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Evaluation of Heavy C-oil from Tanker "Nahodka" on the Early Life Stages of Marine Fish under Laboratory Condition

TADAHISA SEIKAI

Department of Marine Bioscience, Fukui Prefectural University, Obama, Fukui 917-0003, Japan

Abstract - Heavy C-oil spilled from tanker "Nahotoka" polluted seriously the long coast of Japan-Sea in 1997. Laboratory experiments revealed that the effects on the early life stages of Japanese flounder and roundnose flounder. The exposure to the oil suspended seawater (OSW) with unexpectedly low concentration of oil increased the deformation and insufficient growth of larvae. Detergent with spilled oil enhanced the toxicity extremely, and dispersed oil particles were taken easily into the intestine of copepoda, which is one of the important food items for marine fish.

I.Introduction

Russian tanker "Nahodka" caused a serious oil spill pollution from 2nd Jan., 1997 along the wide coastal areas of Japan Sea. Because of the heavy storm in the winter season, the hull was broken to two peaces, and the stern sank down around Oki Island in Shimane prefecture, but the bow drifted to the coast of Mikuni town in Fukui Prefecture. More than 6,000 kl of heavy C-oil was estimated to spill to the ocean from the broken hull. The spilled oil being drifted by the strong winter wind and Tsushima Current, polluted a long shoreline from Tottori Prefecture to Niigata Prefecture [1]. However serious damages were afraid on the various marine organisms in such cases, seabirds seaweed and benthos animals in intertidal zoon and restricted inshore area had been concerned. Thereafter, the laboratory experiments were conducted to evaluate the effects of heavy C-oil spilled from tanker "Nahodka" on the early life stages of marine fish.

II.Materials and Methods

A. Heavy C-oil from "Nahodka"

Heavy C-oil from "Nahodka" which spilled and drifted on the surface was collected by the research vessel "Ryokuyo-maru" of Kyoto University Fisheries Research Station (Maizuru, Kyoto) around Kanmuri Island in western Wakasa-Bay on 13th Jan. 1997. Already this heavy C-oil became very sticky because water in oil emulsion including 50% seawater was formed like chocolate mousse. In addition to this, heavy C-oil from the bow hold of the tanker on the coast of Mikuni town were provided for the present study.

B. Preparation of oil suspended seawater (OSW) and determination of oil content

One gram of drifted oil was added to 500ml seawater in a 1 liter Erlenmeyer glass flask that was shaken vigorously at 10°C for 4 days (200 cycles/minute and 3cm amplitude), assuming stormy water surface in the winter season of this area. After the settling for 30 minutes, the water fraction was collected as an undiluted solution of oil suspended seawater (OSW) for the further experiments and then the oil was extracted with carbon tetrachloride. The oil content was determined as 3.2mg/ml with infrared absorption photometry at Gifu Prefectural Public Health Inspecting Center (Akebonomachi, Gifu City, Gifu). Suspended oil particles in seawater were observed as brilliantly shining particles smaller than several micrometers under fluorescence microscope.

C. *Hatching experiments of marine fish embryos in OSW*

Fertilized eggs of Japanese flounder *Paralichthys olivaceus* and round nose flounder *Eopsetta grigorjewi* were provided from Miyazu Station of Japan Sea Farming Association (Odasyukuno, Miyazu City, Kyoto) for the following hatching experiments. Undiluted solution of OSW was made each time, and then diluted by filtrated seawater to make grading concentrations. Twenty eggs of Japanese flounder and thirty eggs of round nose flounder (about 24 hrs. after fertilization) were incubated at 20°C and 15°C in 100ml beaker each with 100ml of grading concentrations of OSW for 48 hrs., respectively. Each of grading concentrations of OSW was duplicated and triplicated for these two species, respectively. Almost all of eggs in the experimental lots hatched out after 24 hrs.

from the start of incubation, thereafter embryos existed in the diluted OSW during the late developmental stages of egg for 24 hrs. and then yolksac larval stage for 24 hrs.. At the end of incubation, all of dead and unhatched eggs, survived and dead larvae were counted and fixed in 5% formalin, and then the dead egg rate, the hatching rate and the deformation rate of hatched out larvae were determined.

