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

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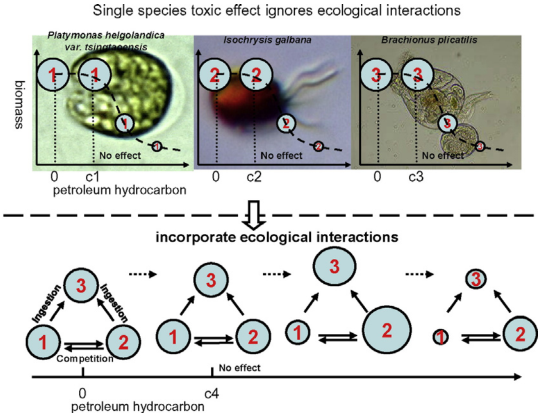
HIGHLIGHTS

- 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^{-1}. 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^{-1} for petroleum hydrocarbons was calculated from the proposed model, which was higher than the no-effect concentration of 0.056 mg L^{-1} derived from the single-species toxicity tests and the predicted no-effect concentration of 0.076 mg L^{-1} 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.

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, Unico (Shanghai) Instrument Co., Ltd.) at 225 nm. The analytical limit of detection was 0.002 mg L^{-1} 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^{-2} s^{-1} (Wang et al., 2010). The initial algal incubation density was 5×10^6 cells mL^{-1} for *P. helgolandica* var. *tsingtaoensis* and 12×10^6 cells mL^{-1} 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^{-1}, 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

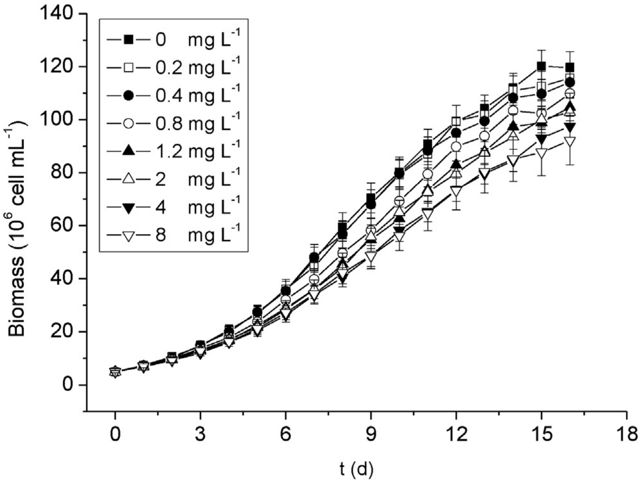

Fig. 1. Biomasses changes of *Platymonas helgolandica* var. *tsingtaoensis* over time at different concentrations of petroleum hydrocarbon.

the experimental period, 5 mL water samples were extracted from the culture flasks every day and fixed with Lugol's solution. The algae species composition and cell numbers in the samples were determined by light microscopy (XLE-2, 3DFAMILY Technology Co., Ltd., Nanjing, China); the measurements were replicated three times.

2.2.2. Algal bi-species competition test

In the bi-species algal competition experiment, the initial algal incubation densities were 5 × 10^6 cells mL^{-1} for *P. helgolandica* var. *tsingtaoensis* and 12 × 10^6 cells mL^{-1} for *I. galbana*, which were the same biomass values as those determined using their single cell volumes (Li and Wang, 2012). The culture conditions, experimental design and duration, and concentration gradient of the petroleum hydrocarbons were consistent with those for the algal single-species tests.

2.3. The toxic effects on zooplankton

2.3.1. Rotifer test for mortality rate

The zooplankton rotifer *B. plicatilis* was cultured in 6-well culture plates containing 5 mL of culture solution. The larvae, which were incubated simultaneously, were placed in the culture plates, with one larva per well. The culture plates 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^{-2} s^{-1}. The larvae were fed every 24 h with 0.2 mL of *Chlorella* sp. algae at a density of 1 × 10^6 cells mL^{-1}. Observations of each *B. plicatilis* were taken every 3 h. After recording the appearance of first eggs and the hatching time of the first larvae, the observations were taken every 6 h (Feng, 2006). The number of eggs laid, number of incubated larvae, and survival times of *B. plicatilis* were recorded simultaneously, and were later used to calculate one of the parameters (the mortality rate of *B. plicatilis*) in the simplified plankton ecosystem model. After the measurements were taken, the larvae were removed from the culture solution. The experiment continued until all of the observed *B. plicatilis* died.

2.3.2. Rotifer test for filtration rates

The simultaneously incubated rotifer larvae were fed either *P. helgolandica* var. *tsingtaoensis* or *I. galbana* for three days, and then not fed for one day before the ingestive behavior experiment was conducted. The unfed larvae were placed in 150-mL conical flasks containing 50-mL algal solutions of different densities (Feng, 2006). The test concentrations of the petroleum hydrocarbons were the same as for the algal tests described in Sections 2.2.1 and 2.2.2. The conical flasks were placed in a carton covered with black cloths, then placed on a controlled temperature oscillator for 24 h. The algal cell densities were determined by light microscopy before and after ingestion. Using the equations proposed by Frost (1972), the filtration and ingestion rates for the test concentrations of petroleum hydrocarbons were calculated by the differences in the density of algae between the control and the treatments containing rotifers.

