



# A method linking the toxic effects at community-level with contaminant concentrations



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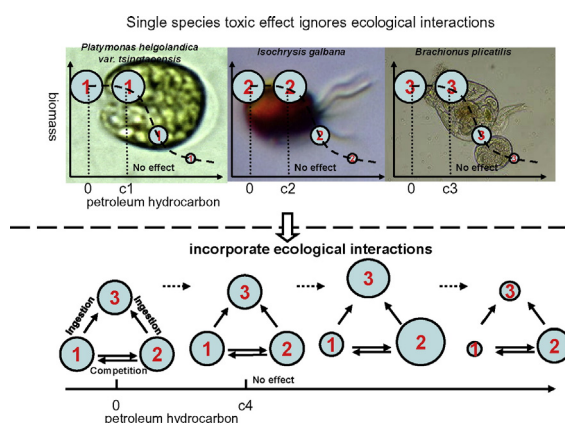
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## HIGHLIGHTS

- We tested petroleum hydrocarbon ecotoxicological effects on a simplified community.
- Concentration-response relationships at a community-level were constructed.
- A deduced no-effect concentration representing ecological interaction was named TCPE.
- TCPE of petroleum hydrocarbons was higher than PNEC calculated from SSD.
- Ecological interactions reduce toxic effect of petroleum hydrocarbons on a community.

## GRAPHICAL ABSTRACT



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## ABSTRACT

In this study, we developed a method to quantify and link the toxic effects in community-level ecosystems with concentrations of petroleum hydrocarbons. The densities of *Platymonas helgolandica* var. *tsingtaoensis*, *Isochrysis galbana*, and *Brachionus plicatilis* in single-species tests and customized ecosystems were examined in response to a concentration gradient of petroleum hydrocarbons ranging from 0 to 8.0 mg L<sup>-1</sup>. A three-population ecological model with interspecies competition-grazing relationships was used to characterize population sizes with concentrations of petroleum hydrocarbons. A threshold concentration of the simplified plankton ecosystem of 0.376 mg L<sup>-1</sup> for petroleum hydrocarbons was calculated from the proposed model, which was higher than the no-effect concentration of 0.056 mg L<sup>-1</sup> derived from the single-species toxicity tests and the predicted no-effect concentration of 0.076 mg L<sup>-1</sup> calculated from the species sensitivity distribution. This finding indicates that interspecies competition and grazing reduced the toxic effect of petroleum hydrocarbons at the community level. The sensitivity analysis for model parameters demonstrates that plankton population biomasses are highly sensitive to filtration rates. Antagonism between interspecies interactions and petroleum hydrocarbon toxicity was attributed to the reduced filtration rate and zooplankton grazing pressure. The proposed method is a simple

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means to address the concern regarding the impacts of ecological interactions on ecological risk assessments of pollutants.

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## 1. Introduction

Over a million tonnes of crude oil have been released into the ocean owing to human economic activities in the past 40 years (ITOPF, 2013). One of the major constituents of crude oil is petroleum hydrocarbons, which produce toxic effects on marine plankton and cause catastrophic damage to the marine environment (Wang et al., 2015a, 2015b). This environmental issue has received enormous attention worldwide. The protection of marine ecosystems relies on accurate understanding and scientific assessment of the effects of oil pollutants. The majority of existing assessments concerning the ecotoxicological effects of pollutants on the structures and functions of ecosystems depend on the extrapolation of single-species effect data to community-level effects (Laender et al., 2008). One of the most sophisticated extrapolation methods, the species sensitivity distribution (SSD), assumes that the sensitivity of an ecosystem can be represented by a set of independent species sensitivities obtained from single-species toxicity tests and the ecological threshold concentrations of all the species in a community follow some form of probability distribution (Laender et al., 2008; Van, 2004). One of the most common approaches that risk managers use to account for any uncertainties or variability in extrapolation is to apply an assessment factor (i.e., a numerical adjustment), which assumes that the factors from 10 to 100 can sufficiently protect the ecosystem (European Commission, 2003; Lau et al., 2013). However, such assumptions ignore the ecological relationships among community populations. The toxic effects on ecosystems at the community-level have been found to be determined by the inherent sensitivities of the species present and the ecological relationships between these species (Chapman et al., 2003; Fleeger et al., 2003; Laender et al., 2008). Therefore, the ecological interactions within communities should be considered during ecological effect assessments to provide accurate estimations of the effects of pollutants (Laender et al., 2008).

