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Systematic Review Protocol

Title
What are the effects of chronic oil exposure on the survival, reproduction, and performance of marine organisms

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Keywords
marine pollution, long-term, oil spills, PAHs, ecotoxicology

Background
Large-scale oil spills are well documented and studied around the world. However, the often-unreported smaller-scale spills are becoming more and more common [1,2]. Continuous spills can lead to chronic exposure of marine biota to hydrocarbon pollutants, such as crude oil and crude oil-derived polycyclic aromatic hydrocarbons (PAHs). PAHs are characterized by high toxicity, high environmental stability, and high hydrophobicity, resulting in their accumulation via the trophic chain, throughout the food web [1]. Long-term exposure to sublethal concentrations of PAHs causes deleterious effects at all biological organization levels, from organisms to communities, by altering survival, growth, and reproduction [3], leading to altered species number, abundance, and biomass [4]. At the organism level, performance is affected through decreased feeding, development, and growth rate [5–7], causing deformities [8] and cellular physiological stress [9][10], and reduction in biomass and lipid stores, potentially leading to increased mortality [11]. In fish, even very low hydrocarbon concentrations can impair reproductive success [12], through delayed hatching time, embryonic toxicity, abnormal sexual differentiation, and prolonged sexual maturity [13]. In copepods, decreased early-stage survivorship, development rate, sexual maturation, and fecundity have been reported under low hydrocarbon levels [14]. Hydrocarbon pollutants at sublethal concentrations may also enter the food web via absorption or ingestion of organisms at low trophic levels and passed on to higher trophic levels through consumption [15]. Hydrocarbon bioaccumulation occurs if the rate of assimilation exceeds the rate of elimination. Biomagnification, the process by which the pollutant is absorbed by an organism through the food chain while its concentrations increase at each trophic level, can lead to bioaccumulation. Long-term exposure to PAH-contaminated food alters metabolic activity, reduces biological activity [16], and compromises innate immune responses, which may lead to disease susceptibility [17].

Theory of change or causal model
Chronic exposure to hydrocarbon pollutants, from continuous small-scale oil spills or from oil transport and exploration activity, may lead to hazardous outcome on the marine environment, from the organism to the community level. The combined effect of hydrocarbon pollution with other stressors, such as temperature elevation, changes in salinity or pH, can push a species or population beyond a critical tolerance threshold. Numerous literature reviews have been conducted on the

effects of acute large-scale oil spills, however reviews assessing the effects of chronic exposure by the more common small-scale oil spills are vital yet lacking.

Stakeholder engagement

This protocol was developed in the framework of the research project "Effects of crude-oil and gas-condensate pollution on the Israeli Mediterranean ecosystems" funded by the Israeli Ministry of Energy. This project aims to systematically assess the direct and indirect impacts of crude-oil and gas-condensate pollution on marine food webs, from plankton to benthos and fish, in the Southeastern Mediterranean Sea. The research results will fill critical knowledge gaps that will aid in developing tools for policy-makers to support the sustainable management and development of marine resources in Israel.

Objectives and review question

The objective of the systematic review is to evaluate and quantify the existing research on the effects of chronic oil exposure on marine species. The review will also ascertain existing knowledge gaps for future primary research. Primary question: What are the toxic levels and magnitude of the effects of chronic exposure to hydrocarbon pollutants on marine organisms? Secondary question: What are the interactive effects (additive, synergistic or antagonistic) of chronic hydrocarbon pollutant exposure and a secondary stressor such as warming, parasitism, UV radiation and changes in salinity on marine species?

Definitions of the question components

Population: • Marine species (excluding water birds and mammals) Exposure or intervention: • Chronic oil/PAHs exposure • 96 hours or longer exposure to oil/PAHs Comparators: • Control group (not exposed to oil/PAHs) • Before exposure (in before-after studies) Outcomes: • Survival/mortality rate • LC50 (lethal concentration 50%) • EC50 (effective concentration 50%) • IC50 (inhibitory concentration 50%) • reproductive traits: egg production rate (EPR), egg hatching success (HS), nauplius survival rate (NSR) • Performance (respiration, photosynthesis, growth, movement, enzymatic activity feces or pseudofeces production, net carbon flux, feeding activity and filtration rate)

Search strategy

The systematic review will follow the Collaboration for Environmental Evidence (CEE) guidelines. The search aims to retrieve a wide range of quantitative scientific evidence, covering the topic of chronic hydrocarbon pollutants and their impact on marine species. Pre-determined keywords will be used to search for relevant studies in three academic databases: ISI Web of Science (all databases), Scopus and GEOBASE. Within each category (population, exposure and outcome) the search terms will be combined in parentheses and separated using the boolean operator 'OR'. These categories will then be combined using the boolean operator 'AND'. In addition, parallel search will be conducted in GeoBase dictionary terms (Thesaurus search). While reviewing the full-text studies, further relevant papers that were missed in the initial search will be identified and added to the systematic review by using the 'snowball' method [18] . Benchmark article screening was done to ensure the comprehensiveness of the search.