2.4. The toxic effects on the simplified plankton ecosystem

The initial algal incubation densities of *P. helgolandica* var. *tsingtaoensis* and *I. galbana* in the simplified plankton ecosystem experiments were 5 × 10^6 cells mL^{-1} and 12 × 10^6 cells mL^{-1}, respectively. In addition, the initial density of the rotifer *B. plicatilis* was 5 individuals mL^{-1}. The two algae species had equal initial biomasses, which were determined using their single cell volumes (Li et al., 2011; Li and Wang, 2012; Wang et al., 2010). The initial rotifers were selected from moving individuals cultured under identical conditions. The experiment was conducted in a 1-L conical flask with 0.5 L of culture solution and was replicated three times for each concentration gradient of the pollutant. The test concentrations of the petroleum hydrocarbons in this experiment were the same as for the algal tests described in Sections 2.2.1 and 2.2.2, as were the culture conditions. The densities of the rotifers and algae were determined each day by light microscopy (XLE-2, 3DFAMILY Technology Co., Ltd., Nanjing, China). The duration of the experiment depended on the occurrence of the stable plankton populations.

2.5. Model construction

A simplified plankton ecological model was constructed by integrating the logistic growth equation and the Lotka-Volterra equation into a competition-grazing model (Volterra, 1926; Wang et al., 2011). In addition, algal density restrictions were accounted for in the model. The simplified plankton ecological model equations are provided below:

$$\frac{dP_1}{dt} = P_1(r_1 - a_{11}P_1 - a_{12}P_2 - a_{13}Z) \tag{1}$$

$$\frac{dP_2}{dt} = P_2(r_2 - a_{21}P_1 - a_{22}P_2 - a_{23}Z) \tag{2}$$

$$\frac{dZ}{dt} = Z(-r_3 + a_{31}P_1 + a_{32}P_2) \tag{3}$$

In this model, P_1 represents the abundance of *P. helgolandica* var. *tsingtaoensis*, P_2 represents the abundance of *I. galbana*, and Z represents

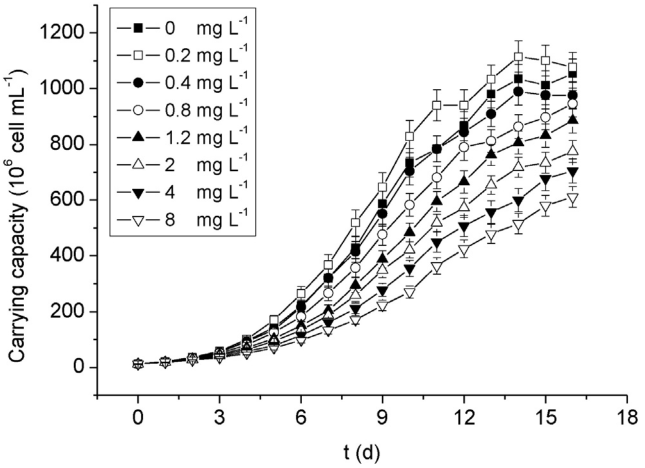

Fig. 2. Biomasses changes of *Isochrysis galbana* over time at different concentrations of petroleum hydrocarbon.

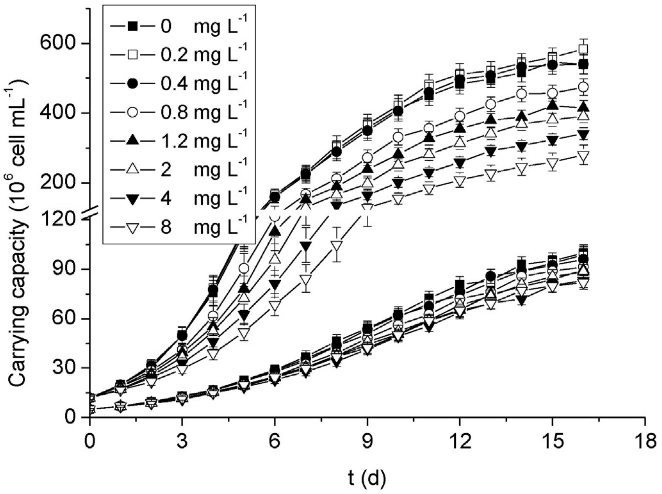

Fig. 3. Biomasses changes of *Platymonas helgolandica* var. *tsingtaoensis* and *Isochrysis galbana* over time at different concentrations of petroleum hydrocarbon in bi-algae competitory experiment.

the abundance of the rotifer *B. plicatilis*. In addition, r_i $(i = 1, 2)$ denotes the instantaneous growth rate under the experimental conditions, with 1 representing *P. helgolandica* var. *tsingtaoensis*, and 2 representing *I. galbana*, while r_3 represents the instantaneous mortality rate of the rotifer *B. plicatilis*. Furthermore, $a_{11} = \frac{r_1}{K_1}$, $a_{12} = \frac{\alpha \cdot r_1}{K_1}$, $a_{13} = F_1$, $a_{21} = \frac{\beta \cdot r_2}{K_2}$, $a_{22} = \frac{r_2}{K_2}$, $a_{23} = F_2$, $a_{31} = F_1 \cdot h_1$, and $a_{32} = F_2 \cdot h_2$, where K_i $(i = 1, 2)$ denotes the carrying capacity of the algae, α is the interspecific competition parameter for *P. helgolandica* var. *tsingtaoensis*, β is the interspecific competition parameter for *I. galbana*, and F_i $(i = 1, 2)$ is the filtration rate from zooplankton to algae (i).

