



Assessing the chemical burden of the North-East Atlantic ecosystem through targeted and untargeted HRMS-based approaches

Georgios Gkotsis^a, Maria-Christina Nika^{a, b}, Nikiforos Alygizakis^{a, b}, Konstantinos Vasilatos^a, Antonia Athanasopoulou^a, Jonathan L. Barber^c, Rob Berbee^d, Thierry Burgeot^e, Susana Galante Oliveira^f, Johan Gustafsson^g, Alejandro Iglesias Campos^h, Ulrike Kammannⁱ, Torben Kirchgeorg^j, Jan Koschorreck^k, Aourell Mauffret^e, Mário Mil-Homens^l, Martin Mørk Larsen^m, Anne Munch Chistensenⁿ, Johan Näslund^o, Peter Oswald^b, Dag Øystein Hjermann^p, Koen Parmentier^q, Ulrike Pirntke^k, Andrew Power^r, Anne L. Soerensen^s, Irene Van der Stap^d, Lucia Viñas^t, Peter von der Ohe^k, Lynda Webster^u, Simon Wilson^v, Jaroslav Slobodnik^b, Nikolaos S. Thomaidis^{a, *}, Brendan McHugh^{w, **}

^a National and Kapodistrian University of Athens, Panepistimioupolis, Zographou, Athens 15771, Greece

^b Environmental Institute s.r.o., Okružna 784/42, Kos 97241, Slovak Republic

^c Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft, Suffolk NR33 0HT, United Kingdom

^d Rijkswaterstaat, Ministry of Infrastructure and Water Management, Rijnstraat 8, The Hague 2515 XP, the Netherlands

^e French Research Institute for Exploitation of the Sea (IFREMER), Chemical Contamination of Marine Ecosystems Unit (CCME) unit, Nantes, France

^f Centre for Environmental and Marine Studies (CESAM), Biology Department, University of Aveiro, Campus de Santiago, Aveiro 3810-123, Portugal

^g Swedish Institute for the Marine Environment, Seminariegatan 1F, Göteborg 413 13, Sweden

^h OSPAR Commission, 12 Finsbury Square, London EC2A 1AS, United Kingdom

ⁱ Johann Heinrich von Thünen Institute, Bundesallee 50, Braunschweig 38116, Germany

^j Federal Maritime and Hydrographic Agency (BSH), Wüstland 2, Hamburg 22589, Germany

^k German Environment Agency (Umweltbundesamt), Colditzstrasse 34, Berlin 12099, Germany

^l Portuguese Institute for Sea and Atmosphere, Rua C do Aeroporto, Lisboa 1749-077, Portugal

^m Aarhus University, Nordre Ringgade 1, Aarhus Centrum 8000, Denmark

ⁿ Danish Ministry of Environment, Holmens Kanal, Copenhagen 421216, Denmark

^o Swedish Environmental Protection Agency, Naturvårdsverket, Stockholm 106 48, Sweden

^p Norwegian Institute for Water Research, Økernveien 94, Oslo 0579, Norway

^q Royal Belgian Institute of Natural Sciences, Rue Vautier 29, Bruxelles 1000, Belgium

^r Marine & Freshwater Research Centre, Department of Natural Sciences, Atlantic Technological University, Dublin Road H91 T8NW, Galway, Ireland

^s Swedish Museum of Natural History, Frescativägen 40, Stockholm 114 18, Sweden

^t Instituto Español de Oceanografía, Centro Oceanográfico de Vigo – Canido, Subida Radio Faro, 50-52, Vigo, Pontevedra 36390, Spain

^u Marine Directorate, Scottish Government, Marine Laboratory, Aberdeen, Scotland AB11 9DB, UK

^v Arctic Monitoring and Assessment Programme (AMAP), Hjalmar Johansens Gate 14, Tromsø 9007, Norway

^w Marine Institute, Co. Rinville, Oranmore, Galway H91 R673, Ireland

* Correspondence to: National and Kapodistrian University of Athens, Department of Chemistry, Panepistimioupolis, Zografou, Athens 15771, Greece.

** Correspondence to: Marine Institute, Rinville, Oranmore, Co. Galway H91 R673, Ireland.

E-mail addresses: geogkotsis@chem.uoa.gr (G. Gkotsis), nikamar@chem.uoa.gr (M.-C. Nika), alygizakis@ei.sk (N. Alygizakis), kvasilat@chem.uoa.gr (K. Vasilatos), antathanasop@chem.uoa.gr (A. Athanasopoulou), jon.barber@cefas.gov.uk (J.L. Barber), berbeerpm@gmail.com (R. Berbee), burgeotte@gmail.com (T. Burgeot), susana.oliveira@ua.pt (S.G. Oliveira), johan.gustafsson@havochvatten.se (J. Gustafsson), Alejandro.IglesiasCampos@ospar.org (A.I. Campos), ulrike.kammann@thuener.de (U. Kammann), Torben.Kirchgeorg@bsh.de (T. Kirchgeorg), jan.koschorreck@uba.de (J. Koschorreck), Aourell.Mauffret@ifremer.fr (A. Mauffret), mario.milhomens@ipma.pt (M. Mil-Homens), mml@ecos.au.dk (M.M. Larsen), anmch@mim.dk (A. Munch Chistensen), johan.naslund@naturvardsverket.se (J. Näslund), oswald@ei.sk (P. Oswald), dag.hjermann@niva.no (D.Ø. Hjermann), kparmentier@naturalsciences.be (K. Parmentier), urlike.pirntke@uba.de (U. Pirntke), powera2@tcd.ie (A. Power), anne.soerensen@nrm.se (A.L. Soerensen), irene.vander.stap@rws.nl (I. Van der Stap), lucia.vinas@ieo.csic.es (L. Viñas), peter.vonderohe@uba.de (P. von der Ohe), Lynda.Webster@gov.scot (L. Webster), simon.wilson@amap.no (S. Wilson), slobodnik@ei.sk (J. Slobodnik), ntho@chem.uoa.gr (N.S. Thomaidis), Brendan.McHugh@Marine.ie (B. McHugh).

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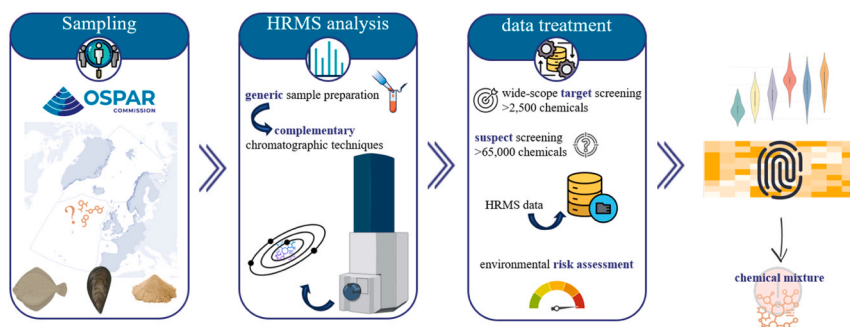
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HIGHLIGHTS

- Extensive HRMS-based monitoring in marine samples from the North-East Atlantic Ocean.
- Wide-scope target screening revealed the presence of 132 organic pollutants.
- Additional substances were identified and semi-quantified through suspect screening.
- Prioritization of chemicals was performed to support pollution policy initiatives.
- The prioritization list included legacy (PAHs, PFAS and PCBs) and emerging chemicals.

GRAPHICAL ABSTRACT



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ABSTRACT

Human activities have introduced significant amounts of anthropogenic chemicals into marine ecosystems, posing threats to aquatic biodiversity and human health. Although, traditional marine monitoring focus primarily on legacy pollutants, the presence and potential risks associated with complex emerging chemical mixtures should not be neglected. In the context of the present study organized via OSPAR Commission and supported by NORMAN network, 52 marine samples were gathered from North-East Atlantic Ocean. State-of-the-art HRMS-based analytical workflows were employed to identify their chemical fingerprint. 132 organic pollutants were identified through wide-scope target screening of more than 2,400 environmentally relevant organic pollutants. The HRMS data were digitally stored in NORMAN DSFP and 134 additional chemicals were tentatively identified through suspect screening of more than 65,000 chemicals. The list included legacy pollutants, along with emerging pollutants, their metabolites and transformation products. A simplified environmental risk assessment was conducted, aiming to prioritize substances based on their potential risks to the marine ecosystem. This study provides a valuable snapshot of the marine pollution, offering insights into chemical occurrence and risks. The findings can support marine scientists, environmental managers and policymakers in identifying pollutant sources, understanding their impacts, and informing regulatory measures to mitigate threats to marine ecosystems.

1. Introduction

The use of man-made chemicals has become deeply integrated into our daily lives. Their widespread use in healthcare, agriculture, food production, and the manufacturing of various consumer products has significantly revolutionised many aspects of society. A significant portion of these chemicals, known as organic pollutants (OPs) may enter the marine environment either directly or indirectly, for instance through direct municipal or urban discharges, terrestrial, aerial or sea-based industrial processes, accidental spills [1–6]. Some of these substances are well known as being hazardous due to their persistent, bio-accumulative, and toxic (PBT) or Carcinogenic, Mutagenic and Reproduction toxic (CMR) or Endocrine Disrupting (ED) properties, potentially leading to harmful effects on marine organisms and/ or human health. Some chemicals with known harmful properties, often referred to as legacy OPs, have been regulated for a number of decades and are routinely monitored in environmental compartments. There are, however, thousands of other substances, known as emerging OPs [7,8], which may enter the marine environment and pose significant threats to these ecosystems. Therefore, it is crucial to supply comprehensive scientific data on the occurrence and on any associated risks of these chemicals, to support the implementation of stringent regulatory measures, a crucial aspect of ensuring proper pollution management and prevention of the potential for adverse effects in ecosystems and human health.

