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Integrated monitoring of chemicals and their effects on four sentinel species, *Limanda limanda, Platichthys flesus, Nucella lapillus* and *Mytilus* sp., in Seine Bay: A key step towards applying biological effects to monitoring

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ABSTRACT

The International workshop on Integrated Assessment of CONtaminants impacts on the North sea (ICON) provided a framework to validate the application of chemical and biological assessment thresholds (BACs and EACs) in the Seine Bay in France. Bioassays (oyster larval anomalies, *Corophium arenarium* toxicity assay and DR Calux) for sediment and biomarkers: ethoxyresorufin-*O*-deethylase (EROD) activity, acetylcholinesterase (AChE) activity, lysosomal membrane stability (LMS), DNA strand breaks using the Comet assay, DNA adducts, micronucleus (MN), PAH metabolites, imposex, intersex and fish external pathologies were analysed in four marine sentinel species (*Platichthys flesus*, *Limanda limanda, Mytilus* sp. and *Nucella lapilus*). Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals were analysed in biota and sediment. Results for sediment and four species in 2008–2009 made it possible to quantify the impact of contaminants using thresholds (Environmental Assessment Criteria/EAC_{2008}: 70% and EAC_{2009}: 60%) and effects (EAC_{2008}: 50% and EAC_{2009}: 40%) in the Seine estuary. The Seine estuary is ranked among Europe's most highly polluted sites.

1. Introduction

The Seine estuary is the largest megatidal estuary in the English Channel and is ranked among Europe's most highly polluted estuaries in terms of chemical contamination (Carpentier et al., 2002; Cachot et al., 2006). The Seine estuary flows into Seine Bay, home to the port of Le Havre, one of Europe's five largest ports. 86% of the total fluvial discharge from adjacent catchments in the English Channel originates from rivers along the French coasts between Calais and Brest, and is dominated by the Seine and its tributaries (Millwards et al., 2015). The Seine catchment area, downstream of the cities of Paris and Rouen and upstream of Le Havre, is highly urbanized and industrialized. The Seine catchment area is a hub for around 40% of France's economic activities. It is influenced by a dense urban population (16 million inhabitants), combined with extensive farming (cereals, oleaginous plants, beetroot and potatoes) at around 100,000 farms. Chemical contaminant drainage to

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the Seine estuary, combined with atmospheric inputs, represent a chronic source of contamination characterized by a wide diversity of contaminants (PAHs, PCBs, heavy metals, phtalates, hormones, PBDEs, EPHEs, alkyphenols, pesticides, nanoparticles, drugs), typical of large European cities.

This French pilot area in the Channel-North Sea OSPAR (The Oslo and Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) zone was therefore selected to validate an integrated approach of contaminants and biological effects in the framework of the ICON programme, conducted on a large geographical scale. The validation of bioassays and biomarkers currently represents a major step towards their future application to monitoring in the framework of the OSPAR Coordinated Environmental Monitoring Programme (CEMP), and of descriptor 8 "Concentrations of contaminants give no effects" of the Marine Strategy Framework Directive (MSFD). Biological effects of contaminants are widely used in many European countries to assess the impact of contaminants within an ecosystem approach, but EU's decision-makers (EC, 2008) remain to be convinced with regards to biological effect indicators. A documented reliability is key to selecting efficient biological and chemical indicators for assessing the ecological health of the marine environment and an integration method suitable for an ecosystem approach. France drew up a legislative decree relating to the MSFD's Good Environmental Status in December 2012 (French Legislative Decree December 2012), incorporating biological and chemical indicators recommended by OSPAR. This legislative decree refers to biomarkers and bioassays (mussel and fish physiology, genotoxicity, reprotoxicity and fish pathologies) for coastal and offshore monitoring. Efforts to date have however focused mainly on coastal areas, which are far more impacted by chemical contamination than offshore areas. The legislative decree takes the biological effects of contaminants into account through a sustainable European monitoring programme, adapted to a national level. Validation of the biological effects of chemical contaminants applied to monitoring within the MSFD remains a major challenge in terms of long-term monitoring data acquisition and aggregation.

