Acute toxicity of antifouling biocides and water-jet derivatives to copepod *Acartia omorii* and *Paracalanus parvus* s. l.

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I. Abstract

Copepods, the dominant member of the zooplankton and major grazers for phytoplankton in pelagic ecosystem, are at risk from exposure to antifouling biocides. This study investigated the toxicity of antifouling biocides (Diuron, Irgarol 1051, Sea-nine 211) and water-jet derivatives [water-jet effluent (WJE) and its MeOH extract (WJEME)] to the copepod *Acartia omorii* and *Paracalanus parvus* s. 1., using egg hatching rate and nauplius mortality. Results showed that Sea-nine 211 had the strongest effects on hatching rates and nauplius mortality of both species, followed by Diruon and Irgarol 1051. In water-jet derivatives, the egg hatching rate of *P. parvus* in WJE was slightly higher than that of WJEME, but no significant difference between the treatments was observed in *A. omorii*. The mortality rate of both species was higher in WJE than in WJEME. Although our study needs to be verified thorough additional experiments, our results can be used for baseline study concerning the toxicity of antifouling biocides against marine environment.

II. Introduction

Biofouling is the result of the accumulation of aquatic organisms on submerged area of ship's hull. There are diverse organisms involved in the gradual development of biofouling burden, such as bacteria, diatoms, barnacles, tubeworms, mussels, tunicates and seaweeds. The accumulation of these organisms on the surface will cause economic lost such as increasing fuel consumption and cleaning costs, but also will induce the ecological risks by increasing the emission of greenhouse gases and introducing invasive species to native environment. In order to prevent biofouling, antifouling paint is applied to ship's hull which will release antifouling biocide in a controlled manner to form a protective film against nearby biofoulers. As a result of their continued used as antifouling paints, their ecological and economic relevance has received increased attention. Therefore, the evaluation of the risk associated with the occurrence of those biocides in the marine environment in urgently needed. Furthermore, there are no data available about the toxicity of waste water from ship's surface during in-water cleaning activities, particularly about their acute toxicity to marine copepod nauplius and egg.

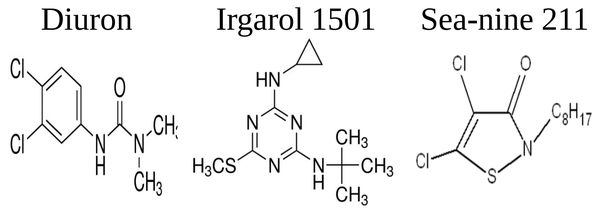
Marine copepod nauplius and eggs have been used for the screening of acute toxicities of booster biocides, because they have short generation time and easy to cultivate. Therefore, in the present study, we tried to investigate nauplius mortality rate of marine copepod *Acartia omorii* and *Paracalanus parvus* s.1. hatched from egg which was exposed to three booster biocides (Irgarol 1051, Diuron, Sea-nine 211), waste and its MeOH extracts from ship's surface during in-water cleaning. This study is quite meaningful in that we firstly attempt to determine the toxicity in waste and its MeOH extracts from ship's surface during in-water cleaning.

II. Material & Methods

❖ Test substances

- Waste released during ship's hull cleaning with high-pressure water-jet : Water-jet effluent, Water-jet MeOH extract
- Antifouling booster biocides – Diuron, Irgarol 1051, Sea-nine 211

Diuron Irgarol 1501 Sea-nine 211



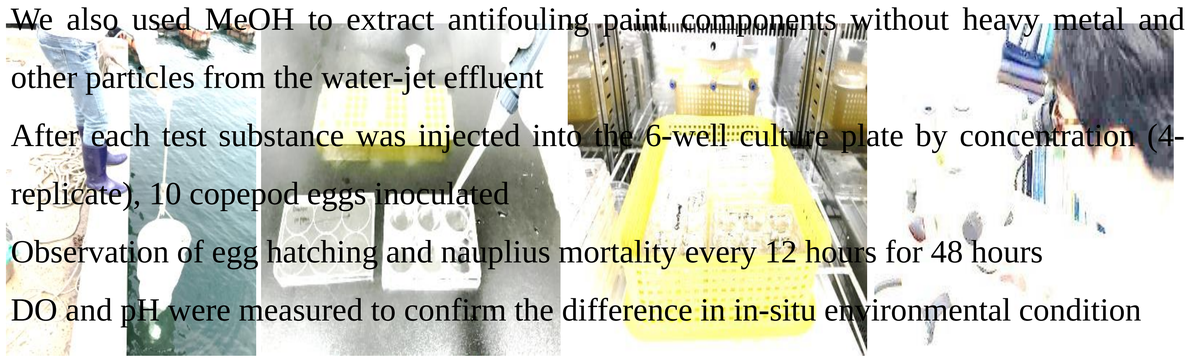
Pictures of ship's hull cleaning with high-pressure water-jet (left) and chemical structure of antifouling booster biocides (right)

