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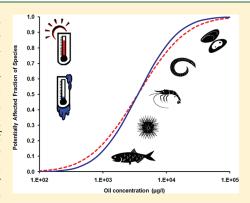


Sensitivity of Polar and Temperate Marine Organisms to Oil Components

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Supporting Information

ABSTRACT: Potential contamination of polar regions due to increasing oil exploitation and transportation poses risks to marine species. Risk assessments for polar marine species or ecosystems are mostly based on toxicity data obtained for temperate species. Yet, it is unclear whether toxicity data of temperate organisms are representative for polar species and ecosystems. The present study compared sensitivities of polar and temperate marine species to crude oil, 2-methylnaphthalene, and naphthalene. Species sensitivity distributions (SSDs) were constructed for polar and temperate species based on acute toxicity data from scientific literature, reports, and databases. Overall, there was a maximum factor of 3 difference in sensitivity to oil and oil components, based on the means of the toxicity data and the hazardous concentrations for 5 and 50% of the species (HC $_5$ and HC $_5$ 0) as derived from the SSDs. Except for chordates and naphthalene, polar and temperate species sensitivities did not differ significantly. The results are



interpreted in the light of physiological characteristics, such as metabolism, lipid fraction, lipid composition, antioxidant levels, and resistance to freezing, that have been suggested to influence the susceptibility of marine species to oil. As a consequence, acute toxicity data obtained for temperate organisms may serve to obtain a first indication of risks in polar regions.

■ INTRODUCTION

The polar regions are subject to contamination supplied by air and water currents emitted in other areas as well as contaminants arising from local activities like tourism, shipping, and infrastructure support for scientific research. 1,2 Local petroleumindustry activities might also introduce contaminants to the polar environment, notably through produced water discharges and accidental spills.³⁻⁵ Effluents, produced daily during oil extraction, discharge alkylphenols, metals, organic acids, and oil components, such as benzene, toluene, xylene, and naphthalene, into the marine environment. 5,6 In addition, oil spills from shipping and drilling activities occur regularly in marine waters.⁷ In the future, oil exploitation and transportation in polar regions is likely to increase due to depleting resources elsewhere and increased exploration opportunities due to melting of sea ice. $^{8-11}$ Eventually, more petroleum-industry activities will potentially contribute to increased contamination of the polar marine ecosystems.^{9,11} Oil may pose a risk to marine species in polar regions due to its persistence in the environment and its tendency to accumulate in biota. 5,12 These risks need to be quantified to support environmental decisions to protect polar ecosystems against impacts of pollution. 13 So far, however, regulatory risk procedures and threshold values specific to the

polar region are lacking. Even more, it is unclear whether such specific values are needed.^{3,14} Polar risk assessments are mostly based on toxicity data obtained for temperate species. Yet, the question rises whether toxicity data of temperate organisms are representative for polar species and ecosystems.^{13,15,16} Adaptations of polar organisms to the harsh conditions of the polar environment have been suggested to influence their sensitivity to contaminants.^{13,14,16} Speculations about differences in sensitivity between polar and temperate marine species are often related to possible differences in physiological characteristics, including their metabolism, lipid fraction and composition, antioxidant defense system, and antifreeze capacity.^{13,14,17} Moreover, characteristics of the polar environment, such as low temperatures and marked seasonality, have been suggested to influence the way contaminants behave.¹⁵

To date, little is known about potential differences in sensitivity to toxicants between polar and temperate species. ¹⁸ Moreover, the few studies available do not allow univocal conclusions.

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toxicant region Annelida Arthropoda Chordata Echinodermata Mollusca total naphthalene 0 11 3 temperate 4 1 19 0 4 0 polar 5 1 10 2-methyl-naphthalene temperate 0 6 3 0 3 12 0 5 3 1 13 polar crude oil-3 types 0 4 2 1 3 10 temperate 0 12 7 0 20 polar 1 crude oil-13 types 4 13 5 1 5 28 temperate 0 13 7 0 2. 22 polar

Table 1. Number of Temperate and Polar Marine Species, Subdivided by Phylum, Used to Derive Species Sensitivity Distributions for Different Toxicants

For example, the Antarctic sea urchin *Sterechinus neumayeri* was less sensitive to zinc but more sensitive to copper and cadmium than eleven urchin species from temperate regions. ¹⁹ Contrastingly, some other polar marine invertebrates, primarily amphipods, were on average equally sensitive to copper and less sensitive to cadmium than numerous temperate marine invertebrates. ²⁰ Five polar marine amphipods were on average equally or less sensitive to zinc and lead than four nonpolar marine amphipods. ^{21,22} Until now, a comparison of polar and temperate marine species regarding their sensitivity to toxicants other than metals is lacking.