According to the non-linear dynamic results of the ecosystem model, the simplified plankton ecological model only yields a positive asymptotic equilibrium point, E^* (P_1^*, P_2^*, Z^*), when the plankton biomass and model coefficient matrix are consistently >0, indicating that the customized plankton ecosystem would continuously survive.

$$P_1* = \frac{a_{12}a_{23}r_3 + a_{13}r_2a_{32} - r_1a_{23}a_{32} - a_{13}a_{22}r_3}{a_{12}a_{23}a_{31} + a_{13}a_{21}a_{32} - a_{11}a_{23}a_{32} - a_{13}a_{22}a_{31}} \tag{4}$$

$$P_2* = \frac{r_1a_{23}a_{31} + a_{13}a_{21}r_3 - a_{11}a_{23}r_3 - a_{13}r_2a_{31}}{a_{12}a_{23}a_{31} + a_{13}a_{21}a_{32} - a_{11}a_{23}a_{32} - a_{13}a_{22}a_{31}} \tag{5}$$

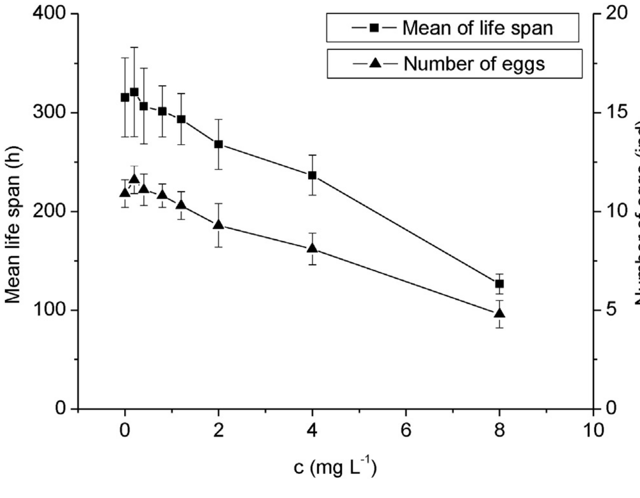

Fig. 4. Changes of life span and number of laid eggs per *Brachionus plicatilis* with concentrations of petroleum hydrocarbon.

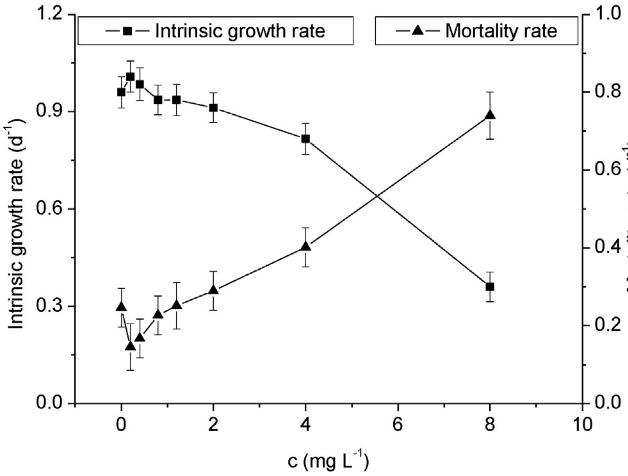

Fig. 5. Changes of intrinsic growth rate and mortality rate of *Brachionus plicatilis* population with concentrations of petroleum hydrocarbon.

$$Z* = \frac{a_{11}a_{22}r_3 + a_{12}r_2a_{31} + r_1a_{21}a_{32} - a_{11}r_2a_{32} - a_{12}a_{21}r_3 - r_1a_{22}a_{31}}{a_{12}a_{23}a_{31} + a_{13}a_{21}a_{32} - a_{11}a_{23}a_{32} - a_{13}a_{22}a_{31}} \tag{6}$$

The equilibrium point values diverged from the original equilibrium point as the pollutant concentrations increased. In the experiment, the maximum pollutant concentration where the equilibrium point did not diverge from the original, i.e., was not significantly different, was defined as the threshold concentration. A hypothesis test approach based on the *t*-test was used to compare the abundances of equilibrium points at the different pollutant concentrations with those in the control group to determine whether divergence occurred. MATLAB 2008b software was used to perform all calculations.