❖ Test organisms

- Two marine copepod species were selected (*Acartia omorii*, *Paracalanus parvus* s.l.)
- Dominant species along the Korean coast

❖ Experimental method

- Test organism was collected with conical net (mesh size: 200 μm)
- The female of healthy adult was sorted using a stereomicroscope and stored in petri-dish containing 20-mL filtered and sterilized sea water for 6 hours in an incubator set to in-situ water temperature, and its egg are secured
- Prepared test substances in accordance with the experimental concentration – antifouling booster biocides dissolved in 0.1% DMSO and waste released from ships hull during water-jet cleaning was diluted with sterilized seawater.
- We also used MeOH to extract antifouling paint components without heavy metal and other particles from the water-jet effluent
- After each test substance was injected into the 6-well culture plate by concentration (4-replicate), 10 copepod eggs inoculated
- Observation of egg hatching and nauplius mortality every 12 hours for 48 hours
- DO and pH were measured to confirm the difference in in-situ environmental condition



Capture of test organism | Prepare test substance and egg inoculation | Incubation | Identification

IV. Results & Discussion

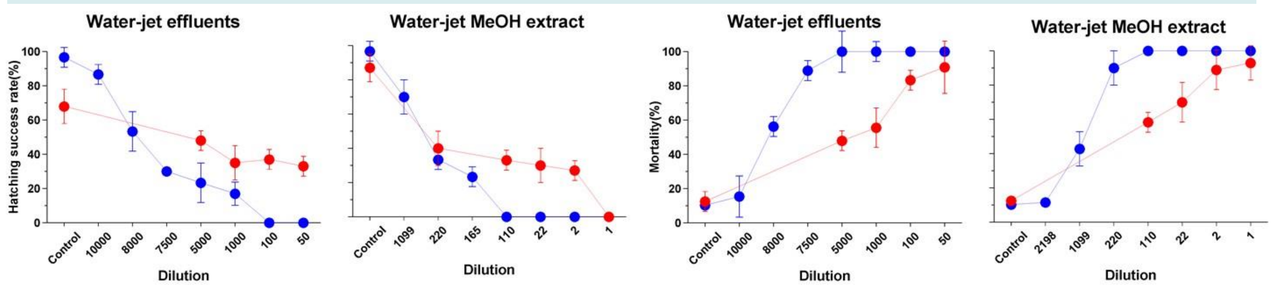
Egg hatching rate and nauplius mortality of marine copepod *A. omoril* and *P. parvus* under two different DMSO concentration

	Hatching Success (%)		nauplius Mortality (%)	
	A. omorii	*P. parvus*	*A. omorii*	*P. parvus*
DMSO 0.1%	86.7 ± 11.5	86.7 ± 5.8	7.7 ± 5.8	8.7 ± 5.0
DMSO 0%	80.0 ± 5.8	93.3 ± 5.8	3.1 ± 5.0	8.0 ± 5.0