The goal of the current study was to compare the sensitivities of polar and temperate marine species to crude oil and individual oil components. To that end, toxicity data available in scientific literature, reports, and databases were collected and species sensitivity distributions (SSDs; cumulative distribution curves) were constructed for each species group (polar and temperate). Possible differences and similarities in sensitivity to oil and oil components between polar and temperate marine species are discussed in relation to their physiological characteristics.

■ METHODS

Data Collection. A literature search provided 14 articles and reports with toxicity experiments on polar marine species ^{14,23-31} and temperate marine species. ³²⁻³⁵ Additional toxicity data on polar and temperate species were obtained from the following databases: RIVM e-toxBase, U.S. EPA ECOTOX, and PAN Pesticide Database. ³⁶⁻³⁸ Marine species were considered polar when meeting one or more of the following criteria: (1) mentioned as such in peer-reviewed scientific literature; (2) included in the Arctic Register of Marine Species; ³⁹ and (3) a minimum of 75% of the distribution records of the species were located within the Arctic or Antarctic marine region. ⁴⁰ The temperate species group included marine organisms from the temperate and subtropical climate zones.

Toxicity data comprised acute LC_{50} , EC_{50} , and TL_m (median tolerance limit) end point values, with mortality or reduced survival effects for 50% of the test organisms. Only toxicity data with short-term test durations (1–8 days) and salt water test conditions were included. To retrieve sufficient toxicity values, data collection included test results from organisms of different developmental stages, both static and flow-through experiments, and both nominal and measured concentrations (respectively 25% and 75% of the 85% available data information). LC_{50} and TL_m values for crude oil were derived from experiments with water-soluble fractions (WSFs) of several oil types. The WSF constituents are dispersed particulate oil, dissolved hydrocarbon,

and soluble contaminants such as metallic ions. 41 WSF is a relatively stable oil-in-water mixture and is, therefore, very useful to assess the toxicity of crude oils for marine organisms. 42

Data Treatment. Toxicity data to derive SSDs were available for crude oil and two oil components: naphthalene and 2-methylnaphthalene (Supporting Information (SI)). Two subsets of the crude oil data were used in the SSD construction. One subset consisted of three types of crude oil with similar compositions, i.e., Alaskan North Slope crude oil, Cook Inlet crude oil, and Prudhoe Bay crude oil. The other subset consisted of these three types of crude oil combined with 10 other types, i.e., Artem Island, Kuwait, Neftyany Kamni, Norman Wells, Sangachaly-More, Shengli, South Louisiana, and Venezuelan Tia Juana crude oil, and Bunker C and No. 2 fuel oil.

Toxicity data were first \log_{10} -transformed. In case of multiple toxicity values for a single substance and a single species, the geometric mean was determined prior to the transformation. For each toxicant and species group (polar and temperate), a sample mean (μ) and standard deviation (σ) were calculated based on the \log_{10} -transformed toxicity data. The parameters μ and σ were used in the integral of the normal distribution (the cumulative distribution function) to derive SSDs, ^{44,45} i.e., the potentially affected fraction (PAF) of species plotted against the environmental concentration of the toxicant. Toxicity is also reported as the hazardous concentration for 5% and 50% of the species (HC₅ and HC₅₀) with a 50% confidence limit. ⁴⁶

Potential differences in sensitivity between the polar and temperate marine species groups were investigated by comparing the means (μ) and variances (σ) of the \log_{10} -transformed toxicity data. If \log_{10} -transformed toxicity values from both species groups were normally distributed according to the Kolmogorov–Smirnov and Shapiro–Wilk tests, the means were compared with the Independent t test. Alternatively, the Mann–Whitney U test was used. The Levene's test was used to compare the variances. All tests were executed with SPSS 15.0 for Windows.

