Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 10, No. 6, p. 201-208, 2017
http://www.innspub.net

INNSPUB

RESEARCH PAPER

OPEN ACCESS

The influence of pH on oil dispersant toxicity to the whiteleg shrimp, *Litopenaeus vannamei*

Muhammad Arif Asadi[1*], Ahmad Didin Khoiruddin[1], Anthon Andrimida[2]

[1]*Department of Marine Science, University of Brawijaya, Malang, Indonesia*

[2]*Department of Marine Affairs and Fisheries, Surabaya, Indonesia*

Article published on June 30, 2017

Key words: Oil dispersant, Toxicity, pH, *L. vannamei*, LC_{50}.

Abstract

Oil spillage accidents lead the use of oil dispersants to break oil into small droplets to prevent oil coming ashore. Meanwhile, recent and rapid drop in surface pH could have devastating consequences to marine environment. However, their combined effects on marine species have not been experimentally evaluated. This study evaluated the toxicity of oil dispersant in the shrimp *Litopenaeus vannamei* under pH 6.5 and 8.5. Healthy post larvae *L. vannamei* were exposed to dispersant solution at concentration of 0%, 3%, 6%, 12% and 24% for 72 hours to determine mortality. Probit analysis was used to determine LC_{50}, while the PAH of dispersant solution was characterized using GCMS method. The dispersant had negative effects to *L. vannamei* and that toxicity of dispersant increased over time and exposure concentration. The 72-h LC_{50} were equivalent to 191.18 mgL^{-1} and 553 mgL^{-1} for pH 6.5 and 8.5 respectively in which that dispersant is practically non-toxic to the shrimps. However, the combination of dispersant and lower pH increases the mortality of the shrimp; thus ocean acidification may increase dispersant accumulation in the *L. vannamei* tissue via surface contacting.

***Corresponding Author:** Muhammad Arif Asadi ✉ asadi@ub.ac.id

Introduction

Oil dispersants are a group of chemicals applied to break up oil slick from the sea surface and disperse it into the water column as tiny droplets allowing microbes to consume the oil (Lessard and DeMarco, 2000). The application of oil dispersants is intended to prevent oil coming ashore in order to protect the vulnerable coastal ecosystem. Nonetheless, it does not reduce the amount of oil but enhances the polycyclic aromatic hydrocarbons in the water column (Lyons *et al.*, 2011).

During Deepwater Horizon oil spill on April 20, 2010, in the Gulf of Mexico, British Petroleum (BP) sprayed about 2.1 million gallons of dispersants called Corexit in deep water and at the sea surface in an attempt to control the spill (Daly *et al.*, 2016). The dispersant application has raised new concern regarding its deleterious effects on the marine ecosystem. It is important to note that dispersants are themselves unpleasant chemical concoctions which in some cases are very toxic to organisms.

The dispersants cause synergistic effect on toxicity of spilled crude oil to exposed organism when applied to control oil spills (Otitoloju, 2005). In laboratory test to macrozooplankton such as *Strombidum sp*, *Spirostrombidium sp*, and *Eutintinnus pectinis*, dispersant concentration as low as 0.5 µL L^{-1} led 100% mortality to those species (Almeda *et al.*, 2014). Furthermore, dispersant, Corexit 12510, is slightly toxic to small crustacean, Artemia and Mysid, which had LC_{50} value 52 ppm and 65 ppm respectively (George-Ares*et al.*, 1999; Wells *et al.*, 1982).

In the toxicity studies on Atlantic herring (*Clupea harengus*) embryos, dispersant alone was toxic but chemically dispersed oil did not cause synergistic toxicity to the embryos *(Adams et al.*, 2014). Thus, marine organisms behave differently in responses to dispersant and dispersant oil exposure, which are depend on physiology and behavior of the individual organisms, and also depend on differences in oil and dispersant composition (Garr *et al.*, 2014; Hook and Osborn, 2012; Jiang *et al.*, 2010).

