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Review

# Emerging studies on oil pollution biomonitoring: A systematic review

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ABSTRACT

In the last decade, several methods were applied to monitor the impact of oil pollution on marine organisms. Recent studies showed an eminent need to standardize these methods to produce comparable results. Here we present the first thorough systematic review of the literature on oil pollution monitoring methods in the last decade. The literature search resulted on 390 selected original articles, categorized according to the analytical method employed. Except for Ecosystem-level analyses, most methods are used on short-term studies. The combination of Biomarker and Bioaccumulation analysis is the most frequently adopted strategy for oil pollution biomonitoring, followed by Omic analyses. This systematic review describes the principles of the most frequently used monitoring tools, presents their advantages, limitations, and main findings and, as such, could be used as a guideline for future researches on the field.

## 1. Introduction

Oil pollution is among the main anthropogenic stressors of marine ecosystems (Michán et al., 2021; Nukapothula et al., 2021). When introduced in the environment, crude oil undergoes weathering processes that change its physical-chemical properties (Bellas et al., 2013). The result of this process is the release of a series of polycyclic aromatic hydrocarbons (PAHs) that tend to spread and impact ecosystems health and functioning (Finch and Stubblefield, 2019; Kottuparambil and Agusti, 2020).

Oil and PAHs cause cytotoxic, genotoxic and teratogenic effects, reducing marine biodiversity (Cerqueda-García et al., 2020; Chitrakar et al., 2019; Fisher et al., 2016, 2014). Due to their lipophilic characteristics, some PAHs can be absorbed by primary producers and bioaccumulate through the food web (Cerezo and Agusti, 2015; Kottuparambil and Agusti, 2020; Lewis et al., 2020; Turner et al., 2020) altering the structure and productivity of marine communities (Moreno et al., 2013).

In fact, the time necessary for marine biota to recover from an oil pollution event is still unclear. For example, twelve years after the Deepwater Horizon Oil Spill in the Gulf of Mexico (DWH, USA) the environmental impact is still perceived (Lewis et al., 2020). Several studies suggest that marine organisms can take decades to recover (Joydas et al., 2012; Montagna et al., 2013). Despite the efforts to minimize the impact, spilled oil can remain available for years on seawater or marine sediment (Finch and Stubblefield, 2019). In this sense, long-term monitoring is necessary to effectively elucidate the post-impact ecosystem recovery trajectory (Finlayson et al., 2015; Girard and Fisher, 2018).

Due to the intensification of marine traffic and the growing number of oil spill incidents in the last decade (Eronat, 2020), a multitude of methods have been developed to monitor the impact of oil spills and accumulation of oil on marine biota, such as toxicity tests (Frometa et al., 2017), biomarkers (Ahmed et al., 2019), bioaccumulation (Caroselli et al., 2020), and ecosystem-level analysis (Gemmell et al., 2018). However, most of these methods have been applied on short-term studies and are frequently invasive or lethal (Sherwood et al., 2019). In this context, there is a prominent need for developing novel tools and standardized protocols that integrate these methods into a long-term environmental monitoring program. This initiative can provide an accurate view of oil impact and post-exposure marine environment resilience (Caroselli et al., 2020; Han et al., 2021).

This article systematically reviews the research on the impact of oil pollution on marine ecosystems health in the last decade. We evaluated the distribution of studies around the world, the most frequently used analytical tools and the preferred organism for each method. We also

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compared the different analytical methods regarding oil exposure time and monitoring time. We highlight knowledge gaps and obstacles in the use of these tools and indicate future directions and best practices in the area. Therefore, this work offers an important contribution to the research on oil spill monitoring and suggests future directions in the field.

## 2. Material and methods

### 2.1. Literature search parameters

The systematic review followed the guidelines proposed by Siddaway et al. (2018). Literature search was performed using three online publication databases: Web of Science, PubMed, and SCOPUS. Considering the high level of redundancy (above 80 %) between the first two databases and the last one, the final search was performed in October 2020 using only SCOPUS, the most complete database accessed. Peer-reviewed articles from the last decade (2011–2021) were recorded.

The following terms were used during search exercises to select for relevant articles:

- Methods: monitor*, assess*, evaluat*, analysis.
- Subject: spill, pollution, stress, impact*, exposure.
- Type of pollutant: "Polycyclic aromatic hydrocarbon*", PAH*, oil.
- Specific environment: marine, seawater, ocean.
- Target: organism*, ecosystem*, communit*.

Searches were limited to keywords, title, and abstract. Terms within each category were combined using the Boolean operator "OR". To combine categories, the operator "AND" was used. The asterisk (*) was used to allow for the presence of any group of characters. The final search string was:

(monitor* OR assess* OR evaluat* OR analysis) AND (spill OR pollution OR stress OR impact* OR exposure) AND ("Polycyclic aromatic hydrocarbon*" OR pah* OR oil) AND (marine OR seawater OR ocean) AND (organism* OR ecosystem* OR communit*)

### 2.2. Screening process

A screening was performed to include the relevant articles found:

Step 1: Study inclusion criteria

The title and abstract of each publication were accessed for relevance according to the following inclusion criteria:

- Subject: analyze the impact of oil or PAH pollution on marine biota.
- Results: present information on methods employed for monitoring the impact of oil pollution on marine organisms.
- Type of study: empirical study published in a peer review journal.