Bibliographic databases

1) ISI WoS 2) SCOPUS 3) Geobase (+Geobase Thesaurus) Languages: English, Hebrew ISI WoS, SCOPUS and Geobase will be search using the following search strings: (marine OR ocean* OR sea OR seas OR bay OR bays OR lagoon* OR cove OR coves OR gulf OR coastal OR brackish OR estuar* OR pelagic OR benth* OR plankt* OR shore OR shores OR subtidal OR intertidal OR harbor* OR harbour*) AND (chronic OR "long term" OR longterm OR persistent) AND (petroleum OR "organic contaminant*" OR "pollution exposure" OR oil OR PAH* OR "Polycyclic aromatic hydrocarbon*").

Geobase Thesaurus will be searched using the following search strings: (coastal waters (index term) OR estuarine environment (index term) OR intertidal environment (index term) OR aquatic environment (index term) OR rocky shore (index term) OR nearshore environment (index term) OR shallow water (index term)) AND ((petroleum (Index Term) OR crude oil (Index Term) OR PAH (index term) OR pollution exposure (index term)) AND (chronic (index term) OR long-term (index term) OR longterm (index term) OR persistent (index term))

Web-based search engines
N/A

Organisational websites
N/A

Comprehensiveness of the search
The comprehensiveness of the search string was examined in the scoping study using five benchmarking articles known to be relevant to the systematic review: 1. Bioaccumulation of polycyclic aromatic compounds: 1. Bioconcentration in two marine species and in semipermeable membrane devices during chronic exposure to dispersed crude oil [19] 2. Sub chronic exposure to crude oil, dispersed oil and dispersant induces histopathological alterations in the gills of the juvenile rabbit fish (Siganus canaliculatus) [20] 3. Effects of chronic crude oil exposure on early developmental stages of the Northern krill (Meganyctiphanes norvegica) [21] 4. Bioaccumulation of polycyclic aromatic compounds: 2. Modeling, bioaccumulation in marine organisms chronically exposed to dispersed oil [12] 5. Chronic exposure of adults and embryos of Pandalus borealis to oil causes PAH accumulation, initiation of biomarker responses and an increase in larval mortality [22]

Search update
If the time exceeds two years between the search and publication submission an update on search strings will be performed.

Screening strategy
The inclusion criteria will be assessed at three levels: title, abstract and full-text. First, articles will be assessed by title to remove citations unrelated to the search. Next, remaining citations will be assessed based on their abstracts to further remove unrelated citations. Finally, full text articles will be assessed according to pre-set inclusion/exclusion criteria.

Eligibility criteria
 Studies on acute exposure alone will be excluded Studies specific to mammals and birds will be excluded Studies referring to chronic effects caused by acute oil exposure will be excluded Studies lacking control will be excluded. Non-replicated studies will be excluded While reviewing abstracts and full texts, studies with intervention time longer than 96 hours will be included [32] even in the absence of chronic/persistent key words. Relevant Habitats: All marine environments worldwide, including estuaries, bays, harbors, coasts, shores, intertidal, reefs, mudflats, sandflats, mangroves, seagrass beds, deep sea. Not including freshwater habitats. Relevant species/population: Phytoplankton, zooplankton, fish, all benthic species, including, but not limited to mollusks, corals, and sponges. Excluding marine mammals and birds. Relevant exposures: Experiments using crude oil, in the form of WAF (water associated fraction, sometimes termed water-soluble fraction (WSF)), slick (a film or layer of oil floating on an expanse of water), or oil droplets.

Consistency checking
Two reviewers will simultaneously and independently assess (include/exclude) studies from a random subset (50 studies) of the search results at the title, abstract and full text levels. Fleiss’

Kappa test will be used for assessing the reliability of agreement between the reviewers. At Kappa <0.6, eligibility and quality criteria and any disagreements will be re-discussed among the reviewers and a new kappa test will be performed.

Reporting screening outcomes
A ROSES flow diagram will be produced to report the outcome of the screening. Articles excluded at title and abstract will be detailed in one file, while another separate file will record articles excluded at full text with explanations of their exclusion. Any deviations from this protocol will be reported and explained.

Study validity assessment
Following inclusion criteria, studies included will be assessed for bias according to the following criteria: • lack of replication • lack of methodological information (e.g. sample size, length of exposure) • uninterpretable outcomes and intervention (oil exposure) data. Based on these criteria, studies will be categorized as having high, medium, or low quality.

Consistency checking
The CEE Critical Appraisal Tool Version 0.3 (Prototype) [24] modified to our review question, will be used for study validity. The CEE Critical Appraisal Tool will be used to assess and categorize each study's susceptibility to bias.

Data extraction strategy
Data will be extracted from charts using WebPlotDigitizer v4.6 (https://automeris.io/WebPlotDigitizer/). If relevant data is either missing or ambiguous, the corresponding authors of those studies will be contacted. In the event of no response, we will indicate no response in our metadata and only report available data in our final analysis. If the amount of missing data is deemed to be substantial (> 50%), the study will be excluded from our final analysis.