Therefore, there is a need for more research into the effects of dispersant on marine organism to better evaluate the impact of using chemical dispersant on marine ecosystem. In this study, the toxicity of dispersant on Whiteleg shrimp (*L. vannamei*) is evaluated. The shrimps are commonly caught in the eastern Pacific Ocean and farmed in many countries. The shrimps are also potentially spreading throughout South-East Asia estuaries and seas which may release from aquaculture ponds. In Thailand, the shrimps are commonly caught in the wild particularly within the coastal lagoon systems where the shrimps are commonly farmed (Senanan *et al.*, 2007).

As decapod crustacean, *L. vannamei* is suitable to be utilized in ecotoxicological testing not only because of its commercially important but also its sensitiveness to chemical and ecologically important (Rand, 1995). *L. vannamei* that forms its shells out of calcium carbonate is also practically suitable for studies of climate change effects on marine organisms. The increasing atmospheric carbon dioxide decreases ocean pH and the level of calcium carbonate saturation. It decreases the ability of the shrimps to maintain their external calcium carbonate skeleton which in turn affects the survival ability of the shrimps (Orr *et al.*, 2005). Thus, in this current study, we would like to evaluate the effect of pH on toxicity of dispersant on *L. vannamei.*

Materials and methods

L. vannamei and its laboratory acclimation

Healthy early post larvae (PL) (6-7 days old) *L. vannamei* shrimps were obtained from a commercial farm in Paciran, Lamongan, Indonesia. Prior to the experiment, the shrimps were acclimated in 150 cm x 60 cm aerated tank with natural seawater adjusted to salinity 20 ‰, pH 7.9, and temperature 26 ± 0.5°C until reached late PL stage (14-15 days old). On toxicological testing of decapod crustacean, adult, juvenile, and PL life stages have been utilized the most as they are less difficult to handle than larval and

megalops stages (Rand, 1995). During the acclimation period, a quarter of the water was replaced daily and the shrimps were fed with both brine shrimp and formulated feed. For the acute toxicity test, the average weight of the shrimps were between 0.06 and 0.1 gram.

Test chemicals and PAH characterization

IPAC-OCD was used as chemical dispersant produced and provided by CV. Pratama Abadi Chemical which is commonly distributed throughout Indonesia. In order to adjust the pH, HCL and NaOH are used to decrease and increase pH respectively.

The dispersant solution was prepared by mixing 400 ml of sterilized natural seawater with 2 ml dispersant liquid. The PAH of dispersant solution was characterized using Supelco Standard QTM PAH Mix 47930 method with Thermo GCMS Trace 1310 ISQ LT instrument and Single Quadrupole Mass Spectrometer (320 ^{o}C) detector at Pusat Penelitian Oceanografi – LIPI.

Preliminary testing

Preliminary testing was performed to assure adequate control survival (≥80%), and to find range exposure concentration before conducting the acute toxicity tests. In triplicate, twelve individuals of 14 days old PL *L. vannamei* were exposed to 0%, 5%, 10%, 20%, 40%, and 80% dispersant solution in 600 mL erlenmeyer flasks with 500 mL of test solution. The erlenmeyers were aerated and kept at room temperature, 16 h light:8 h dark photoperiod. Every 24 h, water quality (temperature, salinity and pH) was measured, mortality was assessed, and the test solutions were renewed. Post larvae mortality was determined at the end of the 72 h exposure.

Acute toxicity test

Two pH were selected for testing; 6.5 and 8.5. The pH range alone should not affect the mortality of the shrimps which could interfere the toxicity test results, and *L. vannamei* has 100 % survival rate under the pH between 5 and 9 (Furtado *et al.*, 2015). The seawater used in the experiments was filtrated at 0.2 µm (fibre filter; Millipore, Billerica, MA, USA). The salinity was kept about 20±1psu which is still within the optimum salinity range for *L. vannamei* (Gao *et al.*, 2016). Standard static 48 h and 72 h acute toxicity tests using *L. vannamei* post larvae were conducted following the methods described by Lee *et al.*, (2013). Briefly, 12shrimps were transferred to each erlenmeyer flask (500ml flask).

The flasks were aerated and the shrimps were not fed during the test periods. The dispersants, which were taken from dispersant solution stocks, were at concentration of 0%, 3%, 6%, 12% and 24%. The experiments were conducted in triplicate which in total 30 erlemenyer flasks were set up. Mortalities of *L. vannamei* following 48 h and 72 h exposures were examined at 6 hour, 12 hour, 24 hour, 36 hour, 48 hour and 72 hour. LC_{50} values were computed using Finney probit analysis.