Step 2: Data extraction and presentation

Selected articles from SCOPUS were read in full and discussed in detail. Articles were organized according to the applied analytical method. Data on indicator organism group, monitoring time, exposure time (hours, days, months, and years), and articles main findings were extracted and discussed in the following section.

## 3. Results and discussion

Searches with the selected terms in the SCOPUS database returned a total of 2096 articles, of which 390 matched the study inclusion criteria (step 1) and were included in this review.

Most of the studies on monitoring marine oil pollution were conducted in the United States of America (n = 89, 22,8 %). China is the second country with the highest number of publications (n = 50, 12,8 %), followed by France (n = 23, 5,9 %), Norway (n = 23, 5,9 %) and Spain (n = 20, 5,1 %). Brazil is in sixth place (n = 19, 4,9 %) with relevant publications on the subject (Supplementary Fig. 1).

The selected studies were categorized according to the analytical method employed: Omics, Ecosystem-Level, Bioaccumulation, Ecotoxicological, Physiological, and Biomarker approaches. Most of the studies (n = 92, 23.6 % of the total) applied a combination of methods for monitoring the biological impact of oil pollution, of which 76.1 % combined only two methods. The most frequently combined methods were Biomarker and Bioaccumulation analysis (30.4 % of combined studies), which allow coupling information on the detection and dosage of oil-derived compounds and its biological effects. Biomarker and Physiological analysis (15.2 % of combined studies) are also often combined, providing an overview of oil biological impact on molecular, metabolic and behavioral level. The majority of single-method studies employed Omics (n = 79, 20.3 %), or Ecosystem-level (n = 71, 18.2 %) analysis (Fig. 1), which are further explored in detail.

Regarding the time of exposure to oil/PAHs, the most frequent time scale was "days" (n = 100, 25.6 %) (Fig. 2a), suggesting that most of the studies are dedicated to evaluate the impact of oil pollution a short time after oil spill incidents in field studies, or after a few days of exposure on bioassays. Besides, biomonitoring is usually carried out for "days" (n = 95, 24.4 %) or "months" (n = 81, 20.8 %) (Fig. 2b), suggesting that short-term (hours, days, or weeks) and medium-term (months) monitoring is widely used, but the long-term effects of oil are often under-researched. Exposure and monitoring times were separately evaluated for each of the analysis methods and the results are discussed in the upcoming subsections (Fig. 3).

### 3.1. Omic analysis

Most of the single method studies here analyzed employed Omic approaches (n = 79, 20.3 %, Fig. 1). Omic technologies were proposed as

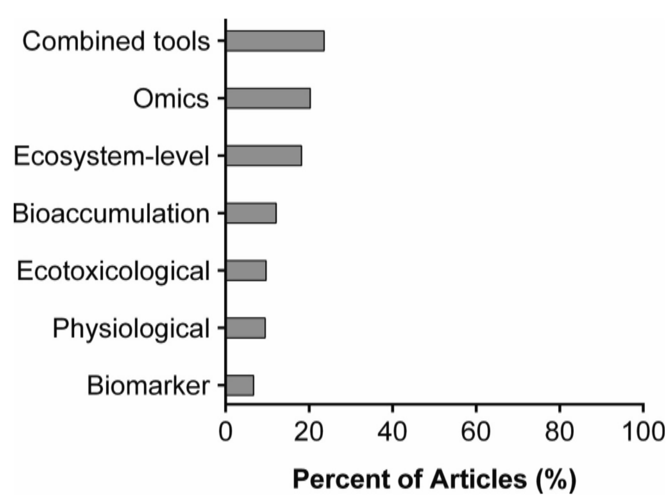


**Fig. 1.** Methods for marine oil pollution biomonitoring employed by the selected publications in "2011–2021", lines 164–165.