The monitoring of OPs in sediments and in biota resident in marine waters is essential for understanding the environmental impact of these chemicals on the marine ecosystem. Pollutant monitoring in sentinel

species may provide insights into the distribution and bioaccumulation of these chemicals in the wider marine environment [9]. Regulatory or convention led monitoring programs [e.g. OSPAR coordinated environmental monitoring programme (CEMP)] enable the assessment of the overall state of ecosystems with regards to pollutants. These programmes support the evaluation of the effectiveness of chemicals management actions, and may act as an early warning system of pollution hotspots and provide crucial assessment of the potential long-term effects of OPs on marine biodiversity and ecosystem health [10]. Moreover, wildlife monitoring can help identify species that are particularly vulnerable to these contaminants, guiding nature conservation efforts and supporting regulatory decisions [11,12].

The OSPAR Commission focuses on identifying, reducing, and ultimately eliminating hazardous substances in the North-East Atlantic to protect the marine ecosystem [13]. The Marine Strategy Framework Directive (MSFD, 2008/56/EC) also plays a crucial role by offering an integrative assessment of the marine environment, covering both coastal and offshore areas. As part of this directive, the identification of potentially hazardous substances not listed as Water Framework Directive (WFD) priority substances (PS) or river basin-specific pollutants (RBSP) is a critical step in addressing emerging risks to the marine environment, as outlined in Commission Decision 2017/848/EU. Therefore, OSPAR and EU operate within the framework of routine monitoring programs, such as those conducted under the WFD (2000/60/EC) and other Regional Sea Conventions (e.g., Baltic, Mediterranean Sea), which should provide safeguards against marine pollution by chemical substances.

Over the past decades, the occurrence of OPs in the marine

environment has primarily been investigated using targeted analytical methodologies aimed to determine a limited number of chemicals with similar chemical properties [14–16]. These methodologies focus on listed or previously prioritized substances selected based on their well-known hazardous properties. It is now well recognised that these may represent a limited fraction of the overall pollutant burden in marine organisms. Many harmful substances, some of which are ubiquitous in the marine environment (e.g. large suites of Per- and Polyfluoroalkyl Substances -PFAS-) and/or have direct detrimental effects, may go undetected or be insufficiently monitored. While conventional targeted approaches provide quantitative results, which are essential for environmental risk assessment, analytical standards are not readily available for many of the tentatively identified candidate substances. High Resolution Mass Spectrometry (HRMS)-based screening strategies can offer a more comprehensive monitoring approach enabling the detection of an unlimited number of known and unknown chemicals with diverse physicochemical properties, through harmonized generic sample preparation protocols and instrumental methods [17–20]. Despite the advantages of HRMS in identifying a broader chemical fingerprint, these approaches face challenges, including the “diminished extraction” of chemicals with certain properties, compromised limits of detection (LOD) and lower quantification accuracy for certain compounds.

One of the major hurdles encountered in HRMS-based methodologies lies with the completion of environmental risk assessment of the identified substances. Aiming to estimate the concentration of the tentatively identified compounds, novel *in-silico* tools have been developed to enable semi-quantification [21–23]. Although these approaches are not yet as precise as traditional more targeted approaches, they are a valuable tool for the assessment of the environmental risk of newly identified OPs in the marine environment. Therefore, a comprehensive evaluation of hazardous substances using HRMS can serve as a complement to the more sensitive low resolution mass spectrometric (LRMS) methods for well-characterized legacy pollutants, providing valuable insights for chemical management [24,25].

The present HRMS-based study aims to bridge the knowledge gap between the relatively limited number of well-known hazardous substances (e.g. PCBs, PAHs, PFOS) [26–32], and the rapidly expanding knowledge of emerging OPs in the environment, including industrial chemicals, pharmaceuticals, plant protection products and personal care products along with their metabolites and transformation products (M&TPs). Moreover, the study seeks to supplement priority substance lists (e.g. OSPAR LCPC list of chemicals for priority action) and inform regulatory decision-making, by providing novel chemical exposure data and applying a simplified risk assessment framework. A unique, “digital” HRMS data library was created, aiming to facilitate future retrospective screening of compounds of regulatory interest. The objectives of the current study are to: (1) identify candidate substances for potential inclusion in OSPAR monitoring programmes and (2) evaluate the effectiveness of an HRMS-based approach for prioritizing hazardous substances in marine biota and sediment samples. Although several studies revealed the occurrence of legacy pollutants in the North-East Atlantic marine ecosystem, the monitoring data on emerging pollutants are notably scarce. Therefore, this monitoring study served as a pioneering collaborative geographically expansive sampling campaign to primarily investigate the occurrence of emerging pollutants within the North-East Atlantic marine ecosystem applying cutting-edge HRMS-based analytical methodologies. The study is part of the CONNECT (CONTaminants of Emerging Concern and Threat in the marine environment) project, organized via OSPAR contracting parties and supported by the NORMAN network.

2. Materials and methods

2.1. Sampling strategy

A total of 50 biota and 2 sediment samples were collected from

diverse marine environments covering nine countries (Denmark, France, Germany, Ireland, the Netherlands, Norway, Spain, Sweden, and United Kingdom) in the North-East Atlantic marine ecosystem. Specifically, soft tissue from 38 mollusc samples (species: *Crassostrea gigas*, *Mytilus edulis*, *Mytilus galloprovincialis*, *Ostrea edulis*), muscle tissue from 10 fish samples (species: *Gadus morhua*, *Platichthys flesus*, *Pleuronectes platessa*), one marine arthropod sample (*Nephrops norvegicus*) and one sea bird egg sample (*Uria aalge*) were gathered by eleven OSPAR commission contracting parties. Individual specimens of the same species which were collected from the same sampling point, were processed into a pooled composite sample. Sampling has been completed in OSPAR designated areas utilising the established OSPAR Coordinated Environmental Monitoring Programme (CEMP) to reduce individual animal variability. It should be noted that samples were representative of a single point in time as distinct to being representative of a wider spatial area. The species used in the present study as well as their sampling distribution are depicted in Fig. 1, whereas details for the sampling (species, sampling station, sampling year) are provided in Table S1.

All samples were shipped either fresh on dry ice or as lyophilized material and delivered to the Laboratory of Analytical Chemistry (National and Kapodistrian University of Athens). Two sediment samples, collected from North Sea, Germany in 2020, were provided as lyophilized and sieved (<63 µm) material. The wet 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 listed in the Table S1.

2.2. Sample preparation and instrumental analysis

Aiming to acquire HRMS data accessible to wide-scope target and untargeted screening, generic sample preparation and instrumental workflows were followed [33]. The enrichment of the final extracts with OPs covering a broad range of physicochemical properties was achieved using two slightly modified and validated generic sample preparation protocols per sample, previously used in other monitoring studies [34–36]. Briefly, the analytes were extracted from the lyophilized biota and sediment samples using Accelerated Solvent Extraction (ASE) and Ultrasonic Assisted Extraction (UAE), respectively, before the purification step using Solid Phase Extraction (SPE). The analysis of the final extracts was conducted using complementary chromatographic (Liquid Chromatography -LC-, Gas Chromatography -GC-) and ionization techniques (Electrospray Ionization -ESI-, Atmospheric Pressure Chemical Ionization -APCI-) coupled to a hybrid Quadrupole-Time-Of-Flight (QTOF) high resolution mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany). Detailed information regarding samples' preparation and instrumental analysis is described as Supplementary Information (Section II).

2.3. HRMS data treatment

The acquired HRMS data were processed using two different post-acquisition data treatment workflows; (i) wide-scope target screening of more than 2,400 environmentally relevant OPs, and (ii) suspect screening of more than 65,000 chemicals.

Wide-scope target screening was conducted using two in-house datasets, built through the analysis of reference standards, including more than 2,400 OPs of environmental relevance, such as pharmaceuticals, plant protection products, industrial chemicals, Per- and Polyfluoroalkyl Substances (PFAS), artificial sweeteners, as well as, their metabolites and transformation products (M&TPs) [37,38]. Post-acquisition data treatment was performed using Bruker TASQ® Software (version 2.1). The wide-scope target screening workflow has been previously discussed in detail. Briefly, the identification of the OPs was based on strict screening thresholds; mass accuracy (<2 mDa), retention time shift (± 0.2 min), isotopic profile matching and the presence of qualifier ions (adduct and fragment ions), which confirmed