■ RESULTS

Toxicity values were found for 41 polar species and 49 temperate species. The full data set is given in the SI (Tables S1 and S2). A total of 10–28 species were available to derive SSDs for naphthalene, 2-methyl-naphthalene, and crude oil (Table 1). Species were categorized according to five phyla: Annelida, Arthropoda, Chordata, Echinodermata, and Mollusca. The number of values per taxon differed between the temperate and polar species groups and between the toxicants (Table 1). Most data were available for Arthropoda, Chordata, and Mollusca,

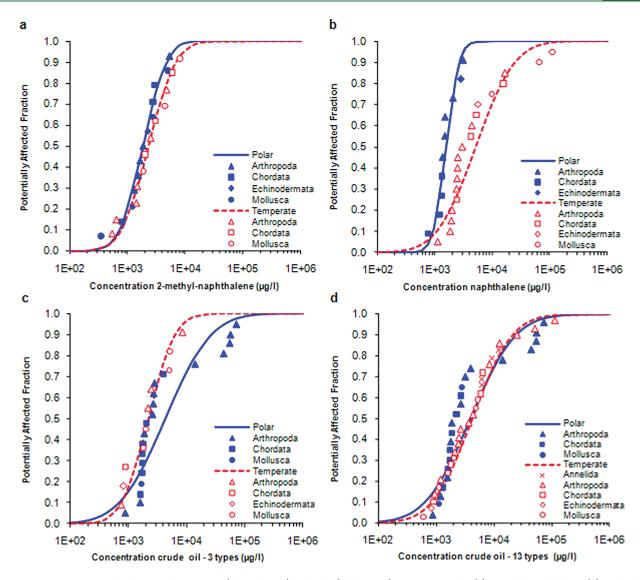


Figure 1. Species sensitivity distributions for temperate (dotted curves) and polar (solid curves) marine species for (a) 2-methyl-naphthalene, (b) naphthalene, (c) 3 types of crude oil, and (d) 13 types of crude oil.

respectively 54%, 32%, and 20% for polar species and 49%, 20%, and 20% for temperate species.

The SSDs of 2-methyl-naphthalene and crude oil showed little difference between polar and temperate species groups (Figure 1). The HC₅ values for 2-methyl-naphthalene and crude oil differed a maximum factor of 1.4 between the two groups, whereas the average toxicities (HC50) were within a factor of 2 (Table 2). Only for naphthalene there was a significant difference in both means (p = 0.002) and variances (p = 0.02) of the SSDs, with a factor of 3 difference between the HC₅₀ values and a factor 1.2 between the HC₅ values of the temperate and polar species groups (Table 2). A comparison of only arthropods, chordates, and echinoderms led to a factor of 2 significant difference (p = 0.006) between the polar and temperate naphthalene HC50 values (Table 2). No significant difference in sensitivity was found between polar and temperate arthropods (Mann—Whitney U test; p = 0.22). The difference between the four polar and four temperate chordates was significant (p = 0.03). The chordates were all fish (Actinopterygii) with exception of the temperate urochordate Ciona intestinalis. Comparing only fish resulted in a p-value of 0.06.

DISCUSSION

Uncertainties Due to Differences in Test Species. Overall, the results indicate that median hazardous concentrations of polar and temperate marine species for oil and oil components differed less than a factor of 3. The significant difference between the mean sensitivities of polar and temperate species to naphthalene could not be explained by differences in taxonomic groups included in the comparison, as a comparison based on the same taxonomic groups (arthropods, chordates, and echinoderms) yielded a significant difference as well. The SSDs for naphthalene suggested that chordates were responsible for the difference between temperate and polar species (Figure 1b).

Uncertainties Due to Test Characteristics. Generally, a higher water temperature in ecotoxicological experiments leads to a lower effect concentration (e.g., LC_{50}), thus to a higher sensitivity of an organism to a toxicant. ^{22,47,48} Although water temperatures were reported in only 60% of the literature sources, we found no distinct indication of a bias in the remaining toxicity data. For instance, test conditions of the most sensitive species in

Table 2. Means (μ) and Standard Deviations (σ) of Polar and Temperate Toxicity Data, Probability on Equality of Means and Variances between Polar and Temperate Marine Species Groups (p-value), HC_{50} $(\mu g/L)$, and HC_{5} $(\mu g/L)$ Values (50% Confidence Limit)