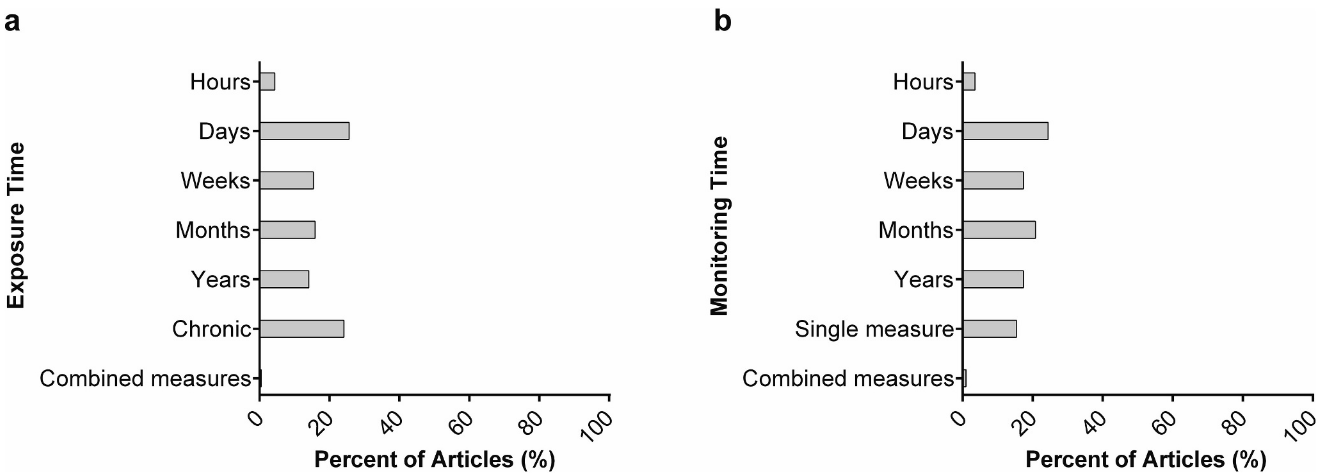


**Fig. 2.** Exposure time (a) and monitoring time (b) for analysis of oil pollution biological impact. Chronic refers to continuous exposure; combined measures comprise studies combining different exposure or monitoring times; hours (0 to 24 h); days (1 to 7 days); weeks (8 to 30 days); months (1 to 12 months); and years (>12 months). Single measure refers to studies performing only one sample collection.

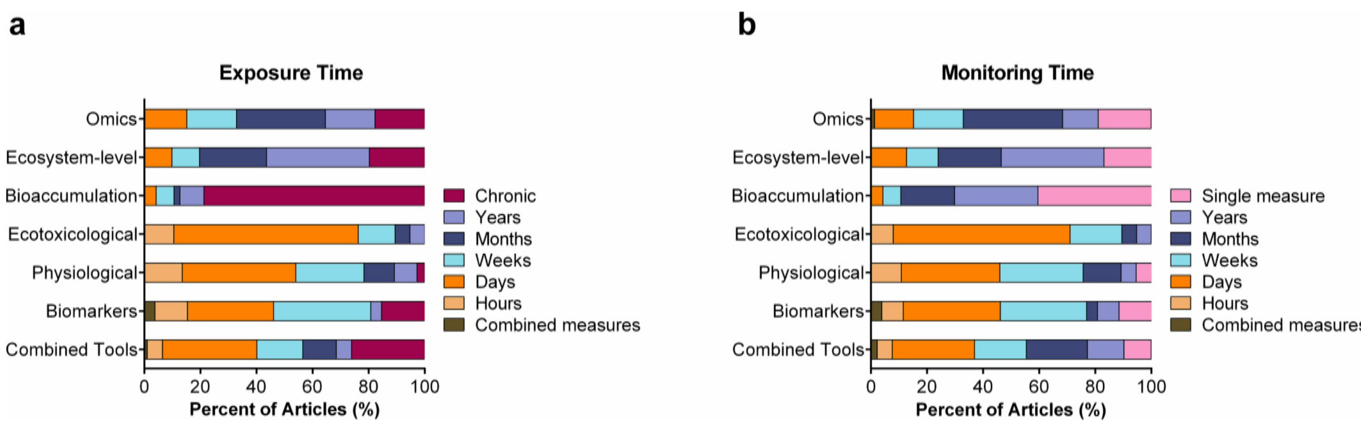


**Fig. 3.** Exposure time (a) and monitoring time scales (b) employed for marine oil pollution biomonitoring. Combined tools refer to studies applying more than one analytical method. Combined measures refer to "studies with different exposure or monitoring time scales", lines 184–186.

an alternative to conventional biomarkers on environmental monitoring. Since the advent of high-throughput sequencing, Omic studies have conquered space on monitoring programs to evaluate the biological impact of PAH or oil. These techniques have the advantage of quantitatively monitoring many biological molecules in a high-throughput manner, providing a broad view of biological responses to pollution. The main Omic technologies are Genomics, Transcriptomics, Proteomics, and Metabolomics. Variations to analyze community-level responses to pollution include Metabarcoding, Metagenome, Metatranscriptome and Metaproteome. In special, multi-omics approaches integrate several omics layers, offering the opportunity to understand the flow of information that underlies biological response to oil contamination and providing information on novel potential biomarkers.

Metabarcoding consists on sequencing taxonomic markers to evaluate changes on community composition and biodiversity. For example, metabarcoding of 16S rRNA, 18S rRNA and COI genes was used to monitor changes in diversity and structure of micro- and macro-biota over a 7-year period following Hebei Spirit oil spill, revealing long-term ecological effects of residual oil (Xie et al., 2018). Through yearly sampling of surface sediments from oil contaminated areas, the authors detected successions of bacterial, protists and metazoan communities, which became dominated by hydrocarbon-degrading bacteria and diatoms, and also oil tolerant arthropod families (Xie et al., 2018). This study revealed that residual oil interfered on the composition of communities from primary producers to higher trophic level organisms.