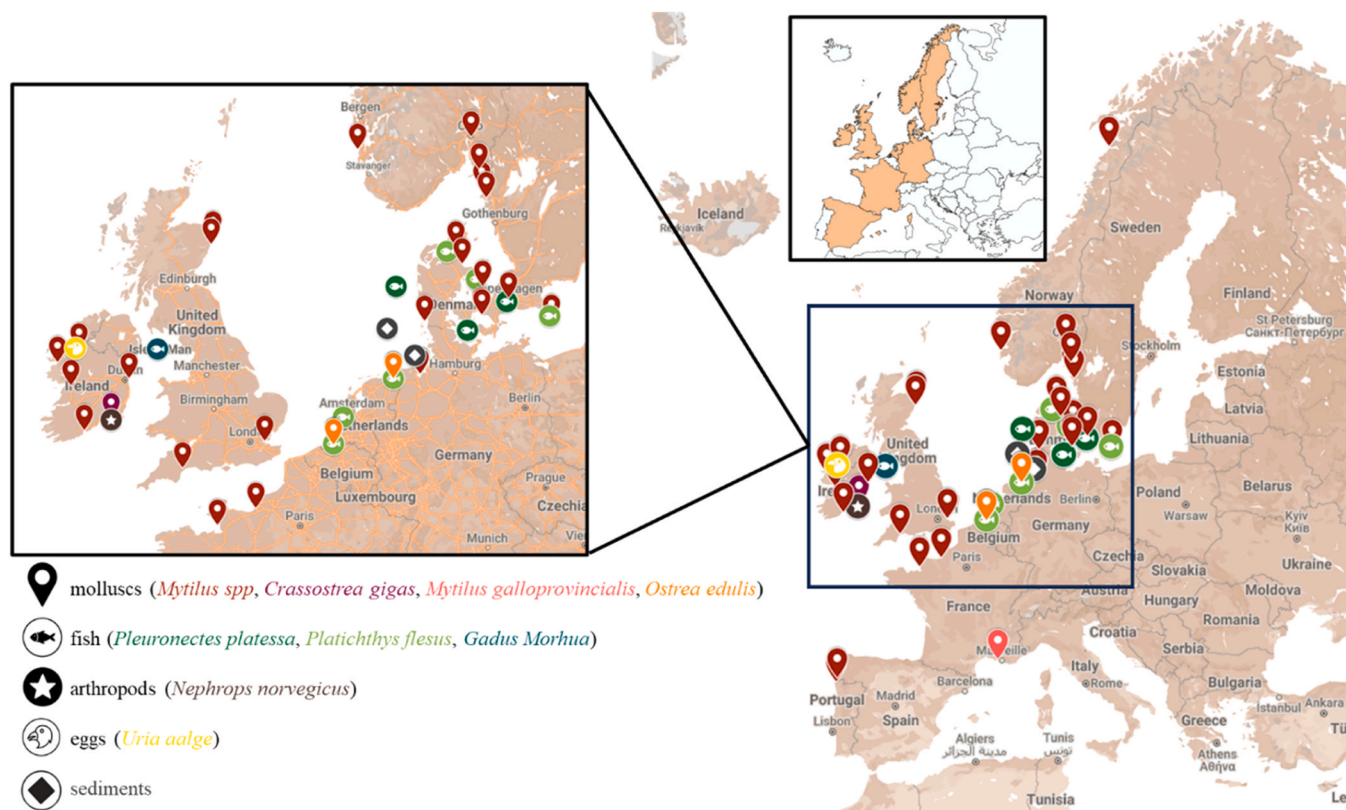


Fig. 1. Geographic distribution of sampling locations and sample type.

the identification of the analytes [18,39].

Suspect screening was performed using the NORMAN Substance Database (SusDat) including more than 65,000 environmentally relevant OPs. Although the NORMAN SusDat contains thousands of chemicals, some are not amenable to detection through suspect screening approaches due to the need for dedicated more targeted analytical methodologies or as a result of compound instability in environmental or biological matrices.

The suspect screening was carried out using a chemometric tool, the NORMAN Digital Sample Freezing Platform (DSFP), which enables the identification of “known unknown chemicals” in environmental samples through suspect screening. Furthermore, it facilitates the retrospective screening of substances of regulatory interest in the uploaded HRMS data, thus, it acts as a digital library of the environmental specimens [40]. This chemometric tool is continuously being updated and new features are added to perform the untargeted screening efficiently. Briefly the HRMS files were exported in mzML format, using CompassXport 3.0.9.2 (Bruker Daltonics, Bremen, Germany). All mzML files along with the sample identity, which include instrumental, sample, matrix-specific meta-data and retention time of retention time indices (RTI) calibrants, were digitally stored in the NORMAN DSFP platform. Harmonized Data Collection Templates were generated through an integrated workflow, which follows standard operating procedure to process the mzML files and all meta-data. The identification criteria of the positive findings were; mass accuracy (<2 mDa), predicted retention time shift, isotopic profile and predicted mass spectrum matching.

2.4. QA/QC

All analyses for OPs were carried out in Laboratory of Analytical Chemistry, National and Kapodistrian University of Athens, which is a member of NORMAN network. This strategy minimized inter-laboratory

variation in the chemical analysis results, which could result from different quality assurance and quality control (QA/QC) procedures and analytical methodologies [41].

A thorough QA/QC protocol was followed during the sample preparation and the instrumental analysis. The efficiency of the extraction was evaluated by spiking each sample with a mixture of representative isotopically labelled internal standards. As described in detail by Gkotsis et al. [34] QC was underpinned by the simultaneous processing and evaluating of solvent blanks, procedural blanks, standards including representative compounds of the analytes included in the target lists, and spiked samples during the analysis of all samples. Minimizing reporting of OPs attributed to laboratory workflow based cross contamination is important in such studies, therefore, the reported concentrations were blank corrected. Two mixtures including 18 RTI calibrant compounds and one mixture of canonical alkanes were analyzed in the sequence for facilitating the additional untargeted post-acquisition data treatment of the acquired LC- and GC- HRMS chromatograms, respectively [42]. A QC sample was analyzed every ten injections to monitor analytical performance and secure the enhanced mass spectrometer sensitivity.

Following wide-scope target screening of the samples, a comprehensive quantification process was completed where all the determined analytes were spiked a low-level polluted sample (i.e. low level occurrence of detected compounds). This was performed as no certified reference material is not commercially available for wide-scope chemical analysis purposes. The quantification was performed using the standard addition curve and representative structurally related isotope-labeled standards [18]. 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 OPs that were detected at below the Limit of

Quantification (LOQ) were reported as Below Quantification Limit (BQL). For the statistical treatment of the results, substitution of BQL findings with the corresponding LOQ/2 values has been performed, as indicated by Directive 2009/90/EC.

2.5. Environmental risk assessment

The detected and tentatively identified OPs were prioritized based on the prioritization scheme proposed by the NORMAN network [43]. The risk associated with the exceedance of toxicity threshold values was assessed by comparing the reported concentrations with available reference points for biota or sediment retrieved from the NORMAN Ecotoxicity database (ECOTOX, last visit: 19/07/2024). Note that most of these are calculated from aquatic ecotoxicity thresholds using information on bioconcentration and sorption. Relevance was ranked in the following order; (i) legislative thresholds - Environmental Quality Standards (EQS), proposed in Directive 2013/39/EU for the legacy OPs; (ii) Predicted No-Effect Concentrations (PNECs) derived from experimental data of reference laboratories; (iii) PNECs predicted using *in-silico* chemometric tools using Quantitative Structure Toxicity Relationship (QSTR) models, when no experimental data are available [44]. The Priority score was calculated for each compound as the sum of three established indicators as follows: (Total) Priority Score = Frequency of Appearance (FoA) + Frequency of Exceedance (FoE) + Extent of Exceedance (EoE), as developed in von der Ohe et al. [45], and previously reported in other monitoring surveys [36,46–49]. The derivation of the metrics is described in detail in Supplementary Information (Annex III). The overall risk ranking of each compound was assigned based on its Priority Score. This approach is a well-established procedure within NORMAN network to prioritize OPs. It should be noted that the present prioritization approach does not represent an assessment of the environmental status or the quality of the environment, as completed under OSPAR, MSFD or WFD frameworks where more in-depth statistical approaches and threshold considerations, including other types of thresholds, such as Environment Assessment Criteria (EAC) derived by OSPAR, are utilized (e.g. Quality Status Report 2024). It is noted that future such assessments will continue to seek to improve

specific thresholds and conversions as such information becomes available.

3. Results and discussion

3.1. Wide-scope target screening results

The wide-scope target screening of more than 2,400 chemicals of environmental relevance revealed that the North-East Atlantic marine ecosystem may be at risk from a “cocktail” comprising up to 132 potentially detrimental OPs. It should be noted that no individual sample contained all 132 OPs. As depicted in Fig. 2a 36% (n = 48) of the determined OPs were classified as Industrial Chemicals, encompassing chemicals known for their persistent, bioaccumulative and toxic properties, such as Per- and Polyfluoroalkyl Substances (PFAS), Polychlorinated Biphenyls (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs). The second most frequently detected chemical class was Pharmaceuticals and their M&TPs (44 OPs, 33%), followed by pesticides and their M&TPs (22 OPs, 17%). The remaining compounds belong to other chemical classes based on their main use, application and legislative status; such as personal care products, tobacco related OPs (6 OPs each, 5%), artificial sweeteners and others (3 compounds each, 2%). The distribution profiles of the OPs determined in the different biota species were comparable in terms of percentages of detected chemical use classes, as illustrated in Fig. 2b, and differed from the profiles determined in the two sediment samples, where only industrial chemicals (62%), pharmaceuticals (24%) and pesticides (14%) were detected. Detailed results from the wide-scope target screening are summarized in Table S2.