2.6. The parameters in the plankton ecosystem model

The growth rates (r_1 and r_2) and carrying capacities (K_1 and K_2) of the phytoplankton were calculated using a combination of the logistic growth model and the experimental data obtained from the algal single species experiment (Wang et al., 2011). The interspecies competition coefficients (α and β) of the phytoplankton were calculated using the Lotka-Volterra competition model and the experimental data obtained

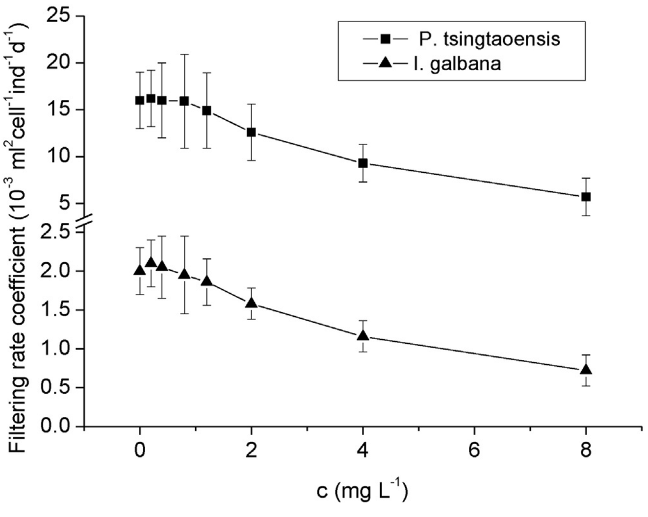

Fig. 6. Changes of filtering rate coefficient of *Brachionus plicatilis* for *Platymonas helgolandica* var. *tsingtaoensis* and *Isochrysis galbana* with concentrations of petroleum hydrocarbon.

Table 1
Model parameters in unexposed simplified plankton ecosystem.

Parameter symbol	Description	Average (standard deviation)
r_{01}	Growth rate of *Platymonas helgolandica* var. *tsingtaoensis* (d^{-1}) in control	0.37 (0.01)
r_{02}	Growth rate of *Isochrysis galbana* (d^{-1}) in control	0.52 (0.02)
K_{01}	Carrying capacity of *Platymonas helgolandica* var. *tsingtaoensis* (10^6 cells mL^{-1}) in control	122.88(2.16)
K_{02}	Carrying capacity of *Isochrysis galbana* (10^6 cells mL^{-1}) in control	1021.73 (40.62)
α	Interspecific competition coefficient of *Platymonas helgolandica* var. *tsingtaoensis*	0.028(0.014)
β	Interspecific competition coefficient of *Isochrysis galbana*	4.5 (0.3)
r_{03}	Mortality rate of *Brachionus plicatilis* (d^{-1}) in control	0.25 (0.05)
Fa_{01}	Filtering rate coefficient for *Platymonas helgolandica* var. *tsingtaoensis* (mL^2 $cell^{-1}$ ind^{-1} d^{-1}) in control	0.016(0.003)
F_{a02}	Filtering rate coefficient for *Isochrysis galbana* (mL^2 $cell^{-1}$ ind^{-1} d^{-1}) in control	0.002 (0.0003)
W_1	Dry weight of *Platymonas helgolandica* var. *tsingtaoensis* (pg $cell^{-1}$)	47.9 (3.4)
W_2	Dry weight of *Isochrysis galbana* (pg $cell^{-1}$)	20.4 (1.5)
W_3	Dry weight of *Brachionus plicatilis* (μg ind^{-1})	0.28 (0.03)
h_1	Transfer efficiency from *Platymonas helgolandica* var. *tsingtaoensis* biomass to *Brachionus plicatilis* biomass (%)	20 (5)
h_2	Transfer efficiency from *Isochrysis galbana* biomass to *Brachionus plicatilis* biomass (%)	20 (5)

from the algal bi-species competition experiment (Volterra, 1926). The filtration rate is proportional to the phytoplankton density when the phytoplankton density is relatively low (Wei, 2009). Therefore, the filtration rate (F) is the product of the filtration rate coefficient (Fa) and phytoplankton density. The *B. plicatilis* mortality rate (r_3) and filtration rate coefficients (Fa_1 and Fa_2) were calculated using the method introduced by Feng (2006) and Wei (2009) and the experimental data obtained from the *B. plicatilis* toxic effect experiment. The data relating to the dry weights (W_1, W_2, and W_3) of the phytoplankton and zooplankton and the transfer efficiencies (h_1 and h_2) from the former to the latter were obtained from the literature (Chen et al., 2007; Feng, 2006; Li et al., 2011) and calibrated within the range of collected data to gain a satisfactory fit.

To account for the variability in the test data, the sensitivities of parameters in the model were calculated using a Monte Carlo setting and characterized with coefficients of variation (CV) of plankton biomass at equilibrium points. The parameters varied within a range of 50% of their averages. While the sensitivity was calculated for a parameter, the others were kept constant. Only the sensitivity parameters were selected to describe the toxic effects with the modified Weibull function recommended by Wang et al. (2011). The modified Weibull functions, representing decreases in the values of sensitivity parameters with concentrations of petroleum hydrocarbons, were used as a toxic effect submodel in the simplified plankton ecosystem model:

$$y = y_0 e^{\left(-a\left(x^b - x_0^b\right)\right)} \tag{7}$$

When characterizing the increase in mortality rate with increased concentrations of petroleum hydrocarbons, Eq. (7) should be replaced with Eq. (8):

$$1 - y = (1 - y_0) e^{\left(-a\left(x^b - x_0^b\right)\right)} \tag{8}$$

where y and y_0 are the values of the parameter at a certain concentration of petroleum hydrocarbons and the control in the simplified plankton ecosystem model, respectively. a and b are the parameters of the Weibull model, x is the concentration of petroleum hydrocarbons (mg L^{-1}) and x_0 is the no-effect concentration at which y does not show a significant difference from y_0. The no-detected toxic effect concentration (NDEC) with a 95% confidence interval, proposed by Wang et al. (2011), was calculated by averaging > 200 values of x_0 estimated with a nonlinear regression in a bootstrap procedure.