Genomic and Metagenomic approaches consist, respectively, on sequencing the complete genome of one particular organism, or multiple organisms on environmental samples As such, these tools provide not only taxonomic relevant data, but also information on organism's metabolic potential. Through metagenomics, Rezaei Somee et al. (2021) observed that a similar shift on microbial community composition towards higher dominance of oil-degrading species occurs both in chronic exposure and oil spill events. Moreover, Hu et al. (2017) used a laboratory-based method to recreate the conditions that were present in the 2010 DWH event and detected, through metagenomics combined with hydrocarbon composition analysis, the same successional pattern observed in the field. These authors observed an early increase on the relative abundance of *Oceanospirillales* and *Pseudomonas* taxa related to the highest concentrations of linear alkanes, followed by *Colwellia* and *Cycloclasticus* in the intermediate stage related to single-ring aromatic hydrocarbon degradation, and finally, the increase on the relative abundance of Rhodobacterales and Flavobacteriales genomes capable of degrading polycyclic aromatics during late-stage hydrocarbon degradation.

Transcriptomic and Metatranscriptomic analysis study the whole set of expressed genes in one organism or a community, shedding light on the metabolic pathways disturbed by oil exposition. As an example, the transcriptome of juvenile fish exposed to WAF for 48 h exhibited altered expression of well-established biomarkers of PAH exposure, such as cytochrome P450 enzymes. Moreover, other metabolic changes could be inferred due to the downregulation of immune response genes and the altered expression of genes related to cardiotoxicity and calcium homeostasis (Greer et al., 2019). Likewise, Zamora-Briseño et al. (2021) compared the transcriptomic changes in liver and gill of adults lined sole fish (*Achirus lineatus*) exposed to a sublethal acute concentration of WAF of light crude oil for 48 h and detected the up-regulation of genes related to xenobiotic metabolism, redox metabolism and DNA repair mechanisms. Furthermore, Knapik et al. (2020) identified through metatranscriptomics the overexpression of several alkane monooxygenases and aromatic ring-hydroxylating dioxygenases in oil exposed seawater bacterial communities. The authors suggest that these genes could be better investigated as appropriate targets for integration into genosensor technologies. These nucleic acid-based devices are promising tools for in situ detection of oil pollution by monitoring the expression of genes related to petroleum degradation specific pathways.

Proteomic and Metaproteomic approaches analyze all proteins and offer information on post-transcription and post-translation modifications. Metabolomic studies simultaneously quantify multiple small molecule types, such as amino acids, fatty acids, carbohydrates, or other products of cellular metabolic functions. By combining Proteomic and Metabolomic analyses, Chen et al. (2016) demonstrated that PAH disturbs energy metabolism, reduces the production of cytoskeleton related proteins suggesting cellular injury or apoptosis, and represses antioxidant metabolism in pearl oysters. However, it is important to mention that metabolomic biomarkers are especially sensitive to organism nutritional status, which should be considered mainly on field studies when several food conditions coexist with pollution levels. In fact, Campillo et al. (2019) exposed mussels under different nutritive conditions to fluoranthene and demonstrated that under food deprivation, the toxic effect of HPA on mussels metabolomic profiles is masked.

Most of the Omic studies analyzed here evaluated the effect of oil pollution on marine microbial communities (81.0 % of omics studies in this review). Also, most articles apply both exposure time (n = 25; 31.6 %) and monitoring time (n = 28, 35.5 %) in the scale of “months”, suggesting that these tools are especially used in medium-term monitoring studies (Fig. 3a, b).

Despite the relatively high cost of omics technologies, when combined to physiological, ecotoxicological and bioaccumulation analysis, they allow a holistic view of the biological impact of oil pollution at different biological levels (from molecules to organisms). Finally, when meta-omics are associated to ecosystem-level analytical methods, it is possible to understand the large-scale effects of oil pollution on community structure and associated functional diversity, such as the reduction on photosynthetic potential or filter-feeding functional traits, followed by the increase of heterotrophic metabolism, bacterial oil degradation and scavenging activity. Omic techniques also allow determining toxicological pathways or routes of effects of different oil compounds.

### *3.2. Ecosystem-level analysis*

Ecosystem-wide impacts of oil spill are often observed as changes on species composition and the reduction of species richness, biodiversity, and biomass. In this sense, ecological indices are frequently used to represent and compare these parameters between pristine and polluted sites.

Community structure changes are often observed in response to oil. As an example, a recent study demonstrated that pollution by crude oil can reduce phytoplankton biomass in >75 %, affecting the primary production rate by up to 96 %, while the abundance of heterotrophic bacteria increased up to 68 % (Shai et al., 2021). Similarly, on a simulated oil spill experiment, benthic macrofaunal assemblage structures were dramatically altered in species number, abundance and biomass, and the community recovery time was directly proportional to oil concentration (Zhou et al., 2019). Biomass measurement of macrobenthic organisms was also the method of choice for a long-term monitoring program in China, indicating the apparent habitat recovery in Bohai Sea five years after an oil spill incident (Wang et al., 2020).

Moreover, ecosystem-level analytical methods present evidence on ecosystem resilience, indicating resistant and sensitive species. In a recent study, it was possible to observe immediate impacts of the oil spill that occurred in Brazil in 2019 on the structure of epifaunal communities associated with two macroalgal species, resulting on the reduction of species richness and abundance, while opportunistic taxa increased (Craveiro et al., 2021).

Changes on community composition can lead to a reorganization of the food web. For example, the removal of key grazers in response to oil and dispersants in the northern Gulf of Mexico disrupted the predator-prey controls, allowing dinoflagellates with higher tolerance to these pollutants to grow and form blooms (Almeda et al., 2018).