		μ^a	σ^a	$HC_{50} (\mu g/L)$	$HC_5 (\mu g/L)$
naphthalene	$temperate^b$	3.70^{d}	0.53	5.0×10^{3}	0.67×10^{3}
	temperate ^c	3.53	0.37	3.4×10^{3}	0.85×10^{3}
	polar	3.20	0.18	1.6×10^{3}	0.79×10^{3}
	<i>p</i> -value ^b	0.002	0.02		
	p -value c	0.006	0.10		
2-methyl-naphthalene	temperate	3.36	0.37	2.3×10^{3}	0.57×10^{3}
	polar	3.27	0.32	1.9×10^{3}	0.55×10^{3}
	<i>p</i> -value	0.49	0.54		
crude oil-3 types	temperate	3.34	0.36	2.2×10^3	0.55×10^{3}
	polar	3.63 ^d	0.62	4.3×10^{3}	0.40×10^{3}
	p-value	0.47	0.08		
crude oil-13 types	temperate	3.61	0.54	4.1×10^{3}	0.52×10^{3}
	polar	3.58^{d}	0.61	3.8×10^{3}	0.37×10^{3}
	p-value	0.51	0.59		

^a Of the \log_{10} -transformed LC_{50} , EC_{50} , and TL_m values. ^b Including all taxonomic groups. ^c Without temperate molluscs. ^d Toxicity values were not normally distributed according to the Kolmogorov–Smirnov and Shapiro–Wilk tests.

the data set included both high and low water temperatures (see Tables S1 and S2, SI). In addition, the 10 species with multiple toxicity and temperature values for a single substance showed no distinct increase or decrease in effect concentrations with an increasing temperature. For example, LC $_{\rm 50}$ values for naphthalene of the pink salmon $\it Oncorhynchus gorbuscha$ both increased and decreased within a temperature rise of 8 °C. 49

Sensitivity to toxicants may vary with life history stages, of which larvae are generally expected to be the most sensitive to oil. Furthermore, static tests may give higher estimates of species sensitivities to oil than flow-through tests. These uncertainties should be assessed when more data become available. The type of concentration probably generated little uncertainty because means and standard deviations of measured concentrations did not differ significantly from data including nominal concentrations.

Comparison with Other Studies. Crude oil, naphthalene, and 2-methyl-naphthalene are expected to exhibit a nonpolar narcotic toxicity mode of action. $^{18,51-53}$ Nonpolar narcotics penetrate the lipid bilayer region of membranes and thereby alter lipid properties, such as fluidity, thickness, and surface tension, as well as fatty acid composition. Ultimately, the disturbance of the membrane function leads to death of the organism. $^{54-56}$ Based on a generic critical body burden of 0.10 mol/kg wet weight, we derived LC $_{50}$ values for the compounds as described in the Supporting Information (text section 1). All measured HC $_{50}$ values were in the same order of magnitude with the estimated LC $_{50}$ values for a narcotic toxicity mode of action of naphthalene and 2-methyl-naphthalene (5.7 \times 10 3 and 1.4 \times 10 3 $\mu g/L$, respectively).

Except for chordates and naphthalene, our comparison of SSDs revealed equal sensitivity to oil for polar and temperate species. This is consistent with a qualitative comparison by McFarlin et al. which showed that three polar species were equally sensitive to one type of crude oil type as seven temperate species. ⁵⁷ A quantitative comparison of metal toxicity revealed also equal sensitivities to copper and even higher sensitivities of temperate species to cadmium, zinc, and lead than polar

species.²⁰ By comparing SSDs for two metals, three pesticides, and a narcotic chemical, Kwok et al. found a higher sensitivity of tropical freshwater species than temperate freshwater species.⁵⁸ Yet, for other metals the reverse was shown, indicating that there is no consistent latitudinal pattern.⁵⁸

Physiological Characteristics. Differences in sensitivity to oil between polar and temperate marine species may be influenced by differences in physiological characteristics, including metabolism, lipid fraction, lipid composition, antioxidant levels, and resistance to freezing. 13,14,17 Generally, the rate of standard metabolism in aquatic organisms decreases 2- to 3-fold with a 10 $^{\circ}$ C decrease in water temperature. Whereas this decrease is likely to reduce uptake, 13 elimination and growth rates are expected to be decreased as well. In addition, the tendency of polar organisms toward gigantism, which causes a lower surface-area-to-volume ratio, has been associated with a reduced contaminant uptake.¹³ Reduced uptake because of lower temperatures or larger size may explain why polar organisms have been perceived as more tolerant to short-term toxicant exposure than temperate organisms. Yet, sublethal end points such as growth and reproduction may not be indicative of mortality. 13,20,50 In long-term toxicity studies on survival, the reduction in uptake may be compensated by a decrease of elimination, yielding similar sensitivities in both types of species. Unfortunately, chronic toxicity data could be obtained for a few polar species only. Furthermore, polar species generally have longer life spans than species from lower latitudes. ^{13,15,61} So, reduced uptake over short periods due to lower temperatures and larger size might be compensated by a more or less proportional decrease of elimination and an increase of life span. Hence, differences in metabolism provide no a priori reason for expecting polar species to be more or less sensitive than temperate species.