Data analysis

Microsoft Excel was used for mortality data calculations which are presented as mean ± standard error (SE). Median lethal concentrations (72 h LC_{50} values) with 95% confidence interval (CI) were determined using probit analysis with Minitab 17.1.0 (Minitab® Inc.). Data on the toxicity of dispersant concentration and pH were analyzed using two-way ANOVA with the number of individual mortality as dependent variable. It is followed by Tukey HSD test to compare the mortality of each dispersant concentration.

Result and discussion

PAH Characterization

The dispersant solution stock, which was made by diluting 5 ml dispersant with 1 L seawater, contained 14.33 mgL^{-1} PAHs. Anthracene was the most dominant PAHs in the stock (9.5 mgL^{-1}), followed by Fluorene and chrysene (2.06 and 1.25 mgL^{-1} respectively). In total, 8 PAHs were extracted and characterized from dispersant the solution stock (Table 1).

Table 1. PAHs concentration and characterization from the dispersant solution stock.

PAHs	Concentration (mgL^{-1})
Acenaphthylene	0.1425
Acenaphthene	0.8008
Fluorene	2.0612
Anthracene	9.5166
Phenanthrene	0.2321
Fluoranthene	0.2732
Pyrene	0.0582
Chrysene	1.2516
Total PAHs	14.3362

LC_{50} of dispersant in L. vannamei

The dispersant toxicity to *L. vannamei* increased over time and exposure concentration which the lower pH (6.5) had higher number of mortality than that of higher pH (8.5) ($p<0.01$). On pH 6.5, dispersant concentrations of 6% and 12% yielded 66.67% and 94.4% mortality respectively. Meanwhile, on pH 8.5, dispersant at the same concentrations yielded 41.67% and 55.56% mortality respectively (Fig.1 and 2).

The LC_{50} value of dispersant on pH 6.5 decreased over time in which the24-h to 48-h LC_{50} had dropped radically from 11.98 ± 1.23% to 4.63 ± 0.65%. Meanwhile, the 48-h to72-h LC_{50} only decreased slightly that 72-h LC_{50} was 3.7536 ± 0.61 %. There was a significant difference between LC_{50} values at pH 6.5 and 8.5 on all time exposure ($p<0.001$) that lower pH had higher mortality rate and lower LC_{50} value. The dispersant exposure to *L. vannamei* on pH 8.5 resulted much lower 72-h LC_{50} (10.85 ± 1.99 %) than that of on pH 6.5 (3.75 ± 0.61 %). However, on pH 8.5, the LC_{50} were gradually decreasing over time from 18.87 ± 2.92 (24-h exposure) to 10.8559 ± 1.99 % (72-h exposure) (Table 2 and 3).

Table 2. LC_{50} value of dispersant under pH 6.5 for *L. vannamei.*

Replicate	LC_{50} (95% CI, %)		
	24 h	48 h	72 h
1	10.6891 (6.04124-16.8641)	5.30948 (1.46287-8.62097)	4.19892 (2.46189-6.19629)
2	12.1252 (5.94859-23.7008)	4.01969 (0.820212-6.52852	3.05323 (0.335442-5.03204)
3	13.1368 (8.82853-20.2473)	4.56118 (-0.893413-8.33701)	4.00891 (-1.91693-7.74993)
Mean ± S.D.	11.9837 ± 1.23	4.6301 ± 0.65	3.7536 ± 0.61

Table 3. LC_{50} value of dispersant under pH 8.5 for *L. vannamei.*

Replicate	LC_{50} (95% CI, %)		
	24 h	48 h	72 h
1	21.6852 (15.7545-39.5217)	13.8001 (8.71172-24.2722)	11.3575 (6.45989-19.0828)
2	19.0797 (13.2333-37.1883)	12.5550 (5.20050-31.8822)	12.5550 (5.20050-31.8822)
3	15.8614 (11.4832-24.1515)	9.86435 (5.21744-15.8177)	8.65543 (3.38041-14.4005)
Mean ± S.D.	18.8754 ± 2.92	12.0732 ± 2.01	10.8559 ± 1.99