Meta-data extraction and coding strategy
For each screened study that fits the inclusion criteria and meets the study validity criteria, data will be extracted according to predetermined codes. The following data will be coded for: • Bibliographic information (author, year, title, source of publication) • Study site (name) • Study location (coordinates) • Study setting (field/laboratory) • Study design (BA, BACI) • Taxonomic group • Tested species (species name) • Hydrocarbon pollutant type (crude-oil, PAHs) • Hydrocarbon pollutant name (PAH compound) • Experimental procedure (slick, WAF, droplets) • Experimental duration (days) • Crossed treatment (warming, dispersant addition, UV, parasites, none) • Outcome category (survival, reproduction, performance) • Outcome (survival rate, mortality rate, LC50, EC50, IC50, egg production rate (EPR), egg hatching success (HS), nauplius survival rate (NSR), respiration rate, photosynthetic rate, growth rate, movement, enzymatic activity, feces or pseudo-feces production, net carbon flux, feeding activity, filtration rate • Reported mean (exposed/impacted group) • Number of replications (exposed/impacted group) • Number of individuals per replicate (exposed/impacted group) • Sample variance and variance type SD/SE/CI (exposed/impacted group) • Reported mean (control group) • Number of replications (control group) • Number of individuals per replicate (control group) • Sample variance and variance type SD/SE/CI (control group)

Consistency checking
Coding will be performed by the first author, with a subset of 5% of studies checked for coding consistency by the last author. Should there be substantial disagreement, the coding criteria will be reviewed and a further subset of 5% of studies will be rechecked for consistency. This will be

repeated until the consistency reaches a minimum of 90% similarity. Other missing relevant information will be coded as “Not specified”, because the timeframe does not allow for contacting authors for information, and information that does not apply to a specific study will be coded as “NA”.

Potential effect modifiers/reasons for heterogeneity
The following effect moderators/modifiers and the sources of heterogeneity were identified, and their analysis methods are detailed in brackets: • Hydrocarbon pollutant type (crude-oil, PAHs) [subgroup analysis] • Hydrocarbon pollutant level (concentration) [meta-regression] • Exposure duration [meta-regression] • Direct/indirect exposure [subgroup analysis] • Study setting (field, lab) [subgroup analysis] • Exposure method (WAF, slick, droplets) [subgroup analysis] • Taxonomic group [subgroup analysis] • Habitat type [subgroup analysis] • Interactive factor (warming, UV radiation, dispersant treatment, parasites) [meta-regression for continuous factors, subgroup analysis for discrete factors]

Type of synthesis
Narrative and quantitative (systematic review with meta-analysis).

Narrative synthesis methods
The narrative synthesis will report basic summary of the studies that have been conducted so far and data availability. Based on the representation in the meta-data of the studies, we can identify sub-topics and research gaps that warrant further research.

Quantitative synthesis methods
A narrative descriptive synthesis will be conducted on eligible studies demonstrating a low and medium risk of bias (based on study validity assessment), summarizing information in tables and figures. Summaries will outline bibliographic information, study location, settings, design, experimental methods and conditions, pollutant types, taxonomic groups, and outcome categories. Where evidence allows, efforts will be made to estimate the effect size of the outcome (i.e. survival, reproduction or performance measures after exposure to a hydrocarbon pollutant relative to the comparator). The effect size will be evaluated by converting the data from primary studies to standardized mean difference (SMD) in form of Hedge's g. The effect size estimates from individual studies will be calculated using the ‘metafor’ package in R [25] and presented in forest plots. Since the data will contain multiple levels and different types of non-independence, a phylogenetic three-level meta-analytic model will be considered (following Cinar et al., 2022), accounting for inherent dependencies between effect sizes (i.e., allowing multiple effect sizes from the same study), and considering that the variance is distributed over multiple levels. To assess the relationship between potential effect-moderators and the effect size within each category, subgroup analyses will be performed using mixed-effects models. Meta-regression analysis will be performed to understand the relationship between continuous effect moderators and effect-size.

Qualitative synthesis methods
N/A

Other synthesis methods
N/A

Assessment of risk of publication bias
To assess the risk of publication bias, effects from individual studies will be visualized in funnel plots and tested for asymmetry using Egger’s test using ‘metafor’ package.

Knowledge gap identification strategy

The research gaps will be identified by analysing the representation of the meta-data, which can be visually shown as a table or figure.

Demonstrating procedural independence

Authors of research studies included in this review will not be involved in any decisions regarding their own work. Procedural independence is guaranteed as none of the authors has (co-)authored studies which could be included in this review.

Competing interests

The authors declare that they have no competing interests.

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Author’s contributions

NPT and TGH conceptualized the idea. NPT conducted the scoping search, designed the protocol and wrote the manuscript. TGH supervised the study, designed the protocol and wrote the manuscript. PA, GR and NS reviewed the protocol design and the manuscript. All authors read and approved the final manuscript.

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