Marine polar organisms generally have higher lipid contents than temperate organisms. ^{13,14,61} This has been thought to result in a higher uptake of lipophilic contaminants by polar species, possibly leading to increased sensitivity. ¹³ Yet, the binding of lipophilic toxicants may leave a smaller amount of oil components to interfere with cell membranes. ⁶¹ The larger amount of storage lipids in the Arctic copepod *Calanus glacialis*, for example, was used to explain

its lower sensitivity to oil components (PAHs; polycyclic aromatic hydrocarbons) in comparison to the copepod *Calanus finmarchicus* from a lower latitude. Following equilibrium fugacity theory, however, one would expect concentrations in fat tissues to be independent of the fraction of lipids. Therefore, a higher lipid content is expected to have little influence on the susceptibility of marine organisms to oil.

Differences in lipid composition have also been expected to cause a changed sensitivity of polar marine species to oil as compared to temperate species. ¹⁷ Lipid composition is thought to influence the distribution of a toxicant in an organism at equilibrium, and thereby the concentration at the target site for nonpolar narcotics, i.e., membranes.¹⁷ Elevated levels of polyunsaturated fatty acids (PUFA) in cell membranes, which appear to be a special adaptation of polar fish and invertebrates to low temperatures, 63-65 may indirectly contribute to a higher sensitivity of polar species to oil. PUFA are primary targets for reactive oxygen species (ROS). Additional production of ROS stimulated by the biotransformation of oil constituents taken up by organisms may lead to an imbalance and thus oxidative damage.⁶⁴ Yet, levels of antioxidant enzymes, such as vitamins A, C, and E, were found to be higher in polar species than temperate species. 63,66 This may lead to a higher tolerance of polar species to the oxidative effects of oil. With few exceptions, 63 this may counteract the negative effects of PUFA in polar cell membranes, possibly leading to equal sensitivities of polar and temperate species.

Finally, the production of antifreeze peptides (AFPs) and antifreeze glycopeptides (AFGPs) is another adaptation to the cold environment noted in several polar species. These proteins prevent fish such as Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) from freezing by interacting with the cell membranes to protect these against cold damage. It is unknown whether and to what extent the effect of antifreeze proteins on membranes may influence the susceptibility of organisms to oil. The low LC50 values noted for *B. saida* for all toxicants (Table S1) can be understood from its specialized excretion process to prevent the loss of antifreeze proteins. Due to the lack of glomeruli in the kidneys, toxicants may be excreted via the bile rather than the urine. Said Excretion via the bile may be disadvantageous, possibly due to a longer retention time of toxicants due to intestinal microflora and reabsorption into the duodenum.

Summarizing, our SSDs showed that the sensitivity of polar and temperate marine species to oil and oil components differed on average less than a factor of 3. In addition, most of the differences were not statistically significant and there was no taxonomic group that was consistently more sensitive than the other groups. Apparently, physiological mechanisms suggested to cause differences between polar and temperate species may have little impact on sensitivity to oil. As a consequence, toxicity data obtained for temperate organisms may serve to obtain a first indication of risks in polar regions. Exceptions due to specific mechanisms can be present, however, as noted for example in *B. saida*. In addition, toxicity data on polar species are limited in terms of quantity and quality. Basic conditions, such as temperature have often not been reported. Chronic toxicity data are largely absent. So, more empirical confirmation is definitely needed.

■ ASSOCIATED CONTENT

Supporting Information. A description of the comparison between measured HC_{50} and calculated LC_{50} concentrations in relation to a narcotic toxicity mode of action in text

section 1; Polar and temperate marine toxicity data in Tables S1 and S2. This information is available free of charge via the Internet at http://pubs.acs.org/.

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