D. Hatching experiment with the seawater from oil polluted site

Hatching experiment with the seawater from oil-polluted site was conducted together with the former experiment of round nose flounder. On 12th and 14th of Feb. 1997, the seawater was collected from the surf zone of Mikuni Town where the bow was washed up and the coastal area was heavily polluted. The climate condition there on 12th was so stormy that the water sampling was dangerous. Oil film spread over the surface of coastal water, and oil droplets in the sampled water were visible to the naked eye. On the other hand, the climate condition on 14th was calm, and the sampled water looked clean. Five times and two times dilutions of the water from 12th were also examined because of the foreboding of the high contents of oil. At the end of experiment, all of dead and unhatched eggs, survived and dead larvae were counted and fixed in 5% formalin, and then the dead egg rate, the hatching rate and the deformation rate of hatched out larvae were determined as stated above.

E. Effects of detergent with oil

Sprinkling of the detergent to the spilled crude oil or heavy oil is popularly conducted to disperse and enhance microbial or physical degradation. Because the former hatching experiments revealed the lethal effects of suspended oil droplets on the early life stages of marine fish, the effects of the detergent on the early life stages of marine fish were examined together with heavy C-oil. Heavy C-oil for this experiment was collected from the bow hold of "Nahodka". The detergent was one of the most popular one (Neos AB-3000, Neos Co. Ltd. Kobe), which was mostly used for this oil spill accident and have the certificate of the Ministry of Transport.

Stock solutions of detergent only (0.5ml/1liter seawater) and detergent and heavy C-oil mix (0.5ml of detergent and 2g of heavy C-oil/1liter seawater) were prepared for this experiment. Two stock solutions were diluted by clean seawater to make grading concentration of detergent only or detergent and oil mixed seawater. Each thirty fertilized eggs of round nose flounder (about 24 hrs. after fertilization) were incubated at 15°C in 100ml beaker with grading concentrations of detergent only or detergent and oil mixed seawater for 48 hrs. Each concentration of grading detergent only or detergent and oil mixed seawater was single. Almost all of eggs in the experimental lots hatched out after 24 hrs. from the start of experiment, thereafter embryos exposed in mixed seawater as late developmental stages of eggs for 24 hrs. and then as newly hatched larvae for 24 hrs. At the end of experiment, all of dead and unhatched eggs, survived and dead larvae were counted and fixed in 5% formalin, and then the dead egg rate, the hatching rate and the deformation rate of hatched out larvae were determined as stated above, and total length of larvae without deformation were measured.

F. Observation of oil intake by fish larvae and zooplankton

Yolksac larvae, which did not open their mouths were immersed in the OSW for 2-3 hours, and then observed under fluorescent microscope.

Wild zooplankton Copepoda *Paracalanus* sp., rotifers *Brachionus plicatilis* and *Artemia* salina nauplii also immersed in OSW for 3 hrs., and then observed under fluorescent microscope.

III.Results

A. Development of marine fish embryos in OSW

For Japanese flounder embryos, effects of OSW were detected from the 0.25 % of stock solution (8ppb of oil in seawater) as a slight increase of unhatched egg rate and deformation rate of hatched out larvae. But 2.5% solution showed a remarkable increase of deformation rate in hatched out larvae drastically (data is not shown).

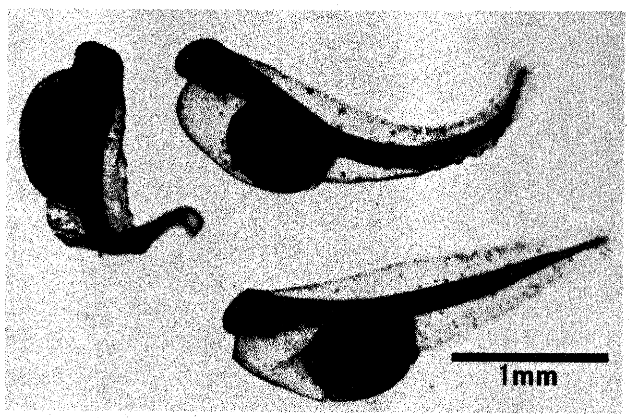

Fig.1. Deformed larvae of roudnose flounder *Eopsetta grigorjewi* after the exposure to 0.5% of OSW stock solution for 48hrs. during egg and newly hatched out larval stages.

