Effect of WAF crude oil exposure to larvae development of the black-scar oyster Crassostrea iredalei

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Abstract. Oil spills potentially effect exposed organisms at various stage of life. This work aimed to access health risk of crude oil to larva development of a sessile organism the Black scar oyster Crassostrea iredalei by using water accommodate fraction (WAF) of crude oil. Male and female gametes was collect and fertilized to obtained larvae at cleavage stage. The larvae were then incubate in various concentrations of WAF (0, 6.25, 12.5, 25, 50, and 100 %). After 24 hour of exposure, normal D-shaped veliger larva (D-larva) was observe. The result showed that WAF crude oil affected the development and the successful of D-larva development. Severity of WAF effect was increasing with dosages of exposure. The abnormal larva developments were increasing in the high concentrations. The information obtaining from current work is important for health risk assessment of crude oil contamination incident in marine ecosystem. This study will also contribute valuable knowledge needed for aquaculture to know effect of crude oil spill to oyster farming area.

1. Introduction

Pollution from anthropogenic activity is the main issue in Asia and in the country’s regions [1], [2]. The effect of exploration, production and transportation activities of oil industry in the coastal area has been the cause of great concern in recent times. Since Southeast Asia has a higher marine biodiversity, including marine diatoms, algae, fishes, coral reefs and invertebrates [3], [4], [5], [6], [7], [8], the management of marine and coastal areas in the region is urgent to be taken as priority. Among a huge number of marine biotas, black-scar oyster in Southeast Asia is economical, healthy, and ecologically important.

Black-scar oyster or slipper-shaped oyster Crassostrea iredalei (Faustino, 1932) is a member of the Family Ostreoidea. The coastal inhabitants traditionally consume the black-scar oyster collected from the wild [9]. In the Philippines, this oyster is one of the famous seafood [10]. Farming of this species has found in many regions such as in the south of Thailand [11].

Oil spill is one of the sources of marine pollution due to the operating procedures of oil exploitation and transportation activities [12]. However, the effect of crude oil spills on fertilization and larvae development in the oyster is limited. The present study, therefore, aimed to investigate the impact of crude oil to the fertilization success and larvae development of the black-scar oyster Crassostrea
iredalei (Faustino, 1932) because of its availability, economic and ecological importance. Oysters have also used as model for marine toxicological study due to their wide geographical distribution, tolerance to chemicals, and easy handling [13]. The water accommodate fraction (WAF) of crude oil was used in the study based on the fact that crude oil composed of various chemical components, only certain molecules will be accommodated in the water once the crude oil spills into the water.

2. Methods

2.1 Oyster conditioning
Twenty of adult oyster C. iredalei (Faustino, 1932) were collected from a local farm in Angsila, Chonburi Province and immediately transported to the Marine Aquaculture Laboratory, Burapha University, Thailand. Oysters were scrubbed and washed to eliminate other biota on shells and acclimatized for one week prior to the onset of the experiment [14]. Briefly, seawater with salinity of 30 ppt was changed daily and food was also supplied daily by adding of algae Nannochloropsis sp. at a ratio of algae to oyster of 2:100 and maintained in 40 L aquarium at 19°C in order to obtain gonad maturation [15]. Aeration was provide by bubbling air connected via plastic tubes to an air pump.

2.2 Gamete collection and larvae preparation
Male and female gametes were collected separately from the gonad of oyster by the dry stripping method [16]. Briefly, gonads were dissected and stripped in order to collected gametes and placed in a glass bowl filled with 50 ml of 1 μm filtered seawater (30 ppt). For male gamete, the sperm suspension was filtered with 20 μm nylon mesh to remove the large chunks of gonad material. The oocyte suspension was also successively filtered at 110 and 30 μm to remove the large and small chunks of gonads, respectively. Sperm viability and oocyte morphology were observed under a light microscope and diluted in seawater in order to determine gamete concentration. Fertilization was achieved by mixing oocyt stock and sperm stock in seawater to obtain a ratio of 1 oocyte to 400 sperms (1:400) according to [8]. A larva in cleavage stage was used for the investigation of WAF exposure.

2.3 WAF crude oil preparation
WAF was prepared according to [17] by filling 4 L glass mixing in a chamber with 2.7 L of seawater (30 ppt) and 0.3 L of crude oil with a ratio of 1:9 (crude oil: sea water). Mixing and creating a vortex was performed by using a magnetic stirrer. A vortex was adjusted to no more than a third of the height of the mixture from the oil-water interface [18]. Stirring was done for 20 hours and at 25°C ± 1°C. After mixing, the oil-water mixture was allowed to stand for 4 hours [18,19]. The lower phase was collected and used as the WAF stock solution (100% WAF). The stock WAF will be stored in dark bottles prior to use.