Three PAHs (fluorene, pyrene and phenanthrene) and the transformation product of the organochlorine pesticide 4,4'-dichlorodiphenyltrichloroethane (DDT), 4,4'-dichlorodiphenyldichloroethylene (DDE), all of which are currently subject to routine monitoring, were detected in all analyzed matrices (Fig. 3). Webster and Fryer (2022) [50] have already reported the need to keep PAHs under surveillance in the North-East Atlantic ecosystem. This report further notes that concentrations for some PAH congeners were above shellfish Environmental

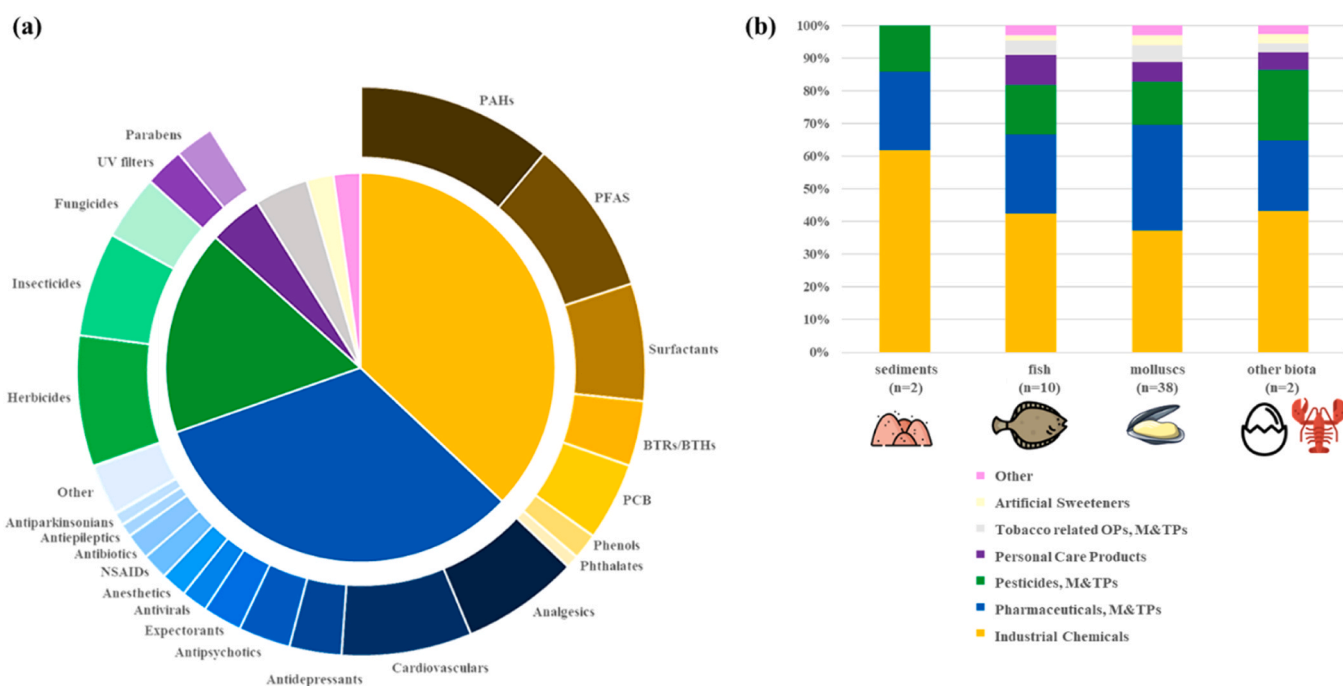


Fig. 2. (a) Chemical use classes and sub-classes of the detected organic pollutants (OPs) through wide-scope target screening of more than 2,400 chemicals, based on their main use, application, or regulatory context class; (b) distribution of the detected OPs in the different analyzed environmental compartments.

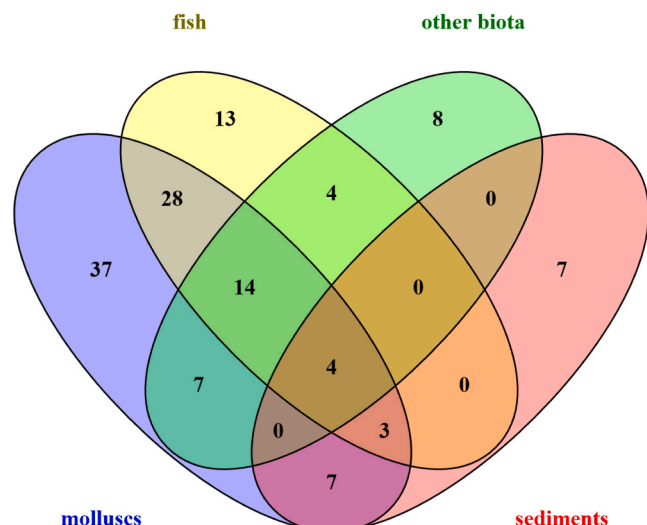


Fig. 3. Venn diagram on the 132 detected chemicals through wide-scope target analysis in different analyzed marine matrices (the graph was created using Venny 2.1.0).

Assessment Criteria (EACs) thresholds employed by OSPAR. The ubiquitous presence of PAHs and DDT-related chemicals in the Atlantic Ocean compartments has already been reported in previous surveys [29,51,52].

Another seventeen OPs were determined in 3 out of the 4 analyzed matrices. The list encompasses the monitored Priority Organic Pollutants (POPs), such as the PAHs (naphthalene, and anthracene) and compounds included in the Stockholm Convention list, including the main PCBs congeners (PCB 52, PCB 101, PCB 138, and PCB 153),

perfluorooctanesulfonic acid (PFOS) and hexachlorobenzene. Additional to these 9 emerging pollutants, including 3 pharmaceuticals, their M&TPs (i.e. 4-acetamido-antipyrine, salicylic acid, and tramadol), 2 personal care products (i.e. benzophenone-4, and methylparaben) and 4 industrial chemicals (i.e. 2,4-dinitrophenol, didecyltrimethylammonium, lauryl diethanolamide, tributylamine) were detected. While forty-six compounds were detected in half of the analyzed samples, the remaining 65 chemicals occurred exclusively in only one type of matrix; i.e. 37 were found in only molluscs, 13 only in fish, 8 in other biota, and 7 only found in sediments.

3.1.1. Molluscs

Specifically, in molluscs, HRMS-based wide-scope target screening revealed the occurrence of 100 OPs in the 38 samples analyzed. Although the dominant chemical class in terms of number of detected compounds, was industrial chemicals (39 compounds with proxy cumulative concentration ($\sum \text{conc}$) range 0.649–282 ng/g w.w.), followed by pharmaceuticals (32 compounds, $\sum \text{conc}$ range 0.201–115 ng/g w.w.) and pesticides (13 compounds, $\sum \text{conc}$ range 0.090 – 65.3 ng/g w.w.), the average cumulative concentration (aver. $\sum \text{conc}$ =128 ng/g w.w.) of personal care products was 3 times higher compared to that of industrial chemicals (aver. $\sum \text{conc}$ =44.8 ng/g w.w.), as illustrated in Fig. 4a.

On average 6 OPs were detected in molluscs, with an average $\sum \text{conc}$ of the determined OPs of 216 ng/g w.w. The mollusc samples, which were least exposed to anthropogenic chemicals were gathered from Eckwarderhörne, Germany (15 OPs, 25.3 ng/g w.w.) and Great Belt, Denmark (3 OPs, 73.4 ng/g w.w.), while the highest number of detected substances was observed in samples collected from Carteau, France (32 OPs, 224 ng/g w.w.), as well as from the Western Scheldt (Knuitershoek), the Netherlands (32 OPs, 109 ng/g w.w.). The highest cumulative concentration was observed for a blue mussel sample from

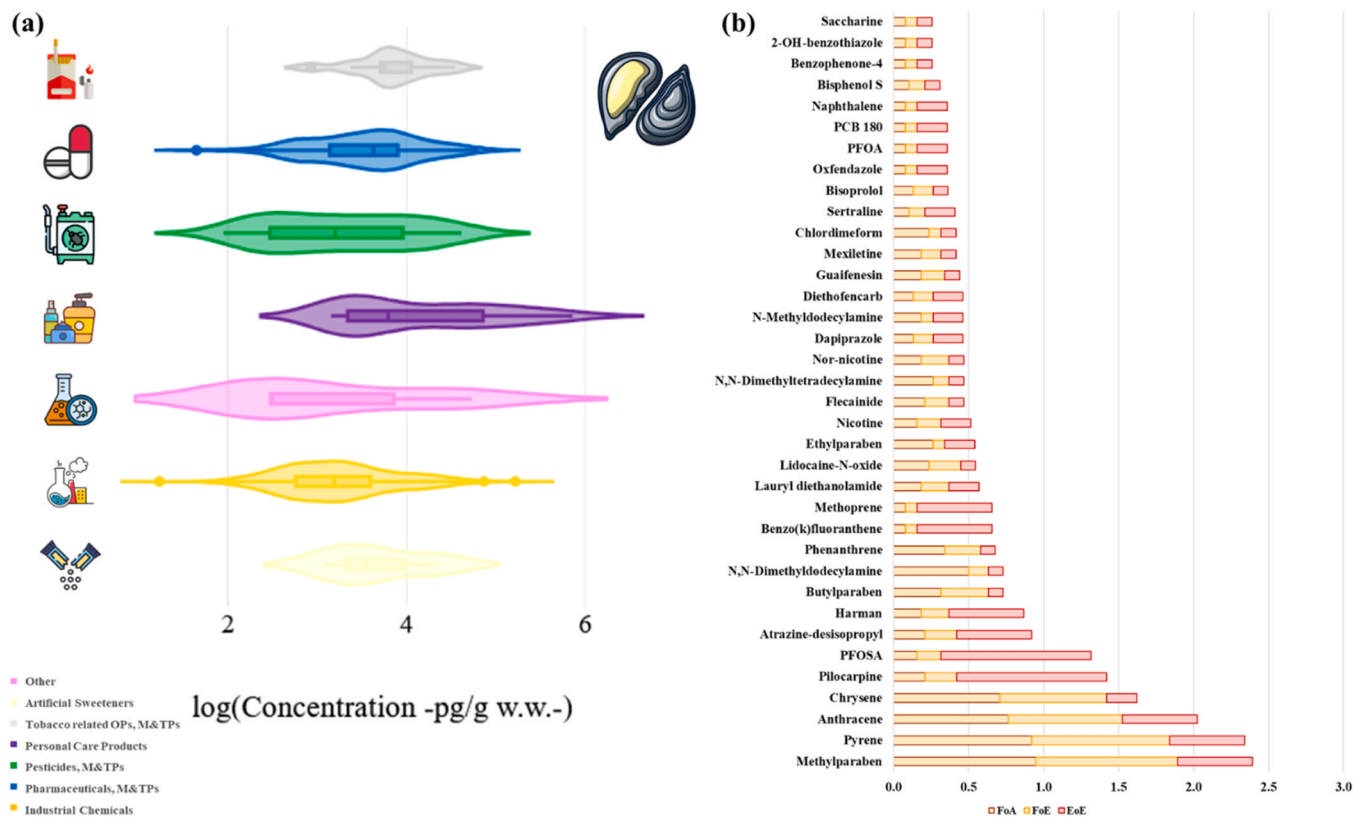


Fig. 4. (a) Violin plot highlighting median logarithmic concentration per chemical class for the OPs detected in the molluscs and the distribution of individual concentration values. (b) Bar chart depicting the contribution of each factor in the priority score for the compounds detected in the molluscs and exceeding their respective ecotoxicological values more than 5 %.