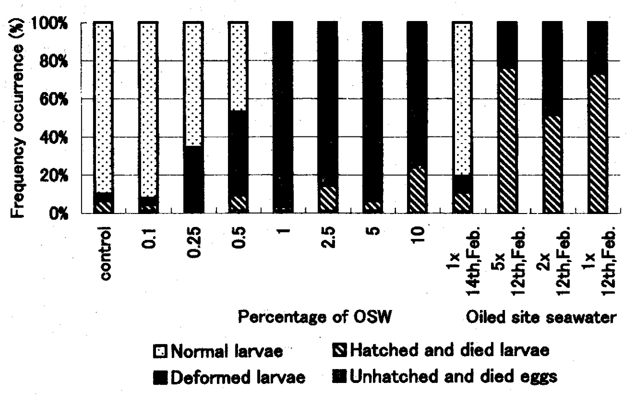

Fig.2. Effects of OSW on the early life stages of round nose flounder *Eopsetta grigorjewi* after the exposure to diluted OSW for 48hrs. during egg and larval stages. Oiled-site seawater was collected on 14th, Feb1997 at the heavily polluted coast of Mikuni Town (calm condition). That was also collected on 12th, Feb., 1997 (stormy condition) at the same place, but that water was diluted 5 times, 2 times and 1 times (no dilution), respectively.

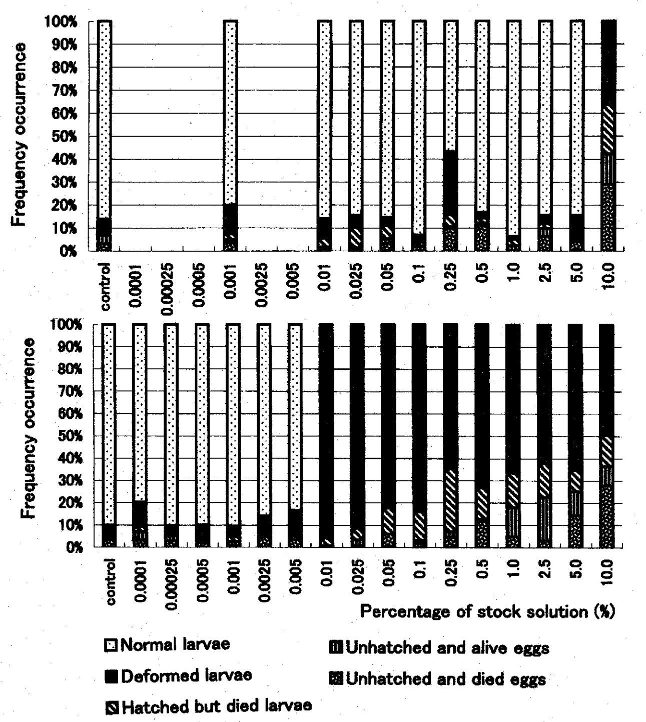

Fig. 3. Effects of detargent (upper) and detargent + heavy c-oil (lower) on the early life stages of round nose flounder. *Eopsetta grigorjewi* after the exposure to the dispersant of each materials for 48hrs. during egg and larval stages.

Stock solution of detergent (0.5ml/1liter seawater) and detergent + heavy C-oil mix (0.5ml of detergent + 2g of heavy C-oil/1liter seawater) were prepared just before this experiment.

For round nose flounder embryos, deformation of hatched out larvae increased remarkably from 0.25% of OSW, and all of hatched out larvae died or deformed at 1% of OSW (Figs.1 and 2). 0.25% of stock solution corresponded to 8ppb of heavy-C oil in the seawater.

B. Effects the seawater from oil polluted site

The effects of the surface water collected on 12th and 14th, Feb. from oiled-site in Mikuni Town were quite different on the early life stages (Fig.2). The water on 14th showed the intermediate effects between 0.1% of OSW (3.2ppb) and 0.25% of OSW (8ppb) of oil in seawater, but the water on 12th showed a extremely stronger effect on the early life stages than 10% of OSW (corresponding 320ppb of oil in sea water). Even in 5 times dilution of the seawater, 70% of newly hatched larvae died immediately after hatching or all of survived larvae were deformed.

C. Effects of detergent with oil

The effects of detergent only were quite moderate, because only 10% of stock solution of detergent (corresponding 50ppm) showed the harmful effects on the early life stages of round nose flounder (Fig.3). Diluted mix solution of detergent and heavy C-oil showed very serious effects on the early life stages at lower concentration. In 0.01% of stock solution (corresponding 50ppb of detergent and 200 ppb of C heavy-oil) almost all of larvae deformed after hatching.