Large-scale experimental studies are complex and expensive, and their reproducibility is low, making them unsuitable for routine practices. Therefore, the development of other methodologies that require fewer resources to extrapolate single-species toxic effect data to ecosystem-level responses is necessary. Ecosystem models can be used as alternative, practical solutions to these extrapolation problems. However, the results obtained by ecosystem models are difficult to validate with experimental data having multi-trophic levels (Meng et al., 2009). One model, the simplified ecosystem model, has few parameters (only the first and second trophic levels are used); thus, it can be validated by ecological experiments. Simplified models are promising as ecotoxicology research tools because they incorporate the essential constituents and principal ecological relationships in ecosystems (Feng, 2006; Steele, 1974; Tang, 1999; Xu, 2008). In the present study, the toxic effects of petroleum hydrocarbons at the community-level were examined using a simplified ecological scenario consisting of representative species of *Platymonas helgolandica* var. *tsingtaoensis*, *Isochrysis galbana*, and *Brachionus plicatilis* from coastal waters of China. The data sets obtained were used to parameterize a plankton ecosystem model with interspecies competition-grazing relationships, which was proposed to quantify and link the toxic effects in community-level ecosystems with the concentrations of petroleum hydrocarbons. In addition, a new indicator representing interspecies interactions was introduced to assess the ecological effects of petroleum hydrocarbons on ocean ecosystems.

## 2. Materials and methods

### 2.1. Plankton species and petroleum hydrocarbon used in experiment

The selection of experimental plankton species referred to an approach for the development of ecological scenarios (Rico et al., 2016), with representative and functional species expected to be impacted by petroleum hydrocarbon exposure considered. *P. helgolandica* var. *tsingtaoensis* and *I. galbana* are planktonic single cell algae found widely in coastal waters of the China Sea, especially in mariculture regions. They can be rapidly cultured and are nutritious for marine animal larvae of economic value, acting as a basic food source. Detailed information exists in the literature on their response to culture conditions, including light, temperature, salinity, and nutrients, and they have been extensively and successfully cultured in laboratories and aquafarms (Hao et al., 2008; Sun et al., 2005). The rotifer *B. plicatilis* occurs worldwide, is extensively found in coastal waters, and is an adequate first feed for larval rearing of marine fish. The adaptable *B. plicatilis* propagates rapidly, having a short life cycle, and its dormant eggs can be commercially obtained (Fang et al., 2013). *B. plicatilis* is often used as a test organism in environmental monitoring and ecotoxicology studies (Fang et al., 2013; OECD, 2002; Snell and Janssen, 1995). For these reasons, *P. helgolandica* var. *tsingtaoensis*, *I. galbana*, and *B. plicatilis* were chosen as the experimental species in the present study.

The water-accommodated fractions of crude oil collected from the SZ36 oil well in Bohai, China were prepared for the petroleum hydrocarbon solution used in this study. The crude oil sample contained 0.2% sulfur, 0.4% nitrogen, 11.6% hydrogen, and 87.6% carbon in element composition of organics, and 2.8% paraffin, 21.4% colloid, 2.0% asphaltene, 0.3% water, 0.02% ash, 9.0% carbon residue, and 63% sodium chloride in chemical composition, placing it in the low sulfur naphthene base oil category.

The petroleum hydrocarbon concentrations in the prepared solution were measured by ultraviolet spectrophotometry (UV-2102PCS, Unicop (Shanghai) Instrument Co., Ltd.) at 225 nm. The analytical limit of detection was 0.002 mg L<sup>-1</sup> and the average relative standard deviation was <2%. The experiments were performed and controlled according to the Chinese standard GB/T 21805-2008 (General Administration of Quality Supervision, Inspection and Quarantine of China, 2008) and (ASTM E1440-91, (2012)).

### 2.2. The toxic effects on phytoplankton

#### 2.2.1. Algal single-species tests

The algae (*P. helgolandica* var. *tsingtaoensis* and *I. galbana*) were cultivated in three 1-L conical flasks containing natural, filtered (<20 µm) autoclaved seawater. The seawater was obtained from the East China Sea near Qidong County, where the salinity was approximately 30 psu. Nitrate, phosphate, vitamins, and trace elements were added in accordance with the f/2 medium recipe (Guillard and Ryther, 1962). The conical flasks were placed in an incubator at a constant temperature of 23 °C for a photoperiod of 12 h light and 12 h dark, and a photon irradiance of approximately 60 µmol m<sup>-2</sup> s<sup>-1</sup> (Wang et al., 2010). The initial algal incubation density was 5 × 10<sup>6</sup> cells mL<sup>-1</sup> for *P. helgolandica* var. *tsingtaoensis* and 12 × 10<sup>6</sup> cells mL<sup>-1</sup> for *I. galbana*. The concentrations of the petroleum hydrocarbons for the toxicity tests were 0, 0.2, 0.4, 0.8, 1.2, 2.0, 4.0, and 8.0 mg L<sup>-1</sup>, which were determined in a preliminary experiment. The algal single-species toxicity tests ran for up to 16 days until maximum densities were approached or attained. During

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