2.4 Experimental exposure of larva to the WAF crude oil
WAF concentrations of 0 (control), 6.25, 12.5, 25, 50 and 100% were prepared from WAF stock solution. For each concentration, 100 ml was filled in a 200 ml-beaker. Larvae in cleavage stage from section 2.2 were transferred into each WAF concentration. Three replicates were performed for each concentration. Temperature was set at 25 ± 1°C.

3. Result and discussion

3.1 Fertilization and larva development of the black-scar oyster
In control group, the first polar body was observed at approximately 30 min after fertilization (Figure 1A) and cleavage stage was achieved after 2 hours of fertilization (Figure 1B). After 16-20 h the
embryo developed into a trochophore larva which similar to other studies [20,21,22,23]. D-shaped veliger larvae (D-larvae) was observed under a light microscope after 24 hour of exposure. The development of the black-scar oyster embryos to D-shaped larvae in control group is generally similar to the previous study [21]. In this study, larvae in cleavage stage (Figure 1B) was used for the investigation of WAF exposure.

3.2 The effect of WAF crude oil exposure on larvae development
WAF affected the development of oyster larvae in concentration-dependent manner resulting in the decreasing of normal D-larva after 24 hour of exposure. In control group (0% WAF), the average percentage of normal D-larva reached 96.43±7.14. In WAF treatments, the average percentages of normal D-larva decreased to 79.17±25.00, 35.42±29.17, 35.42±20.83, 16.25±19.74, 5.00±10.00 following exposure to 6.25, 12.5, 25.0, 50.0 and 100.0 % WAF, respectively. It is noted that the development of larvae in WAT exposure group were still undertaken but most of them exhibited abnormal characteristics (Figure 1D) and some of them were damaged, especially at the higher concentrations.

The study of WAF crude oil and the combination of WAF and dispersant exposures to oyster larva has been reported in the eastern oyster Crassostrea virginica [24] which stated that there is no significantly effect to the veliger larva after 24 hours of exposure. However, the effect could later be observed at peldiveliger larva and settlement. Compared to the present results, the black-scar oyster Crassostrea iredalei is more sensitive to the WAF exposure. Our results clearly demonstrated that this species could potentially be heavily affected by an oil spill and may lead to the mortality of oyster larva. Also, this study improves current knowledge of potential effect of WAF crude oil exposure to this economically important species.

**Figure 1.** Larvae characteristics of the black-scar oyster Crassostrea iredalei. A) Fertilization was indicated by the first polar body. B) Cleavage stage at 2 hours after fertilization was used for exposure. C) Normal D-larvae composed of D-shaped shells covering entire soft-part tissue after 24 hours of fertilization. D) Abnormal larvae from WAF exposed group failed to develop to D-shaped shells after 24 hours of exposure.
4. Conclusion
In this study, the exposure was performed when the larvae begin to develop and we demonstrated that WAF exposure affected the development of larvae to the D-larva stage. Our results revealed that severity of WAF effect was increasing with dosages of exposure. The abnormal larva developments were increasing in the high concentrations.

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References
[1] Leung, K M Y, Yeung K W Y, You J, Choi K, Zhang X, Smith R, Zhou G J, Yung M M N, Arias- Barreiro C, An Y J, Burket S R, Dwyer R, Goodkin N, Hii Y S, Hoang T, Humphrey C, Iwai C B, Jeong S W, Juhel G, Karami A, Huber K K, Lee K C, Lin B L, Lu B, Martin P, Nillos M G, Oginawati K, Rathnayake I V N, Risjani Y, Shoeb M, Tan C H, Tsuchiya M C, Ankley G T, Boxall A B A, Rudd M A, Brooks B W 2020. “Toward Sustainable Environmental Quality: Priority Research Questions for Asia.” Environmental Toxicology and Chemistry 39(8): 1485–1505. doi: 10.1002/etc.4788.

[2] Risjani Y, Santoso D R, Couteau J, Hermawati A, Widowati I, Minier C 2020 Impact of anthropogenic activity and lusi-mud volcano on fish biodiversity at the Brantas Delta, Indonesia. In IOP Conference Series: Earth and Environmental Science 493, 1, p. 012007). IOP Publishing.

[3] Risjani Y, Loppion G, Couteau J 2020 Genotoxicity in the rivers from the Brantas catchment (East Java, Indonesia): occurrence in sediments and effects in Oreochromis niloticus (Linnaeus 1758). Environ Sci Pollut Res 27, 21905–21913 (2020). https://doi.org/10.1007/s11356-020-08575-w.