D. Effects of oil, detergent and their mixture on the larval size after hatching

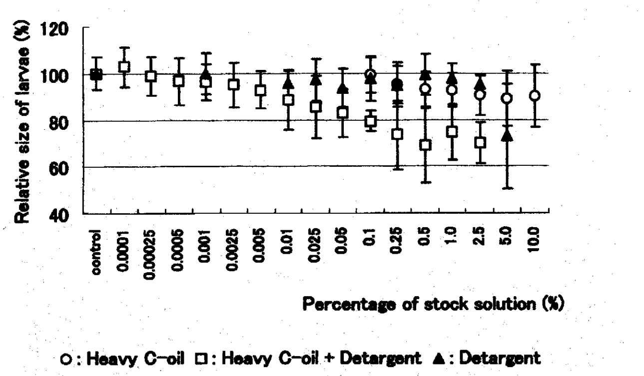

Fig. 4. Effects of oil, detergent and their mixture on the newly hatched larval size of round nose flounder *Eopsetta grigorjewi* after the exposure of their dispersant in seawater.

The effects of these materials on the size of newly hatched larvae were more sensitive indicator than the appearance of deformation. As shown in Fig.4, significant differences of larval length were detected from the lower concentration of these materials in the seawater ($p<0.01$).

D. Observation of oil intake by fish larvae and zooplankton

Fluorescent microscope observation found small particles of oil attaching on the surface of larval skin and fin fold, and moreover larval body emitted week fluorescence later. These phenomena suggested the intake of oil to the larval body through skin suface.

Wild zooplankton Copepoda *Paracalanus* sp. also was immersed in the OSW for 3 hrs. (Fig.5). Then several particles of oil droplets were observed in the intestine under fluorescent microscope. Also rotifers *Brachionus plicatilis* and *Artemia* salina nauplii had taken oil droplets into their intestine.

IV. Discussion

A. Effects of dispersed oil in seawater on the early life stage of fish

In the present study, 0.25% dilution of OSW, corresponding to 8ppb of C heavy oil in seawater, started to affect on the early life stages of Japanese flounder and round nose flounder. This concentration is much lower than the detectable level of officially authorized method[2], and 1/5 lower than that of more sensitive infrared method.

Hatching experiment in the seawater from oiled-site was conducted together with the hatching experiment of round nose flounder in diluted OSW. Depending on the collection dates, effects of sampled seawater on the early life stages of round nose flounder were quite different. If we consider the dilution rate, the concentration of oil in seawater on 12^{th} from the oiled-site was

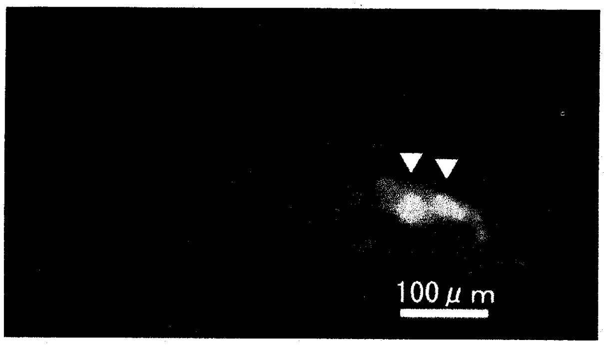

Fig.5. Oil particles intake by copepada. Dispersed oil particles were took into the intestine of copepoda. These particles were observed with the bright light under fluorescent microscope.

estimated to be higher than 1.6ppm. Another day's seawater (14^{th}) corresponded to 3.2-8.0 ppb. From these results, the condition of the water surface along the sea-side is changing every moment, and under stormy conditions, surprisingly large amounts of oil should be suspending in the water.

Remarkable things from these experiments were as follows; the ratios of unhatched eggs were lower than 1.2% within the examined rage of oil concentrations. More than 50% mortality of collected egg of sardine *Sardinella aurita* and herring *Clupea harengus* from the polluted site at the time of oil spill accident of Torrey Canyon in 1967 [3] suggests the much higher concentration of oil in the seawater than the examined revel of present study. Another report revealed the high concentration of oil (several to 20 ppm) from relatively wide area from the polluted site of Julianna oil spill accident off Niigata in 1971 [4]. Regarding to the present accident, relating Prefectures and Fisheries Agency conducted surveys to detect the range of oil from ND to 104ppb in Wakasa Bay and surrounding areas [5]. The survey of egg and fish larvae by Fisheries Agency found 60-80% of anomalies in egg development maximally [6].