2.7. Method for the calculation of threshold concentrations of petroleum hydrocarbons

The parameters changed with the concentrations of petroleum hydrocarbons, which were expressed by the toxic effect sub-model, with these changes in parameters consequentially causing a variation in the outputs from the plankton ecosystem model. The Monte Carlo method was used to account for the variability of the obtained parameters. First, 1000 of the parameter data sets used in the simplified plankton ecosystem model were obtained with Latin hypercube sampling. Typically, it was found that the standard deviations of all parameters stabilized after 100 samplings. Next, five data sets were randomly selected for replacement from the aforementioned 1000 parameter data sets. The concentration value (c_1) was calculated by averaging 0 (no-effect concentration) and c_0 (a certain toxic effect concentration). Then, five simulations were conducted using the five randomly selected data sets at c_1. The results of every population biomass were compared to the corresponding reference experimental data (five control replications), and their significance values were determined using a two-sample *t*-test. c_2 was calculated by averaging c_1 and c_0 when c_1 was determined to be a no-effect concentration, or by averaging 0 and c_1 when c_1 was determined to be an effect concentration. Subsequently, five simulations were conducted using the five randomly selected data

Table 2
Sensitivity for parameters characterized with coefficients of variation of plankton biomasses in equilibrium points.

Parameter	Average	Range	CV			
			Platymonas helgolandica var. *tsingtaoensis*	*Isochrysis galbana*	*Brachionus plicatilis*	equilibrium point
F_{a1}	0.016	0.008–0.024	0.88	0.06	0.06	0.09
F_{a2}	0.002	0.001–0.003	0.27	0.46	0.37	0.45
h_1	0.2	0.1–0.3	0.03	0.03	0.03	0.03
h_2	0.2	0.1–0.3	0.33	0.33	0.25	0.32
K_1	12,288	6144–18,432	0.0002	0.00003	0.00003	0.00004
α	0.028	0.014–0.042	0.00002	0.000004	0.000004	0.000004
K_2	10,273	51,086.5–153,259.5	0.0003	0.00005	0.0003	0.0001
β	4.5	2.25–6.75	0.00003	0.000004	0.000004	0.000004
r_1	0.37	0.185–0.555	0.47	0.07	0.07	0.08
r_2	0.52	0.26–0.78	0.70	0.15	0.45	0.20
r_3	0.25	0.125–0.375	0.30	0.30	0.41	0.31
W_1	0.48	0.24–0.72	0.03	0.03	0.03	0.03
W_2	0.204	0.102–0.306	0.33	0.33	0.25	0.32
W_3	0.28	0.14–0.42	0.29	0.29	0.41	0.31

sets at c_2 again. The significance values between the simulations and reference experimental data were again determined, and c_3 was calculated. These steps were repeated until c_m was determined to be a no-effect concentration and the difference between c_{m-1} and c_m was small enough to satisfy the required precision (0.001 was used in present study).

The confidence intervals of c_m were estimated using a bootstrap technique. Applying the same methodology as described in the previous paragraph, another five data sets were randomly selected for replacement from 1000 parameter data sets, yielding c_m. These procedures were conducted n times, yielding $n \times c_m$ values. The average value of c_m was calculated as the threshold concentration of the simplified plankton ecosystem (TCPE); its confidence interval was estimated using the resulting frequency distributions of the pseudo-values (the 2.5th and 97.5th percentile values).

3. Results and discussion

3.1. The toxic effects of petroleum hydrocarbons on plankton and sensitivity analysis for parameters

The experimental results showed that densities of *P. helgolandica* var. *tsingtaoensis* and *I. galbana* decreased in the whole with increasing concentrations of petroleum hydrocarbons in the single alga test, as can be seen in Figs. 1 and 2, while *I. galbana* showed a greater decrease in density than *P. helgolandica* var. *tsingtaoensis* in the bi-species algal experiment (Fig. 3), indicating a competitive disadvantage. The petroleum hydrocarbon-related exposures also caused a clear reduction in the life span of *B. plicatilis*, number of eggs laid, intrinsic growth rate, and filtration rate coefficient when concentrations were above 1.2 mg L^{-1} (Figs. 4–6). From the data measured in this study, the parameters used in the simplified plankton ecosystem were calculated and are listed in Table 1.