Community functional aspects can also be altered by exposure to oil. Nunnally et al. (2020) observed the loss of deep-sea filter-feeding sessile invertebrates, such as corals and sponges, especially sensitive to the DWH oil spill. Moreover, habitat destruction, which brings about death, led to the observed increase on the abundance of scavengers at this normally food-limited deep-sea environment. The loss of biological traits that should improve ecosystem resilience hampers the recovery of benthic communities impacted by oil spills. In the opposite direction, when adapted subtidal macrobenthic community from historically petroleum contaminated sites were exposed to a simulated acute oil spill, the vertical distribution of organisms along sediment was altered in a way that could help ecosystem recovery (Gilbert et al., 2015). The authors observed a deeper burial of some polychaete species, possibly as a strategy to avoid the direct impacts of oil. The presence of high amounts of oil in the surface leads to a reduction of the oxygen diffusion to the deeper layers of sediments, which can be critical to many infaunal species. In this case, the deeper burial of particularly resistant polychaete species can alter sediment bioturbation, improve the oxygenation of subsuperficial sediments and stimulate aerobic oil-degrading bacteria (Gilbert et al., 2015).

Exposure time (n = 26, 36.6 %; Fig. 3a) and monitoring time (n = 26, 36.6 %; Fig. 3b) for ecosystem-level analyses are usually in the scale of “years”, indicating that this is an important tool for long-term monitoring. Also, ecosystem-level analyses were applied mainly to the benthic community (40.8 % of ecosystem-level studies) since these bottom-associated and usually reduced motility organisms are more suitable for long-term analyses.

When working with natural communities, it is important to take into account that the initial species composition and the experimental approach adopted determine the degree of response (González et al., 2013). Ecosystem connectivity, fecundity rate, life-cycle length (Daly et al., 2021) and seasonal timing of the oil spill (Parsons et al., 2015) are also factors that can interfere on the results and should be considered on experimental or sampling design.

### *3.3. Bioaccumulation analysis*

Bioaccumulation is defined as the accumulation of chemical substances in organisms through feeding, direct contact or breathing (Wang et al., 2017). When applied to oil pollution situations, bioaccumulation analysis typically quantifies PAH compounds in marine organisms' whole tissue or specific organs. The main analytical techniques applied for bioaccumulation analyses are Gas Chromatography coupled to Mass Spectrometry (GC–MS), High Performance Liquid Chromatography (HPLC) and the Fixed Wavelength Fluorescence (FF) technique. GC–MS and HPLC are highly sensitive techniques with high resolution for defining the compounds to be quantified. Both techniques have consolidated use in the literature for the most diverse organisms, mainly bivalves (Elmamy et al., 2021; Wang et al., 2017) and fishes (Ahmed et al., 2019; Sun et al., 2019). In contrast, FF is a rapid detection method that does not perform exact quantification, but can be used for screening specific molecules (Beg et al., 2018; Gravato et al., 2014).

Bioaccumulation analyses are necessary, for example, to evaluate if human consumption of edible species should be allowed (Ranjbar Jafarabadi et al., 2019). Besides, PAH quantitation can be used on biomagnification researches, by comparing PAH concentration in organisms from different trophic levels (Akhbarizadeh et al., 2019). Apparently, low molecular weight compounds (with 2 or 3 rings) are easier to concentrate when compared to medium (4 rings) or high (5 or 6 rings) molecular weight compounds (Keshavarzifard et al., 2017; Ranjbar Jafarabadi et al., 2019).

This analytical method is also employed to investigate toxicokinetic and depuration rates for specific compounds (Wang et al., 2017; Yakan et al., 2017). For example, Wang et al. (2017) observed that the mussel *Perna viridis* releases the PAH benzo[*a*]pyrene (B[*a*]P) faster than the clam *Pinctada martensii*. This suggests that the mussel species could be less vulnerable to oil pollution, while the clam should be a better choice as indicator organism on oil pollution studies, due to its ability to accumulate higher concentrations of HPA. Therefore, toxicokinetic evaluation is especially valuable when choosing sensitive organisms for ecotoxicological assays and to understand bioaccumulation of pollutants along food webs.

One of the advantages of this analysis is that it provides early warnings about the presence of pollutants on marine biota, a key issue for oil pollution (Gravato et al., 2014). Since the detection of chemical compounds itself does not account for its biological effects, bioaccumulation analyses are often combined to other analytical methods to provide ecologically meaningful information (53.9 % of bioaccumulation studies). In this sense, Lehtonen et al. (2019) proposed a monitoring program based on the integration of data on water chemistry, bioaccumulation and biomarkers in caged mussels as well as benthic community status to elaborate a weight of evidence (WOE) model. Moreira et al. (2020) computed an integrated biomarker response index (IBR), which combined to bioaccumulation analysis indicated the contribution of several anthropogenic disturbances on sediment pollution of Mucuripe Bay (Brazil). Also, Oladi and Shokri (2021) combined PAH bioaccumulation in coral tissues, the percent of live coral cover, and the Sediment Constituent (SEDCON) Index to study the influence of oil-related activities on northwestern Persian Gulf reef's health.