Discussion

Oil dispersants are mainly used to remedy oil spill incidents in the marine environment. However, the dispersant toxicity test to *L. Vannamei* showed that dispersant pollution reduced the shrimps' activity and ability to keep balance. Thus, the shrimps became unconscious and even died which were obvious at the 6% concentration onward. The PAHs of dispersant used in the experiment may accumulate in the shrimps' tissue via surface contacting (Jiang *et al.*, 2010), and disrupt the respiratory cell resulting osmotic imbalance (Singer *et al.*, 2001). It might due to the fact that high lipid contents of *L. vannamei* (Chen *et al.*, 2014) could easily bounded by the organic compounds of dispersant in the form of PAHs (Carls*et al.*, 2016).

Table 4. LC_{50} dispersant (mgL^{-1}) and LC_{50} PAH of dispersant (mgL^{-1}).

pH	LC_{50} dispersant (mgL^{-1})			LC_{50} PAH of dispersant (mgL^{-1})		
	24 h	48 h	72 h	24 h	48 h	72 h
6.5	608 ± 61	235 ± 32	191 ± 32	1.72 ± 0.17	0.66 ± 0.09	0.54 ± 0.08
8.5	961 ± 148	613 ± 102	553 ± 101	2.69 ± 0.41	1.72 ± 0.28	1.55 ± 0.28

The dispersant solution stocks used in our experiment contained 14.33 mgL^{-1} PAHs which was mainly in the form of anthracene (9.5 mgL^{-1}). In seawater, anthracene exists in much lower concentration which are in general in the pgL^{-1} range. In Atlantic Ocean, for example, anthracene at concentrations of 67 pgL^{-1} were found in the Atlantic Ocean (Nizzetto *et al.*, 2008). Furthermore, anthracene concentration of 3.32 mgL^{-1} could inhibit 50% of the growth of Green algae *Tetraselmis chuii* (Vieira and Guilhermino, 2012).

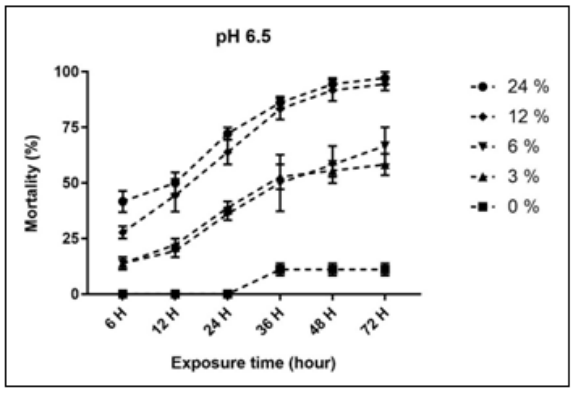

Fig. 1. Mortality rate (%) of *L. vannamei* over time and exposure concentration under pH 6.5.

In our experiment, the anthracene LC_{50} might be 0.3 and 1.03 mgL^{-1} for pH 6.5 and 8.5 respectively which were much lower than that of to *T. chuii* (Vieira and Guilhermino, 2012). However, the dispersant was not contained anthracene alone; there were other PAHs in the solution which might be more toxic and cause the synergistic toxicity effects to the anthracene and other PAHs.

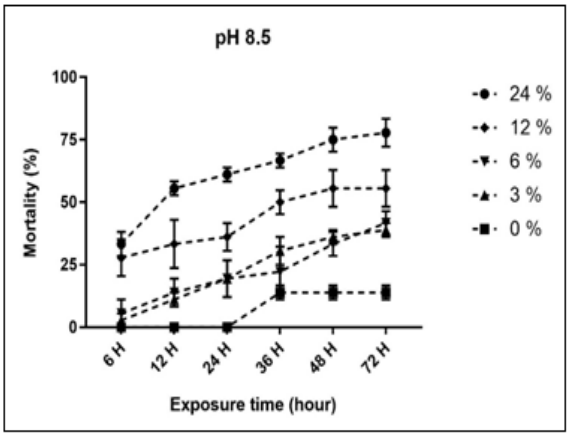

Fig. 2. Mortality rate (%) of *L. vannamei* over time and exposure concentration under pH 8.5.