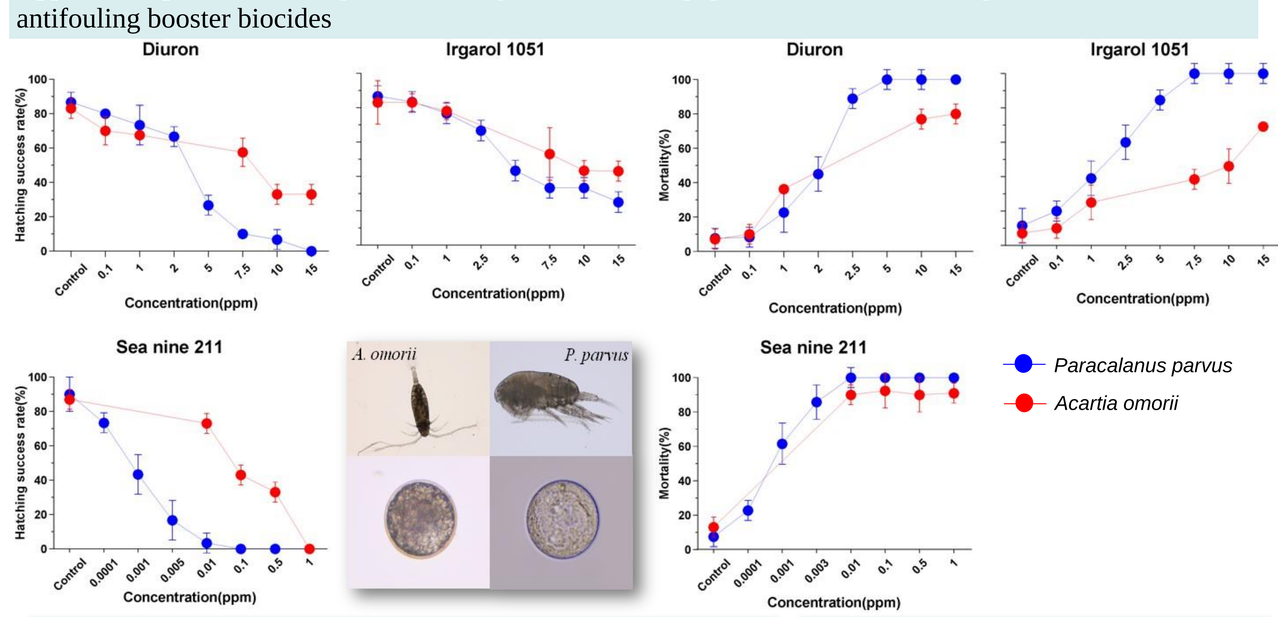
Results of pH value of each test substances

Material	pH
Diuron	8.08 ± 0.01
Irgarol 1051	8.04 ± 0.01
Sea-nine 211	8.09 ± 0.02
Water-jet effluents	8.01 ± 0.03
Water-jet MeOH extract	8.06 ± 0.03

Egg hatching rate and nauplius mortality of marine copepod *A. omoril* and *P. parvus* under water-jet effluents and its MeOH extract



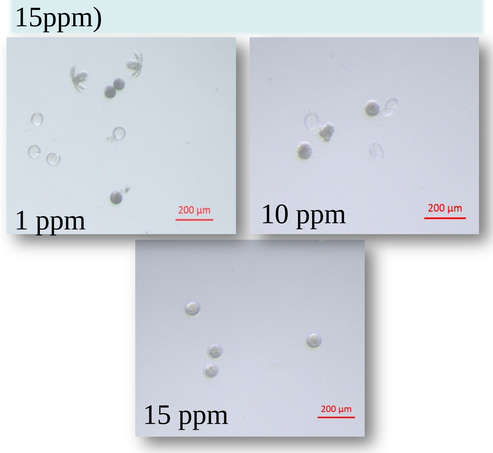
Egg hatching rate and nauplius mortality of marine copepod *A. omoril* and *P. parvus* under different antifouling booster biocides



LC50 values of marine copepod *A. omoril* and *P. parvus* under three different antifouling booster biocides and waste water released during ship's hull cleaning with high-pressure water-jet

Material	24-h LC_{50} (ug/L)	24-h LC_{50} (Dilution factor)
Diuron	2,101	-
Irgarol 1051	2,044	-
Sea-nine 211	0.6	-
Water-jet effluents	-	8,319
Water-jet MeOH extract	-	721

Pictures of marine copepod *P. parvus* eggs treated with Diuron (1ppm, 10ppm, 15ppm)



- Considering lower egg hatching rate and higher nauplius mortality in water-jet effluent than in water-jet MeOH extract, water-jet effluent may confirmed more toxic in both copepods *A. omorii* and *P. parvus*
- Among the antifouling booster biocides, egg hatching rate was higher in order of Irganol 1051, Diuron and Sea-nine 211, and nauplius mortality was higher in order of Sea-nine 211, Diruon and Irganol 1051.
- The LC_{50} values of *P. parvus* were higher in order of Diuron (2.17 ppm), Irgaron 1051 (1.24 ppm) and Sea-nine 211 (0.007 ppm), and water-jet effluent (about 9,000 times diluted) was about 15 times higher than water jet MeOH extract (about 600 times diluted).