Ballisodare, Ireland (24 OPs, 823 ng/g w.w.).

Thirty-nine OPs classified as industrial chemicals were determined in mollusc samples. Among them 13 PAHs (\sum conc range 0.649 – 94.2 ng/g w.w.), 6 PFAS (\sum conc range 0.857 – 11.9 ng/g w.w.) and 5 PCBs (\sum conc range 2.12 – 116 ng/g w.w.) were legacy pollutants that are routinely included in environmental monitoring programs. Pyrene was the dominant PAH and occurred in 92 % of the molluscs at concentrations up to 53.9 ng/g w.w. Anthracene, benzo(a)anthracene, chrysene, and fluoranthene were determined in more than half of the mollusc specimens with the highest concentrations reaching 31 ng/g w.w. The remaining PAHs were less frequently detected in the samples generally at lower concentrations. Although higher detection frequencies have been reported in previous studies in Greece [53] and Spain [31], the cumulative concentration levels were comparable with those reported in the present study. Three Perfluorinated Carboxylic Acids (PFCA_{n = 4,6,8}), 2 Perfluorinated Sulfonic Acids (PFSA_{n = 4,8}) and Perfluorooctanesulfonamide (PFOSA) were found in less than 7 mollusc samples from France, the Netherlands and Ireland at concentration levels less than 10 ng/g w.w. In another monitoring study, Zafeiraki et al. [54] investigated the presence of 7 PFAS congeners in organisms from the Netherlands. Although Perfluoroundecanoic acid (PFUnA), which was not determined in this present study, occurred at the higher concentration levels compared to the other reported PFAS congeners, the average cumulative PFAS concentration were comparable between the two studies at approximately 4 ng/g w.w. Differences in profile can likely be attributed to the different sampling locations of the specimens collected in the two studies. In Flanders, Belgium the mean \sum conc levels of 11 PFCA_{n = 4–14} and 4 PFSA_{n = 4,6,8,10} were slightly higher than 10 ng/g w.w. in 181 mussel samples, indicating a higher PFAS pollution in that specific location in the Atlantic Ocean [55]. The ubiquitous PCBs 52, 101, 138, 153 and 180 were present with high detection frequency in the molluscs gathered from Germany, France and the Netherlands at cumulative concentrations ranging from 2.12 to 116 ng/g w.w. The highest concentration was observed in a mollusc sample collected from Eckwarderhörn in 1985, highlighting their widespread use in the previous decades. The commonly detected industrial chemicals classified as benzotriazoles/benzothiazoles (1-H-benzotriazole, 5-carboxylic acid-benzotriazole, 1-H-benzothiazole, and 2-hydroxy-benzothiazole) and phenols (2,4-dinitrophenol and bisphenol S) were determined in less than five samples at concentrations below 20 ng/g w.w.

Wide-scope target screening revealed the occurrence of 32 pharmaceuticals, their M&TPs which were sparsely distributed in the molluscs (each pharmaceutical occurring in an average of 3 samples) at \sum conc less than 115 ng/g w.w. The majority of the determined pharmaceuticals are generally regarded as being used as cardiovascular and analgesic drugs; these being widely used throughout society. The main metabolite of the analgesic drug metamizole, 4-acetamido-antipyrine, occurred in more than half of the mollusc samples at concentrations ranging from < 1.18 (LOQ) to 20.3 ng/g w.w. The same pattern of detection frequency was observed in the analyzed fish samples. The highest concentration among pharmaceuticals was 65.9 ng/g w.w. and was observed for sotalol in a blue mussel sample from Exeter, United Kingdom. The commonly detected analgesic drug tramadol along with two metabolites, O-desmethyldinor-tramadol and O-desmethylnor-tramadol, were present in approximately 10 % of the analyzed molluscs at concentrations up to 10 ng/g w.w. Three antidepressants (maprotiline, nifoxipam, and sertraline) and one antipsychotic drug (molindone) were found in specimens collected from Ireland (Ringaskiddy, Ballisodare), France (Carteau, Villerville) and the United Kingdom (Canvey Island) at concentrations below 10 ng/g w.w. indicating a widespread prevalence in society, with sertraline being the most widely used antidepressant in Ireland.

Thirteen pesticides, their M&TPs, including legacy substances, were detected in the analyzed mollusc samples from the North-East Atlantic ecosystem at cumulative concentrations up to 65.2 ng/g w.w. Among them, two pesticides included in the Stockholm Convention's list of

POPs, hexachlorobenzene and 4,4-DDE, were detected, however only in Irish blue mussel samples at concentrations below the respective LOQs. The acaricide chlordimeform, which is moderately toxic to mammals and has a high potential for bioconcentration, was the most frequently detected pesticide with concentrations ranging from < 6.59 (LOQ) to 41.0 ng/g w.w. Wide-scope target screening revealed the presence of four M&TPs of pesticides (4,4-DDE, alachlor-OXA, desisopropyl-atrazine and metolachlor CGA 368208) at levels below 30.0 ng/g w.w. This highlights the importance of HRMS-based analytical workflows for the identification of M&TPs, since these compounds may pose threats to marine organisms and, thus, have higher environmental significance than the parent compounds.

Three parabens widely used in cosmetics (methyl-, ethyl- and butylparaben) and two UV filters used in sunscreen agents (benzophenone-4 and octocrylene) were omnipresent (95 % of the analyzed molluscs) at \sum conc ranging from 3.48 to 723 ng/g w.w. The high detection frequency of these chemicals and their observed concentration levels were mainly assigned to methylparaben, which occurred in 36 out of the 38 samples at \sum conc ranging from 3.48 to 719 ng/g w.w. The other two parabens were detected in approximately 30 molluscs at concentrations up to 26.2 ng/g. Similar to our findings, methylparaben was omnipresent in specimens of *Mytilus galloprovincialis* collected from five different areas of the Mediterranean coast in the province of Granada, Spain, determined at concentrations ranging from 11.4 to 93.6 ng/g d. w. [56]. The two UV filters were detected only in the Irish blue mussel samples. It is worth mentioned that benzophenone-4 was determined only in the biota specimens collected from Ireland. The occurrence of UV filters in molluscs collected from Tagus Estuary Natural Reserve, Portugal and French Mediterranean coast has already been noted [57, 58].

Five tobacco related chemicals (anabasine, harman, nicotine and two nicotine's main metabolites nor-nicotine, cotinine) occurred in 20 out of the 38 molluscs of the present monitoring campaign at \sum conc ranging from 0.850 to 45.1 ng/ng w.w. Although methylparaben, nicotine and its derivatives are often found in sewage treatment effluents, it cannot be excluded that the presence of such chemicals may be attributed to cross contamination through the sampling and the initial handling of the specimens. Therefore, the use of a field blank sample is recommended in future studies to correct any background concentrations that may be linked with chemicals commonly used during the sampling process or handling of samples, as recommended by Badry et al. [41]. The artificial sweeteners acesulfame, aspartame and saccharine were determined below their respective LOQs in 3 European flat oyster samples from the Netherlands and in 3 blue mussel samples from Ireland.

A simplified risk assessment approach of individual OPs based on their exceedance of available toxicity ecotoxicological thresholds was used due to the relatively limited number of marine biota samples. For several compounds ecotoxicological thresholds were not available and, consequently, no risk assessment could be carried out. In cases where OPs were detected, but not quantified, LOQ/2 concentration was used for risk estimation. Sixty-two OPs, determined through wide-scope target screening, exceeded their ecotoxicological threshold values in at least one mollusc sample (Fig. 4b, Table S3). Although the majority of these compounds exceeded their toxicity ecotoxicological thresholds in less than 9 samples (FoE=0.24), there are a few OPs which are of high environmental concern and may pose significant threat to marine biodiversity. Methylparaben, a commonly used antifungal agent in cosmetics, ranked highest among the studied OPs. Its relatively high detection frequency, combined with high scores for local and frequent exceedances, classifies methylparaben as an OP of high potential environmental concern, as its concentration exceeded the respective toxicity ecotoxicological value in 36 out of 38 mollusc samples. Butylparaben was also detected at levels exceeding the respective PNEC in 12 samples. Taken into consideration the widespread use of parabens in personal care products, the possibility of cross-contamination should be considered during risk assessments. Therefore, a more focused evaluation, and

thorough QC examination from sampling until instrumental analysis, as well as a dedicated risk assessment using scientifically derived ecotoxicological threshold values is highly recommended for such cases.