Based on probit and Minitab analysis, the LC_{50} of dispersants on pH 6.5 and 8.5 were 3.75 ± 0.61% and 10.85 ± 1.9% respectively which were equivalent to 191.18 mgL^{-1} and 553 mgL^{-1}. Thus, the dispersant used in the experiments, the IPAC-OCD chemical dispersant produced by CV. Pratama Abadi, is still practically non-toxic to *L. vannamei.*

It is based on the US EPA's LC_{50} aquatic toxicity scale which categorized toxicant >100 mgL^{-1} as practically non-toxic (National Research Council (NRC), 2002). Furthermore, species have different tolerant range to

toxicant. In toxicity of COREXIT9500 to copepod, for example, the 48-h LC_{50} and 96-h LC_{50} of calanoid copepod *Acartia tonsa* and adult *Eurytemora affinis* were 34 and 5.2 mgL^{-1} respectively (George-Ares *et al.*, 1999). In the other hand, harpacticoid copepod, *Tigriopus japonicus*, showed much higher LC_{50} level, 518 and 349 mgL^{-1} for 48 h and 96 h toxicity test (Lee *et al.*, 2013).

Even though *L. vannamei* has 100 % survival rate under the pH between 5 and 9 (S. Furtado *et al.*, 2015), the shrimp was more vulnerable and impacted by dispersant with lower pH.

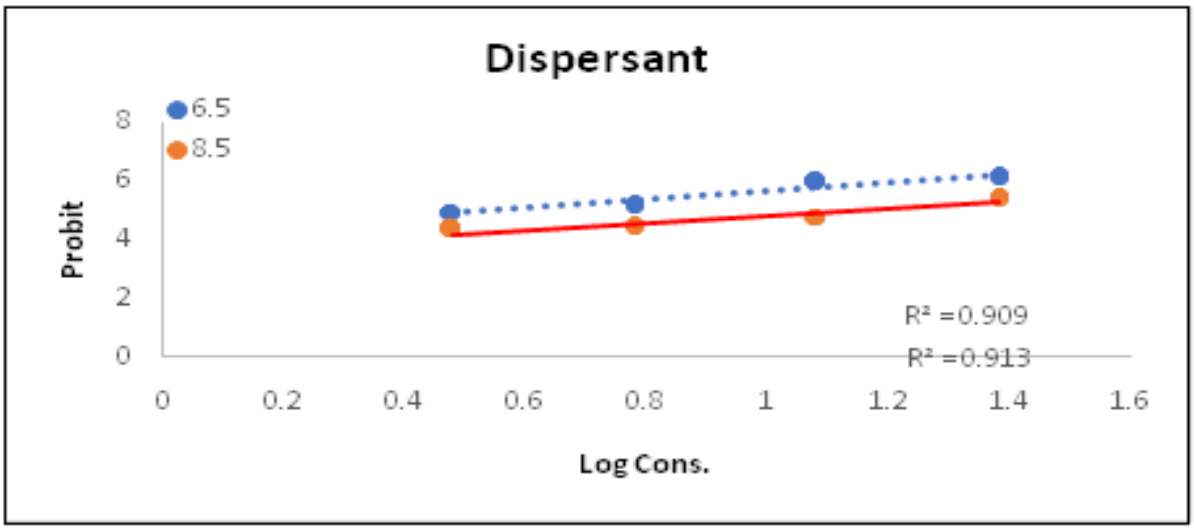

Fig. 3. *L. vannamei* mortality probit curves as function of log concentration of dispersant at both pH (6.5 and 8.5). The R^2 obtained for each pH.

The increasing toxicity of chemical compound on lower pH have also been reported for *Mytilus coruscus* where the combination of nano-TiO_2 and pH reduction increases physiological toxicity of the species (Hu *et al.*, 2017). CO_2-induced pH reduction changes the extracellular acid–base balance of many marine calcifying organisms that can alter the normal metabolic process, impacting relevant biological processes, such as metabolism, growth, fitness, and calcification (Melzner *et al.*, 2009).