The results of the sensitivity analysis for parameters indicated that the population biomass were sensitive to the *P. helgolandica* var. *tsingtaoensis* filtration rate (Fa_1) and the *I. galbana* growth rate (r_2), which had CV values that were higher than 0.7 (Table 2). Following was the *P. helgolandica* var. *tsingtaoensis* growth rate (r_1), with a CV value of approximately 0.5. However, the filtration rate coefficient of *I. galbana* (Fa_2), transfer efficiency from *I. galbana* biomass to *B. plicatilis* biomass (h_2), mortality rate of *B. plicatilis* (r_3), dry weight of *I. galbana* (W_2), and dry weight of *B. plicatilis* (W_3) were less influential, with CV values of approximately 0.3. The carrying capacity of *P. helgolandica* var. *tsingtaoensis* (K_1), carrying capacity of *I. galbana* (K_2), interspecific competition coefficient of *P. helgolandica* var. *tsingtaoensis* (α), interspecific competition coefficient of *I. galbana* (β), transfer efficiency from *P. helgolandica* var. *tsingtaoensis* biomass to *B. plicatilis* biomass (h_1), and dry weight of *P. helgolandica* var. *tsingtaoensis* (W_1) were the least sensitive parameters, with CV values < 0.05 (Table 2). These results indicate that the different parameters affected the excursion of the equilibrium point differently. Interestingly, the CV value for h_2 was higher than that of h_1, suggesting that the contributions of the two algal species to the zooplankton biomasses were significantly different. As a parameter denoting algal maximum population density in the logistic growth model, carrying capacity was an insensitive parameter in the simplified plankton ecosystem, but has been proven to be more sensitive and reliable than the routine ecotoxicological endpoints in the single algal toxic effect test (Wang et al., 2011). This was owing to the fact that standing stocks of the plankton in the simplified plankton ecosystem were much lower than their carrying capacities, which would also account for the insensitivity of α and β.

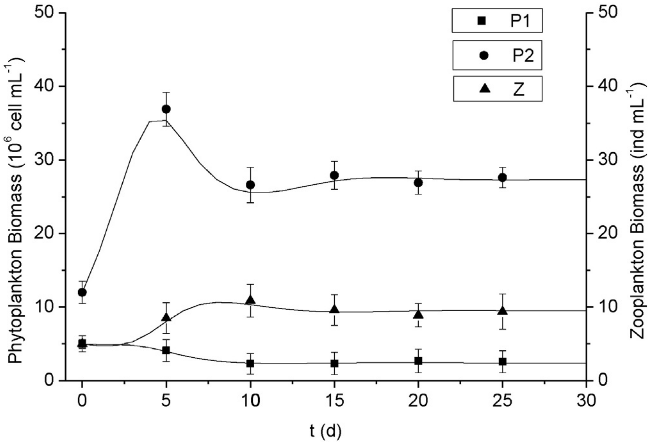

Fig. 7. Biomass changes in the unexposed simplified plankton ecosystem over time (P_1: *Platymonas helgolandica* var. *tsingtaoensis*; P_2: *Isochrysis galbana*; Z: rotifer *Brachionus plicatilis*). Scatter: experimental values; Line: simulated values.

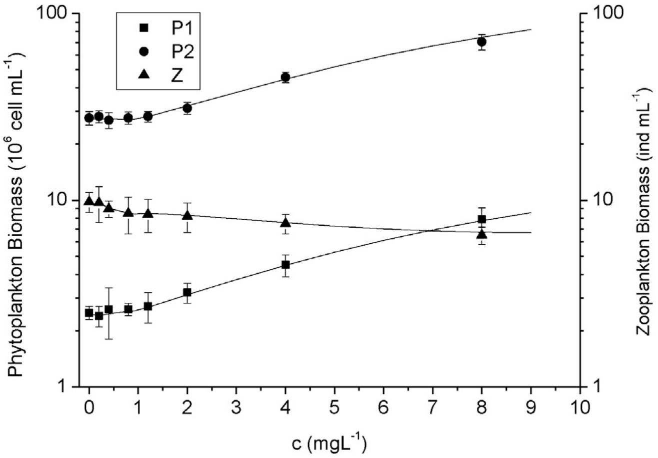

Fig. 8. Changes in the biomasses of the equilibrium points in the simplified plankton ecosystem with concentrations of petroleum hydrocarbon (P_1: *Platymonas helgolandica* var. *tsingtaoensis*; P_2: *Isochrysis galbana*; Z: rotifer *Brachionus plicatilis*). Scatter: experimental values; Line: simulated values.

3.2. The ecotoxicological effects of petroleum hydrocarbons on the simplified plankton ecosystem

As shown in Fig. 7, the densities of the three populations in the unexposed plankton ecosystem fluctuated before day 15 and subsequently became stable at an equilibrium point. When exposed to petroleum hydrocarbons, the densities of algal populations at the equilibrium point showed an a priori unpredictable pattern, which increased with the concentration of petroleum hydrocarbons in the experiment concentration range. Contrary to the algae, densities of *B. plicatilis* at the equilibrium point decreased when concentrations of petroleum hydrocarbons were > 1.2 mg L^{-1} (Fig. 8).

The experimental observations are in agreement with simulation results from the exposed plankton ecosystem dynamics, where toxic effects were expressed by using the selected sensitivity parameters, Fa_1, Fa_2, r_1, r_2, r_3, K_1, and K_2, as endpoints in the toxic effect sub-model

Table 3
Parameters in toxic effect sub-model.

		Parameters of sub-models		
Toxic effects	Endpoints	a	b	x_0 (mg L^{-1})
Growth effect	K_1	0.132 (0.004)	0.325 (0.007)	0.056 (0.002)
	r_1	0.869 (0.079)	0.065 (0.008)	0.233 (0.031)
	K_2	2.70 (0.30)	0.043 (0.003)	0.331 (0.005)
	r_2	3.51 (0.81)	0.029 (0.06)	0.260 (0.053)
Mortality effect	r_3	0.016 (0.005)	1.98 (0.5)	1.18 (0.22)
Sublethal toxic effect	F_{a1}, F_{a2}	0.412 (0.06)	0.592(0.03)	0.904 (0.2)

Table 4
The toxic effects of the petroleum hydrocarbon for various endpoints (mg L^{-1}).