These analyzes are commonly used to evaluate chronically polluted sites (n = 37; 78,7 %; Fig. 3a), and most articles use single measure (n = 19, 40.4 %; Fig. 3b), suggesting that toxicokinetics is often overlooked on oil pollution research. Fish species are the main indicator organisms for PAH quantification (27.7 % of bioaccumulation studies), most likely due to their nutritional and economical value.

Habitat characteristics, organism of choice, tissue type, lipid content and some physicochemical properties of compounds can influence PAH bioaccumulation (Frapiccini et al., 2018). These factors should be considered on experimental design and standardization for comparable results.

### 3.4. Ecotoxicological analysis

Marine ecotoxicology provides information on adverse effects of chemical pollutants on marine organisms, which can be measured as mortality rate or specific sub-lethal changes on physiology and behavior. Acute toxicity tests expose organisms to a single dose of a chemical substance for a short period (usually up to 96 h), while chronic toxicity tests expose organism to the repeated or continuous administration of a pollutant for a major part of the organism's life span.

Ecotoxicological tests are used to evaluate the concentration of a substance causing lethality to 50 % of the organisms tested (LC50). Alternatively, the median effective concentration (EC50) can be used, representing the pollutant concentration causing sub-lethal effects to 50 % of exposed individuals. These values are often applied to environmental risk-assessments and to establish the legally permitted concentration of a specific pollutant in seawater or marine sediment.

Model-species are frequently used for ecotoxicological studies, but the choice of local ecologically relevant species can lead to more significant results. As an example, Echols et al. (2016) used ephyrae of the scyphozoan jellyfish, *Aurelia aurita* on acute toxicity tests for oil from the DWH incident, showing that crude oil alone did not cause significant acute toxicity, but the presence of chemical dispersant resulted in mortality and physical and behavioral abnormalities. The same was observed by Frometa et al. (2017), demonstrating that combinations of oil and dispersants are more toxic to a deep-sea octocoral than exposure to oil alone. Also, Stefansson et al. (2016) used the larvae of two echinoderm species and four bivalve mollusks to test the acute impact of water-accommodated fractions (WAF) and chemically enhanced WAF of the DWH incident.

Instead of working with crude oil and WAF, ecotoxicological tests can also be used to evaluate the biological effect of specific compounds. Indeed, Renegar et al. (2017) evaluated the toxicity of the PAH 1-methylnaphthalene to the coral *Porites divaricata* in a constant exposure toxicity test and detected acute and sub-acute effects, with coral mortality used to estimate LC50. Also, Knap et al. (2017) evaluated the toxicity of this same PAH to five species of deep-sea micronekton crustaceans and the results revealed the high sensitivity of these species.

To assess the toxicity of oil or PAHs in marine biota, short-term exposure (days; n = 25, 65.8 %) and monitoring (days; n = 24, 63.2 %) are massively used (Fig. 3a, b). Also, a combination of organisms is often selected as sentinel, mainly easily cultivable and commercially relevant fish and crustaceans (36.8 % of ecotoxicological studies), which means that other trophic levels and ecologically relevant biological components tend to be neglected.

The advantages of ecotoxicological tests include low-cost, simplicity, easy data interpretation and fast results. However, environmental factors such as temperature and pH can affect the experiment outcomes and require standardization. As an example, under laboratory conditions, the increase of water temperature from 20 to 25 °C significantly increased the toxicity of PAHs to the marine planktonic algae *Tetraselmis chuii* (Vieira and Guilhermino, 2012). Similarly, the cold-water coral *Lophelia pertusa* exhibited a slower recovery from oil dispersant exposure when experienced elevated seawater temperature (12 °C) as compared to the fragments in ambient temperature seawater (8 °C), which indicates that the initial thermal stress can affect coral's ability to cope with additional pollution stress (Weinnig et al., 2020). Moreover, biological factors such as species of choice, nutritional status, age and development stage are also relevant. As an example Yang et al. (2020), reported that B[*a*]P disrupts the steroidogenesis pathway, impairs spermatogenesis and causes histological damage in male scallops in a stage-specific manner. Finally, multi-species toxicity tests can be employed to provide a broader understanding of the biological effects of oil pollution.

### 3.5. Physiological analysis

Several parameters have been monitored to evaluate the impact of oil on marine organisms' physiology, such as histopathological modifications, development indicators, growth and reproduction rates, behavioral changes, and metabolic alterations.

Histopathological changes, accessed through tissue dissection, are common short-term effects of oil spill. As an example, gills and kidney tissues of fish showed hyperplasia of the primary lamellar epithelium and atrophy of the renal tubules, respectively, 48 h after the intraperitoneal injection of the PAH B[*a*]P (Woo, 2021). In mussels, tissues from adductor muscle, digestive glands, and gills showed abnormality after exposure to oil for three days (Al-Subiai et al., 2012).

Also, coral tissue regeneration tests are physiological analyses highly sensitive to short-term oil exposure (6-24 h) and can be related to toxicity (May et al., 2020). In a long-term work, high-definition photographs of deep-sea coral colonies were taken annually after the DWH oil spill to quantify hydroid overgrowth, branch loss, and track recovery patterns. This non-invasive study demonstrated that the effect of initial impact on corals was still visible 7 years after the spill, indicating a long-term, non-acute impact (Girard and Fisher, 2018).