Before Exxon Valdez oil spill accident, few scientists had considered the effects of spilled oil on the early life stages of marine fishes. Because the accident occurred in the Prince William Sound where huge number of the Pacific herring, *Clupea pallasi* migrated and started to spawn, the effects of spilled oil on the herring have been investigated from various points. As herring spawns demersal eggs on the coastal area, being close to the shore line, serious effects were expected on the early life stages of this species. Indeed, chromosome aberration [7], delayed hatching and high percentage occurrence of deformation of newly hatched larvae [8], abnormal cell proliferation of pectoral fin [8], abnormal differentiation of eye during larval stages [9] were observed in oil-water dispersion (OWD) under experimental conditions. Also field surveys revealed the deformation and delayed growth of herring larvae in the polluted area [10], remaining affects on the 3 years later reproduction [11], and accumulation of PAHs in the ovary because of the exposure to the weathered oil [12]. Also, pink salmon *Onchorhnchus gorbusha* which is the target species of stock enhancement around polluted site was examined about the growth and survival rate in the ocean after egg incubation in extremely low concentration of OWD during embryonic period [13], and stray of homing migration was found during oceanic phase [14]. Examined eggs in the present study were of pelagic species, and their spawning grounds are much offshore than that of the herring. Pelagic eggs hatch out earlier at underdeveloped condition than demersal eggs. The past field surveys frequently reported higher revels than the effective dose revel of heavy C-oil in the seawater on the early life stages of the two species in the present study. Thereafter, the effects of oil spill accidents on the species, which spawn pelagic eggs offshore, also not ignorant. On second thought, such pelagic eggs must be useful experimental materials, because they hatch out during short period, and the results could be gotten within 2 days or so. This is the quite useful as a tool to evaluate the urgent effects on the marine ecosystem.

B. Detergent strengthen the toxicity of oil

The present study showed the harmful effects of suspended particles of spilled oil on the early life stages of Japanese flounder and round nose flounder. On the other hands, detergents are popularly used on the spilled oil to disperse into seawater. It has been believed that oil dispersion enhances the bacterial and physical chemistry decomposition. The effects of detergents with spilled oil on the early life stages of fishes were examined according to a great anxiety of the detergents in such case.

The detergent used hear was one of the most popular one, and applied for this accident

From the lesson of the past oil spill accidents, the detergents have been improved to show the extremely lower toxicity on the aquatic organisms than the past ones. In the present study, unexpected high concentration of detergent (50ppm) only showed the harmful effects on the early life stages of round nose flounder, but the toxicity level on the round nose flounder embryos was two digit numbers lower than that on the medaka *Oryzias latipe* which is the popularly used fresh water fish for toxicology [15]. The toxicity of detergent only was still extremely low. But detergents are not used alone, these are used for the oil spill accidents to disperse oil in seawater as small particles. The mixture of oil and detergent, 0.01% of stock solution (50ppb oil and 200ppb detergent) increased the appearance of deformed larvae. It was suggested that dispersed oil droplets formed by the detergent showed harmful effects on the early development of round nose flounder. Ironically, high performance of the present detergents increased the toxicity of spilled oil on the marine organisms. Thereafter, from the viewpoints of acute toxicity, the use of the detergents in the coastal areas should have a strict limitation, especially in the closed inland sea. If the detergents do not contribute on the bacterial degradation of spilled oil, the use of detergents for oil spill accidents will lose the theoretical background.

C. Effects on the ecosystem through oil intake by fish larvae and zooplankton

Newly hatched larvae were estimated to take in the small oil particles through their skin, even when they did not open their mouths. The mechanism of their intake is not uncertain, therefore we should reveal it scientifically. Zooplankton; rotifers, *Artemia* nauplii and wild Copepoda took oil droplets through their mouth like as their foods. As the most of zooplanktons, which are important as food for fish larvae, are filter feeder, ingredients of oil (e.g. polycyclic alomatic hydrocarbones (PAHs)) must be easily involved to the food web of marine ecosystem. PAHs should be traced in the marine ecosystem because these chemicals have the strong mutagenicity and carcinogenicity. As PAHs has revealed the photoenhanced toxicity [16], it must be one of the important subjects how to estimate the fate of spilled oil involved into the feed web by reasonable model [17].

V. Summary and Conclusions

Spilled heavy C-oil from tanker “Nahodka” were dispersed as small particles by the vigorous shake in the seawater (OSW), whose concentration was 3.2mg/ml. Hatching experiments of Japanese flounder and round nose flounder were conducted in diluted OSW. 8ppb of oil in seawater increased deformation of larvae in these two species. The detergent itself was not so toxic but the mixture with oil enhanced the toxicity extremely on the early life stages of these species, because the detergent promoted the dispersion of oil into seawater. The effects on the larval size were more sensitive than the deformation in newly hatched larvae. As dispersed oil particles were easily taken into the intestine of zooplankton, which were the main food items for marine fish larvae and juveniles, we should consider the effects of PAHs on the marine ecosystem through food web.

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