		NDEC			TCPE		
Algal Species	Endpoints	Mean	CI_{95}	R^2	Mean	CI_{95}	R^2
Platymonas helgolandica var. *tsingtaoensis*	K_1	0.056	0.054–0.058	0.99			
	r_1	0.233	0.208–0.258	0.99			
Isochrysis galbana	K_2	0.331	0.327–0.335	0.97			
	r_2	0.260	0.218–0.302	0.99			
Brachionus plicatilis	r_3	1.180	1.004–1.356	0.98			
	F_a	0.904	0.744–1.064	0.99			
Ecosysterm	Equilibrium point				0.376	0.323–0.415	0.9

(Fig. 8). The parameters of the toxic effect sub-model for the customized ecosystem models are summarized in Table 3. According to model calculations, the densities of *B. plicatilis* decreased when exposed to petroleum hydrocarbon concentrations >1.18 mg L^{-1}, and the increase of mortality rate (r_3) with petroleum hydrocarbon concentrations was found to be the main contributor to these observed patterns. As such, the calculated densities of *P. helgolandica* var. *tsingtaoensis* and *I. galbana* increased when exposed to petroleum hydrocarbon concentrations >0.9 mg L^{-1}, at which point Fa_1 and Fa_2 began to decrease, and the decrease in the filtration rate was inferred to contribute to the increased densities of *P. helgolandica* var. *tsingtaoensis* and *I. galbana*. Although r_1 and r_2 decreased when petroleum hydrocarbon concentrations were >0.26 mg L^{-1} (Table 3), the densities of *P. helgolandica* var. *tsingtaoensis* and *I. galbana* remained constant until the petroleum hydrocarbon concentrations were >0.9 mg L^{-1}. For example, at 4 mg L^{-1} petroleum hydrocarbon levels, the modified Weibull function indicated a 14.9% decrease in r_1, a 24.7% decrease in r_2, a 42.2% decrease in the filtration rate coefficient, and a 68.8% increase in K_3. However, at the same petroleum hydrocarbon concentration, the simplified plankton ecosystem model predicted an 84.4% increase in densities of *P. helgolandica* var. *tsingtaoensis*, a 63.0% increase in densities of *I. galbana*, and a 20.2% decrease in rotifer densities, with corresponding changes observed in the experimental data. Therefore, the grazing rate increased by >50%. As such, the reduced filtration rate and rotifer grazing pressure might explain the increased densities of *P. helgolandica* var. *tsingtaoensis* and *I. galbana*. Similarly, the decrease in zooplankton biomass might result from the increased zooplankton grazing rate and decreased survival rate. A reduction in the toxic effects of pyrene on *Daphnia magna* (a small, planktonic crustacean) when combined with predation and competition from rotifers has been observed previously (Viaene et al., 2015), which was attributed to differences in the population structure of *D. magna* and inhibition in the feeding rate of predators. The combination of rotifer competition and exposure to the fungicide carbendazim led to increasing abundances of *D. magna* at all life stages in comparison with the competition controls and was related to the superior grazing capacity of *D. magna* versus rotifers and the uptake of rotifers by *D. magna* in food limiting conditions (Arco et al., 2015). These results indicate that, although the plankton densities in the single-species toxicity test decreased as the toxicant concentrations increased, the densities would not necessarily decrease within a food web owing to their dependencies on ecological interactions.

3.3. Threshold concentration of petroleum hydrocarbons

During the Monte Carlo simulation, the plankton ecosystem model was executed to calculate a TCPE of 0.376 mg L^{-1} for petroleum hydrocarbons with a 95% confidence interval of 0.323–0.415 mg L^{-1}. However, the single-species toxicity model predicted minimal NDEC values of 0.056 mg L^{-1}, 0.260 mg L^{-1}, and 0.904 mg L^{-1} for *P. helgolandica* var. *tsingtaoensis*, *I. galbana*, and *B. plicatilis*, respectively (Table 4). Thus, the TCPE results differed significantly from the NDEC values derived from the single-species toxicity model. Although the NDEC value obtained with endpoint K_1 in the single-species toxicity test was the smallest NDEC value, it was not the threshold concentration for the simplified plankton ecosystem. Owing to the insensitivity of K_1, this did not result in excursion from the equilibrium point in the simplified plankton ecological model. Fa was the most sensitive parameter in the simplified plankton ecological model; however, the NDEC value calculated using this parameter was >0.904 mg L^{-1}. r_1 was a highly sensitive endpoint of the pollutant in the single-species toxicity test, yielding an NDEC value of only 0.233 mg L^{-1}, but was less influential to the excursion about the equilibrium point. These results indicate that the endpoints that were sensitive to the pollutants in the single-species toxicity tests were not necessarily the sensitive parameters in the plankton ecosystem. Thus, owing to the different sensitivities of the parameters, the minimum no-effect concentration determined with the sensitivity endpoints in the single-species toxicity test was not necessarily the threshold concentration causing the excursion from the equilibrium point in the simplified plankton ecological model.