A total of eight regulated PAHs exceeded their ecotoxicological threshold values in at least one sample; pyrene, anthracene and chrysene were also characterized as compounds of high environmental risk, due to their priority score which was higher than 1.5. Concerns regarding the potential adverse effects of PAHs on marine biota, mainly on molluscs, due to their high Octanol-Water Partition Coefficient (K_{ow}) values have been expressed in previous studies [59]. In addition to PAHs, other conventional OPs, such as perfluorooctanoic acid (PFOA), perfluorooctanesulfonamide (PFOSA) and PCB 180 exceeded their PNEC values, indicating the importance of ongoing monitoring for such well known pollutants. It is important to note that the lowest PNECs for the majority of the compounds considered in the risk assessment were at concentration levels ranging from pg/g to ng/g w.w. A thorough examination and ongoing evaluation of marine focused ecotoxicological threshold values, along with further experimental toxicity evidence, is

necessary to strengthen the outcomes of the present and similar risk assessment studies.

3.1.2. Fish

Sixty-six OPs were determined through wide-scope target analysis in the marine fish samples, gathered from Denmark, the Netherlands and Ireland. The cumulative concentrations of determined OPs in the fish samples gathered from the Netherlands ($\sum \text{conc} = 59.1 - 141 \text{ ng/g w.w.}$) exceeded the respective values in other countries [$\sum \text{conc} = 26.6 - 66.6$ (Denmark) and 44.6 (Ireland) ng/g w.w.]. Although the highest number of detected OPs were industrial chemicals ($n = 29$, $\sum \text{conc}$ range $0.593 - 46.8 \text{ ng/g w.w.}$), the proxy highest average of cumulative concentrations (aver. $\sum \text{conc}$) was observed for personal care products ($n = 5$, $\sum \text{conc}$ range $2.69 - 80.0 \text{ ng/g w.w.}$), as depicted in Fig. 5a.

A total of 29 detected industrial chemicals were ubiquitous in fish samples. The highest number and $\sum \text{conc}$ were observed in the fish samples from the Netherlands. The HRMS-based wide-scope target screening unsurprisingly revealed the detection of legacy OPs, including

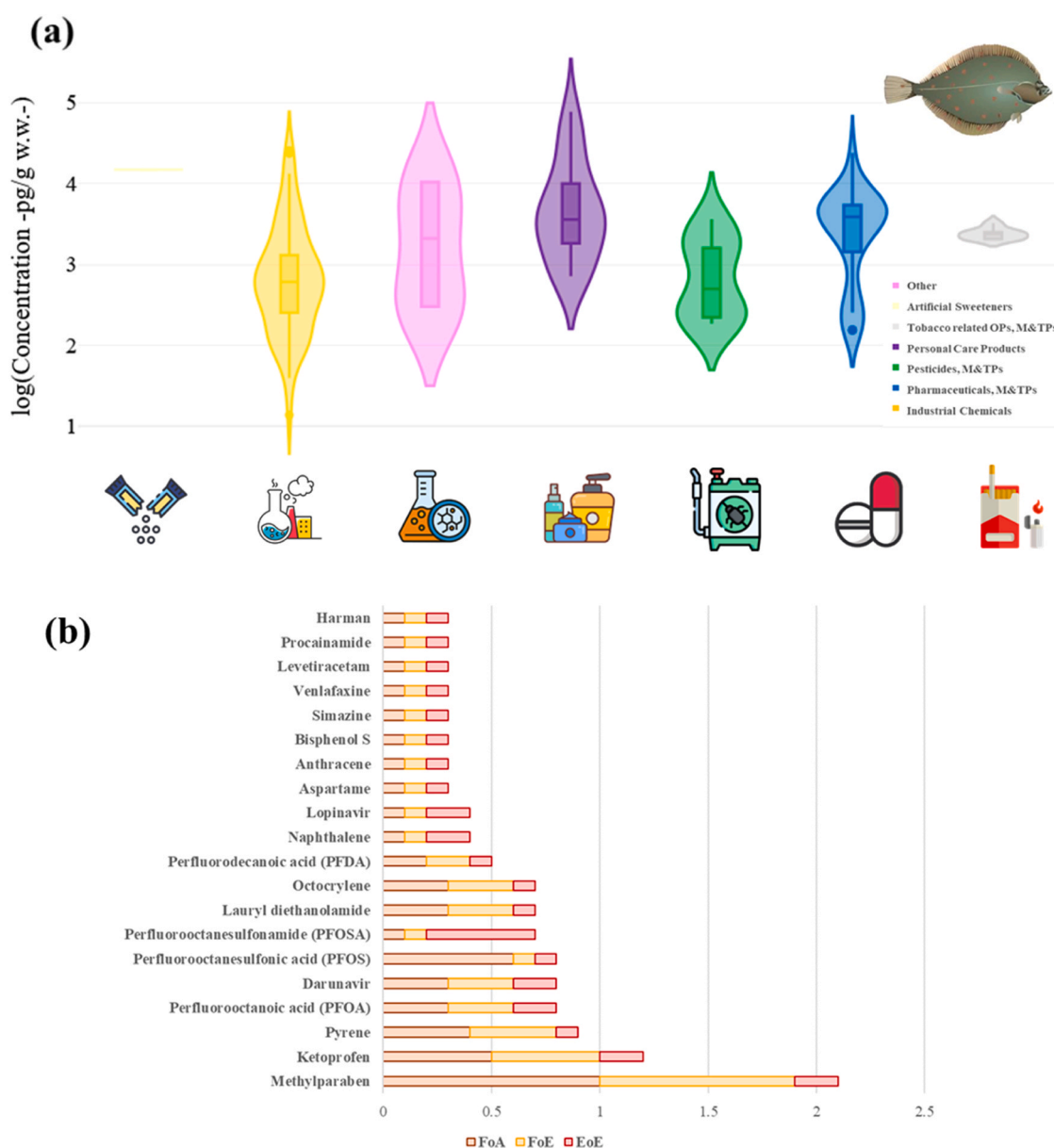


Fig. 5. (a) Violin plot highlighting median logarithmic concentration per chemical class for OPs detected in fish samples and the distribution of individual concentration values. (b) Bar chart depicting the contribution of each factor in the priority score for the compounds detected in the fish samples and exceeding their respective ecotoxicological values.

PFAS, PCBs and PAHs occurred in the fish samples. Specifically, 10 PFAS compounds were detected in less than 30 % of the analyzed fish samples. These include 5 perfluorinated carboxylic acids (PFCA_n = 4,9,10,11), 4 perfluorinated sulfonic acids (PFSA_n = 4,6,7,8, isomers), and a perfluorinated sulfonamide, perfluorooctanesulfonamide (PFOSA). In contrast, perfluorooctanesulfonate (PFOS) was found in 60 % of the samples, with concentrations reaching up to 26 ng/g w.w. Four PCBs (PCBs 52, 101, 138, and 153) were ubiquitous only in Dutch fish samples at concentrations ranging from < 0.235 (LOQ for PCB 52) to 2.13 ng/g w.w. This was not the first report of these chemicals in the ecosystem of the North-East Atlantic Ocean, since Zafeiraki et al. (2019) [54] and Teunen et al. (2021) [55] detected PFAS congeners in marine fish species at comparable concentration levels. Three PAHs (anthracene, phenanthrene, and pyrene) were detected at concentration levels below 4 ng/g w.w., with a detection frequency ranging from 30 % to 40 %, whereas naphthalene occurred only in the Irish fish sample (7.87 ng/g w.w.), noting that these compounds are quickly metabolized in fish tissues. Nine industrial chemicals, mainly surfactants and two phenols (bisphenol S, and 2,4-dinitrophenol), were determined in the samples at cumulative concentrations less than 40 ng/g w.w.

Sixteen pharmaceuticals were determined at \sum conc up to 35 ng/g w.w. The lowest concentrations were observed in the fish samples from the Netherlands, indicating a low exposure to such substances. Among them, the widely prescribed drug for treating depression, anxiety disorders, and other related conditions, venlafaxine, was detected only in the Irish samples at 1.71 ng/g w.w. The occurrence of venlafaxine residues in marine organisms at levels below 2.5 ng/g d.w. has also been reported in literature [15]. The Non-Steroidal Anti-Inflammatory Drug (NSAID) ketoprofen was detected in half of the analyzed fish samples at concentrations ranging from < 9.37 (LOQ) to 24.0 ng/g w.w. This was the highest concentration observed among all pharmaceuticals analyzed. The metabolite of metamizole, 4-acetamido-antipyrine, which is commonly detected in environmental compartments, occurred in 70 % of the analyzed fish samples. The remaining detected pharmaceuticals were sparsely determined at concentrations up to 11 ng/g w.w.

Ten pesticides were determined through wide scope target screening all at concentrations below 4 ng/g w.w. Among them, the legacy insecticides hexachlorobenzene and 4,4-DDE, were found below their respective LOQ in the Irish fish sample only. In contrast, the emerging insecticides isoprocarb and diethyltoluamide (DEET) were found with the highest detection frequencies (50 % and 40 %, respectively) and the highest concentrations, reaching up to 3.64 ng/g w.w., but only in the Danish samples.

The most frequently used antimicrobial preservative in personal care products, methylparaben, was the only OP which was omnipresent in the fish samples, with concentration levels ranging from 1.72 to 77.1 ng/g w.w., while ethylparaben was omnipresent in Dutch fish samples, but below its' LOQ (1.43 ng/g w.w.). Three UV filters commonly used in sunscreen products were determined at below 4 ng/g w.w. levels; octocrylene, benzophenone-4 and benzophenone-3 were found only in Danish, Irish and Dutch fish samples, respectively. The artificial sweetener aspartame, along with three tobacco-related OPs (nicotine, nor-nicotine, and harman), were detected in up to three samples, but only at levels below their respective LOQs.