Moreover, development indicators and juvenile growth rate analysis are often applied since organisms are more vulnerable when exposed during early life stages. Indeed, the exposure to phenanthrene reduced hatching rates, delayed hatching time of embryos, and increased deformity rate of newly-hatched fish larvae (Zheng et al., 2020). Controlled exposure experiments also indicated that the larval growth of *Crassostrea virginica* (Vignier et al., 2019), the development of scallops (Yang et al., 2020), and the growth and development of *Artemia parthenogenetica* nauplii (Cong et al., 2021) may be impaired by oil or PAH. Interestingly, in a post-exposure experiment, Hartmann et al. (2015) moved coral larvae from oil-contaminated to clean seawater and observed a strong reduction on settlement, indicating that oil pollution disrupts coral life cycle in a sublethal manner even after exposure ends.

Reproduction is another trait affected by oil. Duan et al. (2018) observed a transgenerational effect in sea urchins with the transfer of PAH and DNA damage to gametes resulting from maternal and paternal exposure to heavy fuel oil. Yang et al. (2021) reported the effect of PAH on bivalve gonads, causing damages to biological macromolecules in gonadal subcellular fractions, inhibiting gonadal development and ultimately leading to reduction in fertility. Similarly, Yang et al. (2020) demonstrated that B[*a*]P disrupts the steroidogenesis pathway and impair spermatogenesis of male scallops.

Behavioral changes were also observed in response to oil, such as severe polyp retraction in the coral species *Pocillopora damicornis* (May et al., 2020), immobility of *A. parthenogenetica* (Cong et al., 2021) and reduced swimming velocity in the common prawn *Palaemon serratus* (Silva et al., 2013). In the ecosystem-level, the effects of these behavioral changes reduce survival and reproductive success, alter the predator-prey dynamics, interfere in the food web, and, consequently, impair ecosystem's health and stability.

Oil pollution also affected energy metabolism, with the increase of oxygen consumption on polar cod (Nahrgang et al., 2019). Regarding photosynthesis, contrasting results were observed, with the reduction or stimulation of photosynthetic rates depending on oil physical properties (Wegeberg et al., 2020). Similarly, fatty acid stable isotope composition was used to evaluate the physiological state of the macroalga *Ulva pertusa* in response to different types and concentrations of oil, revealing petroleum-induced stress and the feasibility of this tool as an environmental risk assessment method in the intertidal zone (Liu et al., 2020). The immune defense of organisms also seems to be impaired following oil exposure, as demonstrated for organisms as diverse as bivalve mollusks (Luna-Acosta et al., 2015; Mansour et al., 2017) and bottlenose dolphins (White et al., 2017) and, therefore, could influence their ability to resist infectious diseases.

Short-term exposure (days, n = 15, 40.5 %; Fig. 3a) and monitoring (days; n = 13, 35.1 %; Fig. 3b) are frequently employed for physiological analysis, pointing to the need to further investigate long-term physiological effects of oil. Most researches use fish as indicator organism (29.7 %), and studies should be further extended to other taxonomical groups and ecological niches.

Physiological analyses provide relevant information on organisms' health status in response to oil, especially when combined to Bioaccumulation analyses. However, intrinsic factors such as individual nutritional status, age, reproductive and developmental stage can affect the results and should be standardized to avoid masking the effects of oil. Early development stages are especially sensitive to pollutants, as demonstrated by Soysa et al. (2012) that exposed zebra fish embryos to crude oil WAF starting at 3.5 hours post-fertilization (hpf) until a maximum of 5 days post-fertilization (dpf). The authors noticed that earlier exposition resulted on more severe developmental deformations, including changes in head and trunk morphology, compromised cardiovascular system and impairment of proper swimming behavior. Also, combining different indicator species and various exposure and monitoring times are necessary for a broader view of the impact of oil pollution over the marine biota.

### 3.6. Biomarker analysis

Biomarkers are defined as measures of pollutant exposure or effect expressed at sub-organism level (Sherwood et al., 2019). These are sensitive tools that provide information on the bioavailability of contaminants, and integrate the response to various exposure routes, such as through inhalation, feeding or dermal absorption. Biomarkers also provide information about metabolic pathways affected by oil and the activated detoxication mechanisms (Blalock et al., 2020).

Most biomarker analyses can be grouped in two categories: gene expression, usually through Real-time quantitative PCR (RT-qPCR); and enzymatic activity, based on fluorescence or colorimetric analysis. Transcriptional variations tend to be more sensitive to pollution than enzymatic activity, but do not necessarily lead to functional responses. As such, gene expression analyses can be referred as “exposure” biomarkers, while enzymatic activities are reported as “effect” biomarkers.