Furthermore, as a crustacean, *L. vannamei* may dispose of toxic dispersant by dumping their old exoskeletons (Bergey and Weis, 2007); therefore, calcification rate reduction due to lower pH level could adversely affect *L. vannamei* to dispose the dispersant out its tissue. The lower pH also increases dispersant accumulation in *L. vannamei* via surface contacting, leading higher mortality rate of the shrimps (*Jiang et al.*, 2010).

Conclusion

The results of this study indicate that the dispersant has adversely affect to *L. vannamei* and that toxicity of dispersant increases over time and exposure concentration. The toxicity of dispersant is higher on pH 6.5 than that of on pH 8.5. The combination of low pH level and dispersant leads more mortality rate to the decapod crustacean. However, the IPAC-OCD dispersant is still practically non-toxic to the shrimp as it has LC_{50} higher than 100 mgL^{-1}.

Acknowledgments

The authors would like to acknowledge the Rector of UB and the Dean of Faculty of Fisheries and Marine Science, UB as the article publication was financially supported by the University. Oil dispersant (IPAC-OCD) was kindly provided by CV. Pratama Abadi Chemicals. Assistance from students of Brawijaya University, Risris Kemalasari, Ade Rizki Pratama, were greatly appreciated.

References

Adams J, Sweezey M, Hodson PV. 2014. Oil and oil dispersant do not cause synergistic toxicity to fish embryos. Environmental Toxicology and Chemistry **33,** 107–114.
http://dx.doi.org/10.1002/etc.2397

Almeda R, Hyatt C, Buskey EJ. 2014. Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. Ecotoxicology and Environmental Safety **106,** 76–85.
https://doi.org/10.1016/j.ecoenv.2014.04.028

Bergey LL, Weis JS. 2007. Molting as a mechanism of depuration of metals in the fiddler crab, *Uca pugnax*. Marine Environmental Research **64,** 556–562.
https://doi.org/10.1016/j.marenvres.2007.04.009

Carls MG, Holland L, Pihl E, Zaleski MA, Moran J, Rice SD. 2016. Polynuclear Aromatic Hydrocarbons in Port Valdez Shrimp and Sediment. Archives of Environmental Contamination and Toxicology **71,** 48–59.
https://doi.org/10.1007/s00244-016-0279-3

Chen K, Li E, Gan L, Wang X, Xu C, Lin H, Qin JG, Chen L. 2014. Growth and Lipid Metabolism of the Pacific White Shrimp *Litopenaeus vannamei* at Different Salinities. Journal of Shellfish Research **33,** 825–832.
https://doi.org/10.2983/035.033.0317

Daly KL, Passow U, Chanton J, Hollander D. 2016. Assessing the impacts of oil-associated marine snow formation and sedimentation during and after the Deepwater Horizon oil spill. Anthropocene **13**, 18–33.
https://doi.org/10.1016/j.ancene.2016.01.006

Furtado PS, Fugimura MMS, Monserrat JM, Souza DM, Garcia LDO, Wasielesky W. 2015. Acute effects of extreme pH and its influences on the survival and biochemical biomarkers of juvenile White Shrimp, *Litopenaeus vannamei*. Marine and Freshwater Behaviour and Physiology **48,** 417–429.
https://doi.org/10.1080/10236244.2015.1086539

GaoW, Tian L, Huang T, Yao M, Hu W, Xu Q. 2016. Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. Aquaculture Reports **4,** 125–129.
https://doi.org/10.1016/j.aqrep.2016.09.001

Garr A, Laramore S, Krebs W. 2014. Toxic Effects of Oil and Dispersant on Marine Microalgae. Bull. Bulletin of Environmental Contamination and Toxicology **93**, 654–659.
https://doi.org/10.1007/s00128-014-1395-2

George-Ares A, Clark JR, Biddinger GR, Hinman ML. 1999. Comparison of Test Methods and Early Toxicity Characterization for Five Dispersants. Ecotoxicology and Environmental Safety **42**, 138–142.
https://doi.org/10.1006/eesa.1998.1734

Hook SE, Osborn HL. 2012. Comparison of toxicity and transcriptomic profiles in a diatom exposed to oil, dispersants, dispersed oil. Aquatic Toxicology **124–125,** 139–151.
https://doi.org/10.1016/j.aquatox.2012.08.005