A simplified environmental risk assessment approach was followed, aiming to prioritize the determined OPs in the fish samples. In total, 20 substances exceeded their respective ecotoxicological thresholds at least in one sample and thus, can be characterized as compounds of high concern for the biodiversity of the studied marine environment (Fig. 5b). Methylparaben, due to the high detection frequency and the high exceedance of its' respective PNEC value (i.e. 2.56 ng/g w.w.), was the chemical with the highest priority score. PFOS exceeded the Environmental Quality Standard established within the EU Directive 2013/39 in only one sample. In the list of priority substances, which may pose a threat for the marine ecosystem, three PFAS (PFOA, PFOSA, and PFDA), and three PAHs (anthracene, naphthalene and pyrene) are included. The

antidepressant drug venlafaxine, which is included in the EU Watch List of 2022 (EU 2022/1307) was detected in one fish sample at levels exceeding its' PNEC by 5-times. Table S4 summarizes the risk assessment factors and their contribution to the priority score.

3.1.3. Other biota

In the framework of CONnect project, a preliminary wide-scope target screening was carried out in one muscle tissue sample from prawn (*Nephrops norvegicus*) and in one common guillemot (*Uria aalge*) egg sample. In total, 37 OPs were determined in these samples through HRMS-based wide-scope target screening approaches at concentrations below 60 ng/g w.w. Thirty-one out of those 37 compounds occurred in the common guillemot egg sample (\sum conc=338 ng/g w.w.), whereas 17 OPs were determined in prawn muscle tissue sample (\sum conc=45.9 ng/g w.w.). Compounds with known PBT properties such as, pentachlorobenzene (<0.331 ng/g w.w.), six PCB congeners (28, 52, 101, 138, 180, and 153) with a cumulative concentration (\sum conc) of 79.0 ng/g w.w. and two PFAS congeners [perfluorooctanesulfonic acid (PFOS) with 29.0 ng/g w.w. and perfluorohexanoic acid (PFHxA) with 4.41 ng/g w.w.] were detected along with 12 other OPs (\sum conc=45.9 ng/g w.w.) only in the common guillemot egg sample. The highest concentration levels were observed for compounds listed in the Stockholm Convention; hexachlorobenzene (55.9 ng/g w.w.), 4,4-DDE (45.5 ng/g w.w.), PCB 138 (36.9 ng/g w.w.) and PCB 153 (28.1 ng/g w.w.). Although an environmental risk assessment could not be conducted on these samples due to their limited number and the lack of PNEC values for these specific matrices, a comprehensive comparison was performed with the list of OPs that exceeded the PNEC values in fish or molluscs. In total, 14 compounds, which were prioritized as compounds of concern for the North-East Atlantic Ocean marine environment, based on the risk assessment of fish and molluscs (listed in Table S5), were also determined in the other biota samples analyzed in the context of CONnect.

3.1.4. Sediments

In total, 21 OPs were detected in the two North Sea sediment samples. More than half of the detected compounds (n = 13) were classified as industrial chemicals, predominantly PAHs, whereas the rest of the compounds were pharmaceuticals and pesticides. 21 OPs were determined in the silty sample # 33, with \sum conc = 513 ng/g d.w., whereas only 11 industrial chemicals occurred in the sandier sample # 34, with \sum conc = 280 ng/g d.w. This indicates the importance of understanding the potential role played by organic content in marine sediment analysis. Although the number of sediment samples was insufficient for a comprehensive environmental risk assessment, all the reported concentrations were below the PNEC thresholds for marine sediments, as retrieved from the NORMAN Ecotoxicology Database. As summarized in Table S2, 9 PAHs were found in both analyzed sediment samples, highlighting their strong tendency to accumulate in this compartment and their high environmental significance for the marine ecosystem. Accumulation in sediments is generally attributed to PAHs high K_{ow} values [which exceed a value of 3.30 (naphthalene)] [60] and the sediment organic carbon content. The cumulative concentrations in the analyzed sediment samples were consistent with findings in the existing literature on the North-East Atlantic Ocean ecosystem [61–65]. However, slightly higher concentrations were observed for anthracene, chrysene, fluoranthene, and pyrene in this study, exceeding 35 ng/g d.w. It is well documented that as a consequence of high industrial activity the North Sea marine ecosystem has had a history of PAHs exposure.

Two benzotriazoles (1-H-benzotriazole and tolytriazole) and one phthalate (dimethyl phthalate) were determined below the LOQs of the applied HRMS-based analytical methodology. 4,4-DDE, the most common transformation product of the banned insecticide DDT, occurred in one sediment sample (0.587 ng/g d.w.). Five pharmaceuticals, including the antipsychotics amisulpiride and tiapride, the cardiovasculars flecainide and metoprolol and the analgesic tramadol were present only in

one of the sediment samples, with a cumulative concentration of 133 ng/g d.w. Although this study provides valuable insights into the potential exposure of sediments to a complex mixture of chemicals, more samples are required to estimate a representative picture of the chemical burden of the North-East Atlantic Ocean ecosystem.

3.2. Suspect screening results

The HRMS-based suspect screening of more than 65,000 environmentally relevant OPs included in the NORMAN Substance Database, revealed the presence of 134 additional OPs in the 52 tested samples. Eleven compounds were identified at level 2 A, based on the identification scheme proposed by Schymanski et al. [66], for which a probable structure by library match was proposed, whereas the remainder were only tentatively identified (i.e. level 3). The dominant chemical class was industrial chemicals (86 OPs, 64 %), followed by pharmaceuticals (29 OPs, 22 %), pesticides (10 OPs, 8 %), tobacco related OPs (7 OPs, 5 %) and personal care products (2 OPs, 1 %). The profile of the chemical classes of the tentatively identified OPs followed the same pattern with the compounds determined through wide-scope target screening. The suspect screening results are summarized in Table S6, whereas their estimated concentrations in logarithmic scale are depicted as a heatmap in Figure S1, where compounds with the highest frequency of appearance (FoA) are ranked on the top of the heatmap.

Most of the tentatively identified OPs, classified as industrial chemicals ($n = 86$). The majority of the industrial chemicals identified through suspect screening are included in the European Chemicals Agency (ECHA) database, indicating their use throughout EU. For instance, the occurrence of some of the identified industrial chemicals, which are produced in high volume (10,000–100,000 annual tonnage), such as ethyl 2,4-dimethylbenzoate, hexahydrophthalic anhydride, 12-oxooctadecanoic acid, octanedioic acid and diacetone acrylamide, may be of high concern, due to their high frequency of appearance in the analyzed samples and their continuous release in the environment. Moreover, the presence of some of these compounds has already been reported in organisms and surface water samples collected throughout Europe. Erucamide (identification level 2 A), a lubricant widely used in the plastic manufacturing industry, which was identified in 22 % of the marine samples, with semi-quantitative concentration levels ranging from 0.4 to 128 ng/g w.w., has been previously reported in raptor species from Germany and the Netherlands [35,67]. The presence of the tentatively identified surfactant tetradecylamine, with high frequency of appearance (62 %) and estimated concentration levels (up to 8.6 ng/g w.w.), has previously been highlighted in the ecosystem of Dniester river in Ukraine in 2019 [46]. The tentatively identified plasticizer acetyl tributyl citrate, widely used in packaging films for food, along with the industrial chemicals 2,6-dimethylaniline, benzamide, tris[2-(2-hydroxyethoxy)ethyl]azanium, N,N-bis(2-hydroxyethyl)dimethyloctanamide, and the surfactants nonaethylene glycol and pentaethylene glycol (2 A) have been reported in raptor egg samples collected across Baden-Württemberg state in Germany [35].

The pharmaceutical 4-aminophenol, a substance registered under REACH and reported as Persistent, (very) Mobile and Toxic (PMT/vPvM) in the PMT/vPvM list provided by the German Environment Agency (UBA, Umweltbundesamt) [68], was identified in 50 % of the tested samples, at estimated concentrations ranging from 1.5 to 797 ng/g w.w. This finding indicates that HRMS-based monitoring data may support the *in-silico* data, predicting potential PMT/vPvM properties. Moreover, the antiviral drug used in the treatment of hepatitis B infection, telbivudine (FoA: 74 %, 0.3–600 ng/g w.w.), has been reported in other monitoring campaigns in Europe [35,46,67], whereas N-benzylformamide (FoA: 28 %, 0.9–36 ng/g w.w.) and the metabolite of the commonly used non-steroidal anti-inflammatory drug ibuprofen, ibuprofen-methylester (FoA: 42 %, at estimated concentration levels up to 10.6 ng/g w.w.), were noted to occur in raptor egg specimen collected from Germany [35].

Ten pesticides and their M&TPs including the synthetic pyrethroid emperthrin (FoA: 80 %, 1.4–832 ng/g w.w.) used in insecticides, which is suspected as endocrine disruptor, in addition to the metabolite of the fungicide metalaxyl, metabolite CGA 108906 (FoA: 10 %, 6.4–60 ng/g w.w.) were tentatively identified in the analyzed biota and sediments. Furthermore, the HRMS-based suspect screening of more than 65,000 chemicals revealed the presence of 5 nicotine's metabolites in estimated concentrations up to 193 ng/g w.w. and with a frequency of appearance up to 62 % of the analyzed marine samples. The presence of nicotine metabolite 6–180 (24 %, 0.1–1.8 ng/g w.w.) in biota samples have been previously reported [35]. Moreover, two UV filters, which are commonly used in sunscreen personal care products, were tentatively identified in these North-East Atlantic Ocean ecosystem samples: enzacamene (FoA: 20 %, 0.4–10.7 ng/g w.w.) and phenoxyethyl caprylate (FoA: 38 %, 13 ng/g w.w.–1,63 µg/g w.w.).