The ICON programme aims to demonstrate the pertinence of applying biomarkers and bioassays on both a wide and local geographical scale. Its strength lies in the fact that it is backed by a European consensus built on a framework developed through the International Council for the Exploration of the Sea (ICES) and OSPAR (Hylland et al., 2012; 2017b; Vethaak et al., 2016). Based on the numerous strategies and the different indices already proposed for biological effect data integration with specific tools as for example the multi-biomarker index (Beliaeff and Burgeot, 2002; Broeg and Lethonen, 2006; Viarengo et al., 2007; Devin et al., 2013), a multistep process was proposed which follows on from experience of the assessment of contaminants data for sediment, fish and shellfish in OSPAR contexts (Vethaak et al., 2016). The main difference between the framework used in the ICON programme and other indices is that (1) the current framework is based on internationally agreed threshold criteria for biological responses and chemicals contamination in biota (Environmental Assessment Criteria: EAC and background assessment criteria: BAC) and (2) the framework includes more matrices than most other indices (Hylland et al., 2017b)

Appropriate sites were selected in the ICON programme from the North to the South Atlantic and one in the Mediterranean, spanning Iceland to Spain. The selection of a Mediterranean site was particularly important for France and Spain, which must harmonize monitoring efforts on the Atlantic and Mediterranean coasts. Validation at local sites would allow wide-scale biomarker and bioassay validation at sites with varying characteristics, from the North to the South of the North-East Atlantic and in the Mediterranean.

This paper presents the work conducted in the Seine estuary and Bay: a pilot site particularly suitable for the validation of biomarkers and bioassays and the development of an integrated chemical-biological approach in the East Channel. The Seine estuary has been the focus of research and monitoring campaigns for over 20 years (Burgeot et al., 1994; Minier et al., 2000; Burgeot and Gagné, 2013). High contaminant levels have been identified there on the basis of predominant chemical contaminants in sediment, biota and water (Chiffoleau et al., 2001; Gonzalez et al., 2001; Lafite et al., 2001; Le Hir et al., 2001; Munschy et al., 2003; Cachot et al., 2006). Characterized by a highly diverse fauna, but low numbers of individuals of each taxon (Tecchio et al., 2015), the Seine estuary provides a good illustration of the estuarine quality paradox (Dauvin, 2007). The parameters structuring the various organism populations, such as salinity, substrate and hydrodynamics, are extremely heterogeneous along the freshwater-estuarine-coastal-open marine continuum. The various organisms adapt their metabolism constantly to this variable environment, making it more difficult to detect impacts of other stressors in the estuarine system as a whole. This continual adaptation is nevertheless subject to annual seasonal fluctuations and, in the longer term, to global change, as a combined result of climate change and the interaction of chemical pollutants. Estuaries under continual stress are generally highly productive ecosystems and major nursery and recruitment areas for a wide variety of invertebrates and fish, which are key prey for high trophic level animals (Dauvin, 2007). They hence offer a marine typology characteristic of transitional waters, with biological and physical regulation mechanisms that need to be studied and monitored. The typology of estuaries characterized by fine grain and organic-rich sediments favours a high accumulation and potential bioavailability of chemical contaminants. Estuaries are therefore priority zones for research into bioindicator species and the study of biomarkers and bioassays to determine the biological effects of chemical contaminants. On a legal level, monitoring of estuaries undertaken in the framework of the WFDs six-year cycles has highlighted a lack of indicators suitable for assessing the $good_{environmental}$ ecological status of transitional waters in estuary zones (Boeuf and Fritsch, 2016).

A French consortium contributed to the ICON programme by conducting a sampling campaign on sediment and biota matrices in the Seine Bay, including flounder (*Platichthys flesus*), dab (*Limanda limanda*), dogwhelk (*Nucella lapillus*) and mussels (*Mytilus* sp.). The objective was to 1) validate bioassays and biomarkers in four sentinel species (flounder, dab, dogwhelk and mussels) and for sediment on a local scale in the Seine Bay and estuary 2) interpret biomarkers and bioassays according to the BAC and EAC thresholds determined per species and for sediment, 3) apply the integrated chemical and biological method developed by OSPAR (JAMP, 2012) to assess the environmental status of the Seine Bay and estuary and compare it to other selected European sites.

2. Materials and methods

2.1. Sampling

Sampling took place at seven stations in Seine Bay (Figs. 1 and 2) to collect the four selected species in a polluted area influenced by the Seine panache (Seine estuary/fish, Villerville/dogwhelk, Cap de la Hève/dogwhelk, Honfleur/mussels) around Le Havre (Figs. 1 and 2) and in a zone located to the West of the Seine Bay, uninfluenced by the Seine panache (Parfond/fish, Pointe de la Loge/dogwhelk, Le Moulard/mussels). Sampling was performed in accordance with OSPAR and ICES guidelines (JAMP, 2012).

Sediment samples were collected in 2008 from the Seine