Hu M, Lin D, Shang Y, Hu Y, Lu W, Huang X, Ning K, Chen Y, Wang Y. 2017. CO_2-induced pH reduction increases physiological toxicity of nano-TiO_2 in the mussel *Mytilus coruscus*. Nature Scientific Reports **7,** 40015.
https://doi.org/10.1038/srep40015

Jiang Z, Huang Y, Xu X, Liao Y, Shou L, Liu J, Chen Q, Zeng J. 2010. Advance in the toxic effects of petroleum water accommodated fraction on marine plankton. Acta Ecologica Sinica **30**, 8–15.
https://doi.org/10.1016/j.chnaes.2009.12.002

Lee KW, Shim WJ, Yim UH, Kang JH. 2013. Acute and chronic toxicity study of the water accommodated fraction (WAF), chemically enhanced WAF (CEWAF) of crude oil and dispersant in the rock pool copepod *Tigriopus japonicus*. Chemosphere **92,** 1161–1168.
https://doi.org/10.1016/j.chemosphere.2013.01.080

Lessard R, DeMarco G. 2000. The Significance of Oil Spill Dispersants. Spill Science & Technology Bulletin **6**, 59–68.
https://doi.org/10.1016/S1353-2561(99)00061-4

Lyons MC, Wong DKH, Mulder I, Lee K, Burridge LE. 2011. The influence of water temperature on induced liver EROD activity in Atlantic cod (*Gadus morhua*) exposed to crude oil and oil dispersants. Ecotoxicology and Environmental Safety **74,** 904–910.
https://doi.org/10.1016/j.ecoenv.2010.12.013

Melzner F, Gutowska MA, Langenbuch M, Dupont S. Lucassen M, Thorndyke MC, Bleich M, Pörtner HO. 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?. Biogeosciences **6,** 2313–2331.
https://doi.org/10.5194/bg-6-2313-2009

National Research Council (NRC). 2002. Oil in the sea III: Inputs, fates and effects. National Research Council, Washington DC.

Nizzetto L, Lohmann R, Gioia R, Jahnke A, Temme C, Dachs J, Herckes P, Guardo AD, Jones KC. 2008. PAHs in Air and Seawater along a North–South Atlantic Transect: Trends, Processes and Possible Sources. Environmental Science & Technology **42**, 1580–1585.
https://doi.org/10.1021/es0717414

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner GK, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig MF, Yamanaka Y, Yool A. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature **437,** 681–686.
https://doi.org/10.1038/nature04095

Otitoloju AA. 2005. Crude oil plus dispersant: always a boon or bane? Ecotoxicology and Environmental Safety **60,** 198–202.
https://doi.org/10.1016/j.ecoenv.2003.12.021

Rand G. 1995. Fundamentals of aquatic toxicology : effects, environmental fate, and risk assessment. Taylor &Franchis, Washington DC.

SenananW, Tangkrock-Olan N, Panutrakul S, Barnette P, Wongwiwatanawute C, Niphonkit N, Anderson DJ. 2007. The presense of the Pacific Whiteleg Shrimp (*Litopenaus Vannamei*, BOONE, 1931) in the wild in Thailand. Journal of shellfish research **26,** 1187–1192.
https://doi.org/10.2983/07308000(2007)26[1187:TPOTPW]2.0.CO;2

Singer MM, Aurand DV, Coelho GM, Bragin GE, Clark JR, Sowby M, Tjeerdema RS. 2001. Making, measuring, and using water-accomodated fractions of petroleum for toxicity testing. International Oil Spill Conference Proceedings **2001,** 1269–1274.
https://doi.org/10.7901/2169-3358-2001-2-1269

Vieira LR, Guilhermino L. 2012. Multiple stress effects on marine planktonic organisms: Influence of temperature on the toxicity of polycyclic aromatic hydrocarbons to *Tetraselmis chuii*. Estuaries in a Changing Climate **72**, 94–98.
https://doi.org/10.1016/j.seares.2012.02.004

Wells PG, Abernethy S, Mackay D. 1982. Study of oil-water partitioning of a chemical dispersant using an acute bioassay with marine crustaceans. Chemosphere **11,** 1071–1086.
https://doi.org/10.1016/0045-6535(82)90112-6