Aiming to prioritize the tentatively identified substances, an in-depth comparison of their semi-quantitative concentrations with the ecotoxicological threshold values listed in NORMAN ecotoxicology database was completed. This preliminary environmental risk assessment highlighted that 38 tentatively identified substances were identified in the majority (FoA > 50 %) of the analyzed marine environmental compartments. Moreover, the estimated concentrations exceeded the respective PNEC value for 63 substances. Thirty-one OPs showed a total environmental priority score higher than 1, indicating that these compounds should be considered for greater monitoring effort in the North-East Atlantic Ocean to obtain sufficient monitoring data to better assess both their fate and their potential adverse effects on the marine environment. In Table S7 the tentatively identified substances are sorted based on their priority score, which was determined from the environmental risk assessment study.

4. Conclusions, limitations, and recommendations

This monitoring study applied state-of-the-art analytical screening methods, integrating complementary chromatographic techniques coupled to HRMS, aiming to investigate the occurrence of chemicals in 52 marine samples gathered from 11 countries across the North-East Atlantic Ocean ecosystem. The present HRMS-based screening survey marks a significant state-of-the-art advance by identifying a geographically extensive mixture of organic pollutants which pose potentially serious concerns for the marine ecosystem. These findings underscore the need for broader investigative monitoring efforts, incorporating both seasonal and spatial trends in the studied marine environment. Two post-acquisition data treatment workflows were used to process the HRMS data: (i) wide-scope target analysis of more than 2,400 environmentally relevant OPs out of which 132 chemicals were determined in the samples, and (ii) suspect screening of more than 65,000 chemicals out of which 134 substances were tentatively identified and semi-quantified. The acquired HRMS data are currently stored in the NORMAN and OSPAR repositories, enabling future retrospective suspect screening. Post HRMS analysis, a risk assessment workflow was carried out to identify within these 266 chemicals the ones that exceed ecotoxicological EQS values from Directive 2013/39/EU or PNEC biota/sediment values from the NORMAN Ecotoxicology Database. The prioritization highlighted the potential risks from 67 compounds from the wide-scope target screening and 63 tentatively identified substances form the suspect screening, which exceeded their respective ecotoxicological values at least in one sample. Therefore, some OPs potentially pose a threat to marine biodiversity and/or human consumers of seafood produce. It is recognized that secondary poisoning of marine predators is not considered in the current approach and may be more critical than direct ecotoxicity. It should be noted that the toxicity thresholds used in this study are preliminary values, often derived by translating water-based ecotoxicological risk limits into equivalent concentrations in biota or sediment on the basis of experimental or often predicted bio-concentration factors or partitioning to sediment using K_{oc} values,

respectively. Therefore, further work on derivation of risk limits specifically for marine biota and sediment and concentrations based on target screening is therefore needed to reduce critical uncertainties and improve the risk assessment. The marine environment is a unique ecosystem where resident species can attain higher trophic status than in the terrestrial environment and thus the potential for biomagnification into top level predators needs to be carefully considered. This novel monitoring campaign has uncovered a significant number of previously unknown OPs in the North-East Atlantic Ocean and further supports the long-established pollutant and effects monitoring programmes established by OSPAR. As a consequence of this and future developments in risk assessment, national and regional measures may be required to further investigate sources, fate and risk and mitigation measures against the potential harmful effects. The present collaborative study of OSPAR Commission and NORMAN network lay the foundation for the future marine monitoring, since such collaborations are clearly beneficial to evaluating potential risk to the marine ecosystems.

While this pilot collaborative study successfully identified a wide range of potential pollutant-related threats to the NE Atlantic marine environment and its' resident species, it is important to acknowledge certain limitations. The restricted spatial and temporal sampling coverage limited the statistical power and generalizability of the findings. Future monitoring initiatives would benefit from a more extensive and strategically designed sampling framework, coupled with robust statistical power assessments. Future monitoring studies, particularly those that are more targeted or hypothesis-driven, should aim to include a broader range of sample matrices and increased sample sizes to enhance the reliability and accuracy of risk assessment studies. In parallel, there is a clear need for ongoing evaluation and refinement of ecotoxicological threshold values, supported by experimental toxicity studies, to strengthen the interpretation of detected contaminants in an ecological context. The development and adoption of standardized sampling protocols and sample handling procedures are also critical to minimize the potential for contamination and to improve the reproducibility and comparability of results across studies. Despite these limitations, this study demonstrates the value of integrated collaborative monitoring efforts and provides a foundation for advancing marine environmental assessment. Continued methodological refinement, including improvements in analytical sensitivity and ecotoxicological relevance, will be essential for supporting future regulatory and scientific decision-making.

This study highlights several key recommendations aiming to guide future monitoring and research development. Standardisation of sampling is highly recommended to support long-term monitoring in the North East Atlantic Ocean. Further comprehensive regional assessment, utilizing the full potential of HRMS capabilities coupled with complementary and advanced chemometric tools to investigate the accumulation of OPs within food webs and to identify any connections to specific pollution sources should be considered as a result of the findings of this study. The occurrence of certain substances identified in this study may trigger further research or knowledge gathering to better understand the current chemical potential threats for the marine ecosystem. The development of an HRMS surveillance indicator may form part of a complementary cutting-edge suite of early risk warning mechanisms to provide support to possible policy actions, in complement of target approach monitoring in place. While this study adhered to a unique, comprehensive HRMS-and risk evaluation-based methodology, caution is needed to ensure both analytical methodologies are sufficiently robust and that thresholds have sufficient risk security to avoid both the potential for over- or under estimations of environmental risk. As a focal point, investigative initiatives should consider the lists of specific substances or chemical groups identified within the present study and should consider incorporation of new and existing sources of marine threshold values into risk assessment. Conventional targeted approaches using LRMS techniques, which are more sensitive than the applied HRMS based screening approaches, should continue be carried out for

legacy OPs, such as PCBs and PFAS, in order to decrease the detection limits and provide monitoring data to assess the spatial variability, temporal trends of the pollution and evaluate the efficiency of the established mitigation measures. Moreover, additional QA/QC protocols should be followed during the sampling in the field and the processing in the laboratory to minimize any possible cross-contamination of the samples from compounds which are widely used throughout society.

This study demonstrates the value of such multi-country monitoring OPs campaigns to support knowledge building and assessment at both a regional and national level, even though it is relatively limited in size and scope. To enhance the robustness of such risk assessments and prioritization exercises, further target verification of the presence and wider spatial distribution (incorporating input information) of a wide range of OPs is merited. Expanding this study to include additional matrices, such as water, other sediment types, and possibly higher trophic level species, as well as accounting for mixture toxicity, will strengthen the confidence in the risk assessment. Furthermore, ongoing advancements in the HRMS-based screening methodologies, such as the improved sensitivity of analytical method and the enhancement of the post-acquisition chemoinformatic workflows, as well as the refinement of marine ecotoxicological threshold values, will continue to improve the understanding of chemical mixtures in the marine environment to inform measures to reduce emissions from its sources.

Environmental implication

The present monitoring study highlights the pervasive occurrence of organic pollutants in biota and sediments collected from the North East Atlantic Ocean, raising significant environmental and ecological concerns. The findings indicate that the ecosystem is exposed to a diverse mixture of hundreds of emerging and priority pollutants, some of which exceed their respective ecotoxicological threshold values, posing a potential threat to marine biodiversity. The application of novel HRMS-based methodologies reveals "hidden" chemical mixtures in the marine environment and provides critical insights to trigger regulatory actions, safeguarding marine ecosystems, and ensuring the sustainability of marine resources.

CRediT authorship contribution statement

Mauffret Aourell: Writing – review & editing, Resources, Funding acquisition. **Koschorreck Jan:** Writing – review & editing, Resources. **Kirchgeorg Torben:** Writing – review & editing, Resources. **Kammann Ulrike:** Writing – review & editing, Resources. **Iglesias Campos Alejandro:** Writing – review & editing, Resources. **Thomaidis Nikolaos S.:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Gustafsson Johan:** Writing – review & editing, Resources. **McHugh Brendan:** Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Galante Oliveira Susana:** Writing – review & editing, Resources. **Slobodnik Jaroslav:** Writing – review & editing, Resources, Funding acquisition. **Burgeot Thierry:** Writing – review & editing, Resources. **Wilson Simon:** Writing – review & editing, Resources. **Berbee Rob:** Writing – review & editing, Resources. **Webster Lynda:** Writing – review & editing, Resources. **Barber Jonathan L.:** Writing – review & editing, Resources. **von der Ohe Peter:** Writing – review & editing. **Viñas Lucia:** Writing – review & editing, Resources. **Van der Stap Irene:** Writing – review & editing, Resources. **Athanasopoulou Antonia:** Writing – review & editing, Methodology, Formal analysis. **Vasilatos Konstantinos:** Writing – review & editing, Methodology, Formal analysis. **Alygizakis Nikiforos:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Soerensen Anne L.:** Writing – review & editing, Resources. **Nika Maria-Christina:** Writing – review & editing, Project administration, Investigation, Data curation. **Power Andrew:** Writing – review & editing, Resources. **Gkotsis Georgios:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation.

Pirntke Ulrike: Writing – review & editing, Resources. **Parmentier Koen:** Writing – review & editing, Resources. **Oystein Hjermann Dag:** Writing – review & editing, Resources. **Oswald Peter:** Writing – review & editing, Resources. **Näslund Johan:** Writing – review & editing, Resources. **Munch Chistensen Anne:** Writing – review & editing, Resources. **Mørk Larsen Martin:** Writing – review & editing, Resources. **Mil-Homens Mário:** Writing – review & editing, Resources.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2025.138393](https://doi.org/10.1016/j.jhazmat.2025.138393).

Data availability

Data will be made available on request.

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