Figure 2. Average percentage of normal D-lavae in WAF crude oil exposure.
[4] Risjani Y, Mutmainnah N, Manurung P, Wulan S N, Yunianta 2021 Exopolysaccharide from Porphyridium cruentum (purpureum) is Not Toxic and Stimulates Immune Response against Vibriosis: The Assessment Using Zebrafish and White Shrimp Litopenaeus vannamei. *Marine Drugs*, 19(3), 133. https://doi.org/10.3390/md19030133

[5] Risjani Y, Witkowski A, Kryk A, Yunianta, Görecka E, Krzywda M, Safitri I, Sapar A, Dąbek P, Arsad S, GusevS, Rudiyansyah, Peszek L, Wróbel R J 2021 Indonesian coral reef habitats reveal exceptionally high species richness and biodiversity of diatom assemblages. *Eastuarine, Coastal and Shelf Science*. In press.

[6] Kryk A, Witkowski A, Ribeiro L, Kociolek J P, Mayama S, Wróbel R J, Risjani Y, Yunianta, Bemiasa J, Bemanaja E 2021 Novel diatoms (Bacillariophyta) from tropical and temperate marine littoral with the description of Catenulopsis gen. nov., and two Catenula species. *Diatom Research*. In press.

[7] Risjani Y and Abidin G 2020 Genetic diversity and similarity between green and brown morphotypes of Kappaphycus alvarezii using RAPD. *Journal of Applied Phycology*, 32(4), 2253-2260.

[8] Roem M, Musa M, Risjani Y 2021 Sediment dynamics and depositional environment on Panjang Island reef flat, Indonesia: insight from grain size parameters. *Aquaculture, Aquarium, Conservation & Legislation*, 14(1), 357-370.

[9] Devakiea and Ali A B 2000 Effects of storage temperature and duration on the setting and post-set spat survival of the tropical oyster, Crassostrea iredalei (Faustino). *Aquaculture* 190, 3 69-376.

[10] Ticar R L 2015 pH of Raw Oysters (Crassostrea iredalei) in Capiz, Philippines. *Int. J. Sci. Res. Publ.*, 5, 1–2.

[11] Yoosukh W and Duangdee T 1999 Living Oyster in Thailand. Phuket, Thailand: Phuket Marine Biological Center, 19, 363-370.

[12] Keramea P, Spanoudaki K, Zodiatis G, Gikas G, Sylaios G 2021 Oil Spill Modeling: A Critical Review on Current Trends, Perspectives, and Challenges. *Journal of Marine Science and Engineering*, 9(2), 181. https://doi.org/10.3390/jmse9020181

[13] Kington, Chitramvong Y, Janvilisri T 2007 ATP-binding cassette multidrug transporters in Indian-rock oyster Saccostrea forskali and their role in the export of an environmental organic pollutant tributyltin. *Aquat. Toxicology* 85, 124-132.

[14] Chavez-Villalba J, Pommier J, Andriamiseza J, Pouvereau S, Barret J, Cochard J C, Penne C M 2002 Broodstock conditioning of the oyster Crassostrea gigas: origin and temperature effect. *Aquaculture* 214, 115-130.

[15] Allen S K and Bushek D R 1992 Large scale production of triploid oysters, Crassostrea virginica (Gmelin) using “stripped” gametes. *Aquaculture* 103, 241-251.

[16] Song Y P, Suquet M, Quêau I, Lebrun L 2009 Setting of a procedure for experimental fertilisation of Pacific oyster (Crassostrea gigas) oocytes. *Aquaculture*, 287, 311–314.

[17] Gulec I and Holdway D A 1999. The Toxicity of Laboratory Burned Oil to the Amphipod Allorchestes compressa and the Snail Polinicesconicus. *Spill Science and Technology Bulletin* 5, 135-139.

[18] Singer D Aurand, Bragin G E, Clark J R, Coelho G M, Sowby M L, Tjeerdema R J 2000 Standardization of the preparation and Quantitation of water-accommodated Fraction of Petroleum for Toxicity Testing. *Marine Bulletin*, 40, 11, 1007-1016.

[19] Andersen S I and Speight J G 2001 *Petroleum Science and Technology* 19, 1-34

[20] Idayu N 2015 Early development of tropical oyster Crassostreairedalei (Faustino, 1932). 9, 1–8.

[21] Leverett D and Thain J 2013 Oyster embryo-larval bioassay (revised). (2013).

[22] Galslof P S 1964 The American oyster Crassostrea virginica Gmelin. Fishery Bulletin, Bureau of Commercial Fisheries

[23] Ver L M M 1986 Early development of Crassostrea iredalei Bivalvia Ostreidae with notes on the structure of the larval hinge. *Veliger*, 29, 78–85.
[24] Garcia S M, Clos D, K T, Hawkins O H, Gemmell B J 2020 Sublethal Effects of Crude Oil and Chemical Dispersants on Multiple Life History Stages of the Eastern Oyster, Crassostrea virginica, *J. Mar,Sci.Eng.* 8, 808. https://doi.org/10.3390/jmse8100808.