In this study, the NDEC derived from the single-species toxicity test was more protective than TCPE. Using the SSD method, a predicted no-effect concentration (PNEC, significance level 5%) of 0.076 mg L^{-1} was derived from the single-species toxicity test data collected in literature (Table 5) and those calculated in this study (Table 4), which was also lower than TCPE. The results from this experiment indicate that interspecies competition and grazing reduced the toxic effect of petroleum hydrocarbons at the community level, with similar studies on this topic also being reported (Arco et al., 2015; Viaene et al., 2015).

Table 5
The collected NOEC of petroleum hydrocarbon for single species used in Species Sensitivity Distribution.

Species	Endpoints	NOEC (mg L^{-1})	Reference
Moina mongolica	Growth rate	4.7	Lu and He (2000)
Ruditapes philippinarum	Odours	25	Jiang et al. (2006)
Brachydanio rerio	Survival rate	45	Zheng et al. (2014)
Isochrysis galbana	Cell density	0.5	Zhang (2013)
Scrippsiella trochoidea	Uptake rate of nutrients	8.25	Wang et al. (2013)
Skeleton costatum	Cell density	1.96	Zhang et al. (2002)
Pheodactylum tricornutum	Cell density	1.05	Zhang et al. (2002)
Nitzschia closterium f. minutissima	Chlorophyll-a	0.98	Ma (2013)
Nitzschia longissima	Chlorophyll-a	1.21	Huang et al. (2011)
Hemiceutrotus Pulcherrimus	Fertilization rate of eggs	0.5	Lv (2009)

Contrary to observations in the present study, species interactions have been found to result in greater toxic effects of pollutants (Foit et al., 2012; Gergs et al., 2013). These findings highlight the complexity of the impacts of ecological interactions on the sensitivity of populations together with the toxic effects of the pollutants. Realistic tools with ecological relevance are required to characterize and assess the response of populations to pollutants in a community. The complex ecosystem experiments are more relevant to the ecology of realistic marine ecosystems, but they suffer from poor reproducibility and difficulty in validating the predicated results with experiment data (Carpenter, 1989; Meng et al., 2009).

The community in our experiment was composed of only two species of phytoplankton and one species of zooplankton, which was easily manipulated and reliably reproduced. It is recognized that phytoplankton are the base of the marine food web and zooplankton an important link in the food chain of marine ecosystems and, in combination, they are the main constituents of ocean ecosystems. The interspecies competition and grazing in the plankton also showed the representative ecological relationships occurring in marine organisms (Chang and Ge, 2001; Lalli and Parsons, 1993). The selected species interactions are relevant to entire marine ecosystems. The model proposed in the present study was a three-species coupling model with only a few parameters and, thus, was easily verified. Moreover, a coupling model using only three species possesses a positive asymptotic equilibrium point under certain conditions, which can be used as the endpoint in the calculation of threshold concentrations for pollutants such as petroleum hydrocarbons. This proposed indicator incorporated interspecies interactions and integrated multiple effects into a single measurement to characterize the ecotoxicological effects of the plankton community, which was a response to concern over the impacts of ecological interactions on ecological risk assessments of pollutants. The model proposed in this study provides an improved method for understanding mathematically the key ecological relationships in realistic marine ecosystems and for better predictions of communities' responses to pollutants than can be obtained through extrapolation of single-species effect data.

However, our study is, at most, an advance on the use of SSDs based on single-species tests. The method introduced in this study is only useful for a community where three species can co-exist and inapplicable if there is no equilibrium point in a community, such as when one species dominates and the other/s become extinct. The relevance to the ecology for the performed ecosystem experiment is also limited as only three species were used. The toxic effects of pollutants in complex communities may be less pronounced because of greater ecological interactions and species redundancies. Therefore, indirect effects might have a great impact on ecotoxicological effects in complex communities; it is also possible that the NDEC from the sensitive single species will be over protected for some pollutants. This also demonstrates that the toxic ecological effects at the community-level are determined not only by the sensitive species, but also by the ecological relationships among the species.

4. Conclusions

The performed experiments in this study examined the toxic effects of petroleum hydrocarbons on the densities of three plankton species in a customized ecosystem, and showed that interspecies competition and grazing interacted antagonistically with petroleum hydrocarbons when the toxic effect of petroleum hydrocarbons on algae was pronounced. The three-population ecological model constructed in the present study was conducted to calculate a TCPE of 0.376 mg L^{-1} for petroleum hydrocarbons at the community level, which was higher than the NDEC of 0.056 mg L^{-1} derived from the single-species toxicity test and the PNEC of 0.076 mg L^{-1} calculated from the SSD. This is an example of antagonism between interspecies interactions and pollutants toxicity. Nevertheless, previous reports indicated that species interactions resulted in synergistic effects. These findings highlight the complexity of the impacts of ecological interactions on populations and communities together with the toxic effects of pollutants and the need to consider these toxic effects not only at an individual or population level but also at the community level when assessing the risk of pollutants for communities. A similar simplified experiment in combination with the three-population ecological model proposed in this study could assist with integrating interspecies interactions to assess ecological risk of pollutants and to account for the patterns observed in experiments with ecological interactions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.06.164.

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