges, J.P., Eulaers, I., Persson, S., Roos, A., Bäcklin, B.M., Kauhala, K., Tange Olsen, M., Harding, K.C., Treu, G., Galatius, A., Andersen-Ranberg, E., Gross, S., Lakemeyer, J., Lehnert, K., Lam, S.S., Peng, W., Dietz, R., 2020. Health effects from contaminant exposure in Baltic Sea birds and marine mammals: A review. Environ. Int. 139, 105725 https://doi.org/10.1016/J.ENVINT.2020.105725.
Spaan, K.M., van Noordenburg, C., Plassmann, M.M., Schultes, L., Shaw, S., Berger, M.L., Heide-Jørgensen, M.P., Rosing-Asvid, A., Granquist, S.M., Dietz, R., Sonne, C., Riget, F., Roos, A.M., Benskin, J.P., 2020. Contaminants in Aquatic and Terrestrial Environments Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere. Environ. Sci. Technol. 54, 4046–4058. https://doi.org/10.1021/acs.est.9b06773.
Subedi, B., Du, B., Chambliss, C.K., Koschorreck, J., Rüdel, H., Quack, M., Brooks, B.W., Usenko, S., 2012. Occurrence of pharmaceuticals and personal care products in German fish tissue: A national study. Environ. Sci. Technol. 46, 9047–9054. https://doi.org/10.1021/ES301359T/SUPPL_FILE/ES301359T_SI_001.PDF.
Swiacka, K., Maculewicz, J., Smolarz, K., Szaniawska, A., Caban, M., 2019. Mytilidae as model organisms in the marine ecotoxicology of pharmaceuticals - A review. Environ. Pollut. 254, 113082 https://doi.org/10.1016/j.envpol.2019.113082.
Thomaids, N.S., Asimakopoulos, A.G., Bletsou, A.A., 2012. Emerging contaminants: A tutorial mini-review. Glob. NEST J. 14, 72–79.
Trabalón, L., Nadal, M., Borrull, F., Pocurull, E., 2017. Determination of benzothiazoles in seafood species by subcritical water extraction followed by solid-phase microextraction-gas chromatography-tandem mass spectrometry: estimating the dietary intake. Anal. Bioanal. Chem. 409, 5513–5522. https://doi.org/10.1007/s00216-017-0487-3.
Vargas-Berrones, K., Bernal-jácome, L., De León-Martínez, L.D., Flores-Ramírez, R., 2020. Emerging pollutants (EPs) in Latin América: A critical review of under-studied EPs, case of study -Nonylphenol-. Sci. Total Environ. 726, 138493 https://doi.org/10.1016/j.scitotenv.2020.138493.
Ventura, R., Casasampere, M., Berges, R., Fernandez-Moran, J., Segura, J., 2002. Quantification of perphenazine in Eurasian otter (Lutra lutra) urine samples by gas chromatography–mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 769, 79–87.
Weijs, L., Das, K., Siebert, U., van Elk, N., Jauniaux, T., Neels, H., Blust, R., Covaci, A., 2020. Concentrations of chlorinated and brominated contaminants and their metabolites in serum of harbour seals and harbour porpoises. Environ. Int. 35, 842–850. https://doi.org/10.1016/j.envint.2009.02.001.
Weinberger, Joel, Klaper, Rebecca, 2014. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish Pimephales promelas (fathead minnow). Aquatic Toxicology 151, 77–83. https://doi.org/10.1016/j.aquatox.2013.10.012.
Wiens, J.D., Dilione, E.K., Eagles-Smith, C.A., Herring, G., Lesmeister, D.B., Gabriel, M. W., Wengert, G.M., Simon, D.C., 2019. Anticoagulant rodenticides in Strix owls indicate widespread exposure in west coast forests. Biol. Conserv. 238, 108238 https://doi.org/10.1016/j.biocon.2019.108238.
Wu, Y., Simon, K.L., Best, D.A., Bowerman, W., Venier, M., 2020. Novel and legacy per- and polyfluoroalkyl substances in bald eagle eggs from the Great Lakes region. Environ. Pollut. 260, 113811 https://doi.org/10.1016/j.envpol.2019.113811.
Yeh, A., Marcinek, D.J., Meador, J.P., Gallagher, E.P., 2017. Effect of contaminants of emerging concern on liver mitochondrial function in Chinook salmon. Aquat. Toxicol. 190, 21–31. https://doi.org/10.1016/J.AQUATOX.2017.06.011.
Yin, X., Guo, C., Teng, Y., Xu, J., 2019. Development and application of the analytical method for illicit drugs and metabolites in fish tissues. Chemosphere 233, 532–541. https://doi.org/10.1016/j.chemosphere.2019.06.018.
Yu, L., Luo, X., Zheng, X., Zeng, Y., Chen, D., Wu, J., Mai, B., 2013. Occurrence and biomagnification of organohalogen pollutants in two terrestrial predatory food chains. Chemosphere 93, 506–511. https://doi.org/10.1016/j.chemosphere.2013.06.023.