Biomarkers responding to oil pollution are often part of biotransformation pathways, or related to oxidative stress and neurotoxicity (Table 1). For instance, the up-regulation of aryl hydrocarbon receptor (AhR) was detected in response to PAH and oil exposure (Diaz de Cerio et al., 2017; Du et al., 2015). AhR is a ligand-activated transcription factor that can bind to a variety of chemicals, including PAH, and act as an important receptor in biotransformation. Biotransformation proceeds in a first step of oxidation (phase I), and a second step of conjugation to nontoxic endogenous metabolites, resulting on excretable products (phase II) (Danion et al., 2014). Moreover, exposure to pollutants, including oil, cause oxidative stress through the formation of reactive oxygen species (ROS), which induces antioxidant defense pathways and can be used as a quantitative, although unspecific, biomarker of chemical pollution (Cariello Delunardo et al., 2019; Sherwood et al., 2019).

In general, biomarkers offer quick responses to anthropogenic stressors and, as such, most of the studies encompass short-term exposure (“days”, n = 9, 34.6 %, Fig. 3a) and monitoring (“weeks”, n = 9, 34.6 %, Fig. 3b). The use of biomarkers for long-term oil impact monitoring is still scarce in the literature, and should be carefully evaluated.

Mollusks (46,1 %), especially mussels (*Mytilus* sp.), are often selected as indicator organisms, since bivalves are sessile filtering organisms that tend to accumulate and respond to oil pollution (Sforzini et al., 2018; Soares et al., 2021; Traina et al., 2021; Viñas et al., 2018). In order to obtain ecologically relevant results and establish suitable biomarkers, it is important to choose native species.

Due to the relative technical simplicity, Biomarker analyses have been widely used over the last decade on oil pollution biomonitoring. However, for a deeper understanding of biological impacts, it is advisable to combine both exposure and effect Biomarkers with Bioaccumulation and Physiological analyses. In this sense, several studies have proposed the development of an integrated approach (Casini et al., 2018; Di et al., 2017), including a method to simplify the interpretation

**Table 1**
Main biomarkers for oil pollution environmental monitoring.

| Metabolic pathway | Biomarker |
|---|---|
| Transcription regulation | Aryl hydrocarbon receptor (AhR) |
| Biotransformation | |
| Phase I | 7-Ethoxyresorufin-O-deethylase (EROD) |
| | Cytochrome P450 (CYP) |
| | Flavin-containing monooxygenase (FMO) |
| | Monoamine oxidase (MAO) |
| Phase II | Glutathione S-transferase (GST) |
| Oxidative stress | Peroxidase (POD) |
| | Glutathione peroxidase (GPx) |
| | Glutathione reductase (GR) |
| | Superoxide dismutase (SOD) |
| | Catalase (CAT) |
| Neurotoxicity | Acetylcholinesterase (AChE) |

of multiple biomarkers into a single metric (Blalock et al., 2020; Tsangaris et al., 2011).

Considering that reproductive status, sex-related variability (Blanco-Rayón et al., 2020; Imbery et al., 2019), nutrition state (González-Fernández et al., 2017), lipid content and body size (Hansen et al., 2013) can affect the responsiveness of most biomarkers, these parameters should be standardized on experimental design. Moreover, environmental factors, such as sampling season, salinity and temperature should be surveilled when applying biomarkers on environmental monitoring (Hansen et al., 2013; Lysenko et al., 2015; Osse et al., 2018).

## 4. Conclusion

In the last decade, a large number of studies were dedicated to analyze the biological impact of oil pollution in the marine environment, reflecting the intensification of marine traffic and the growing number of oil spill incidents. This article systematically reviewed the literature on the topic providing a comprehensive summary of the most relevant methods applied and the major findings. The combination of Biomarker and Bioaccumulation analysis is the most frequently adopted strategy, offering information on tissue/cell PAH concentration and molecular-level biological impact. Following a global trend on environmental studies, Omic analyses were also highly employed on high-throughput monitoring of biomolecules to understand the flow of information that underlies biological response to oil. Most analytical methods are used on short-term or medium-term studies. Several tools are known to capture acute responses, which justifies their choice for short-term impact research. However, the chronic effects of oil and the ecosystem capacity to recover tend to be neglected. In this context, our review provides a critical evaluation of the characteristics, advantages and limitations of frequently employed methods. The present review also provides a framework to develop standardized and integrated protocols combining different tools for holistic assessments of oil pollution impact through seawater and sediment analysis. This should enable an articulated response from the scientific community, environmental organizations and government in cases of oil pollution.

### *4.1. Future perspectives*

Despite the global concerns regarding oil pollution impacts on marine environment, there are still some important knowledge gaps that need to be addressed to advance in the field. Most studies have focused on the short-term effects of oil spill over one or a few marine species. To fully understand the impact of oil pollution and HPA bioaccumulation on different trophic levels, it is necessary to carry out multi-species research and combine methods that assess changes at individual organism, population, community and ecosystem levels. This approach will offer a holistic view of oil pollution effects on ecosystems rather than on isolated species. Furthermore, there is an eminent need to understand how abiotic and biotic factors, such as temperature, pH, nutritional status, organisms age and development stage affect the outcomes of the employed analytical tools.

In addition, to elucidate the broad-range impact of oil pollution, it is crucial to consider the connectivity between different environments and how oil spills can affect nearby or even distant areas. Finally, the development of biomonitoring programs that last for years or decades after oil acute exposure should provide valuable information to understand and even predict ecosystem's capacity of resilience to fully recover or reach a different level of ecological balance.

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## 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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