

Ingestion and Effects of Dispersed Oil on Marine Zooplankton

**For Prince William Sound Regional Citizens' Advisory Council (PWSRCAC)
Anchorage, Alaska**

By

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I. Introduction

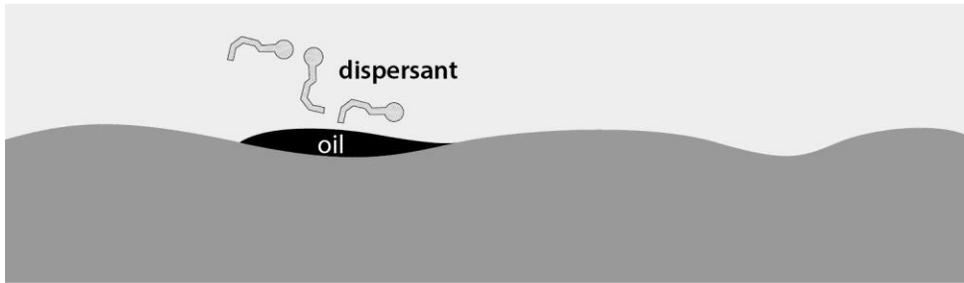
In the past, dispersants have sometimes been applied to oil spills to break up the slicks to help mitigate shoreline oiling. The formation of underwater plumes of dispersed oil after oil slicks are treated with dispersants has important implications for zooplankton populations within these plumes. Zooplankton in such underwater plumes can be exposed to hydrocarbon concentrations as high as 200 ppm, while water concentrations from untreated spills are in the ppb range (Clayton et al., 1993; Lichtenthaler and Daling 1985). Figure 1 diagrams formation of a plume after an oil slick is treated with dispersant followed by ingestion of dispersed oil droplets by herbivorous zooplankton. The predicted movement and hydrocarbon concentrations of a submerged plume of Alaska North Slope crude oil after dispersant application to a slick is shown in Fig. 2. The very uneven distribution of oil after an oil spill, results in zooplankton being exposed to high concentrations of both dispersant and dispersed oil after dispersant is applied to an oil spill.

Since the *Torry Canyon* spill in 1967, when much of the observed environmental damage was due to the toxicity of the applied dispersants (Nelson-Smith, 1968), less toxic dispersants have been developed. Thus, dispersants, such as Corexits 9527 and 9500, are less toxic than the first generation dispersants. The LC_{50} for Corexit 9527 was 40 ppm (Anderson et al., 1985) while the LC_{50} for dispersed oil, at a dispersant-to-oil ration of 1:20 (Corexit 9527:Prudhoe crude oil), was 4 ppm (Anderson et al., 1985). This test, using estuarine mysid shrimp, showed that the dispersed oil was ten times more toxic than the dispersant alone.

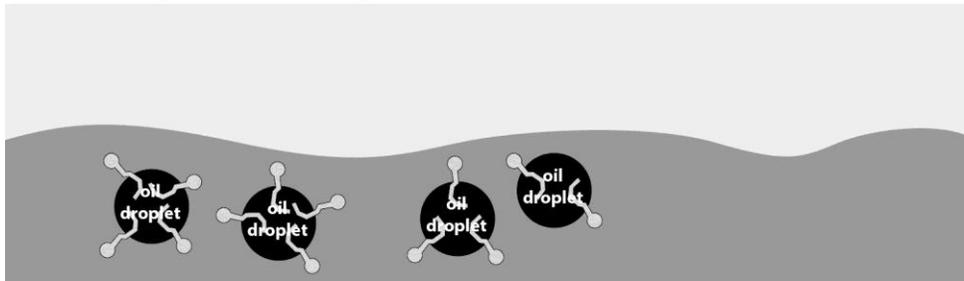
During the release of large amounts of oil by the Deepwater Horizon incident approximately 6.4 million liters of Corexit 9500 were applied. As discussed below, there are reports and papers showing that Deepwater Horizon oil was taken up by Gulf of Mexico zooplankton. In light of the large amount of dispersant used it seems likely that much of this ingested oil was in the form of dispersed oil.

This review discusses the ingestion and effects of dispersed oil on zooplankton and fish larvae, the different types of surfactants used in dispersants with possible effects on zooplankton, gaps in research and priority areas for future research on effects of dispersed oil on zooplankton.

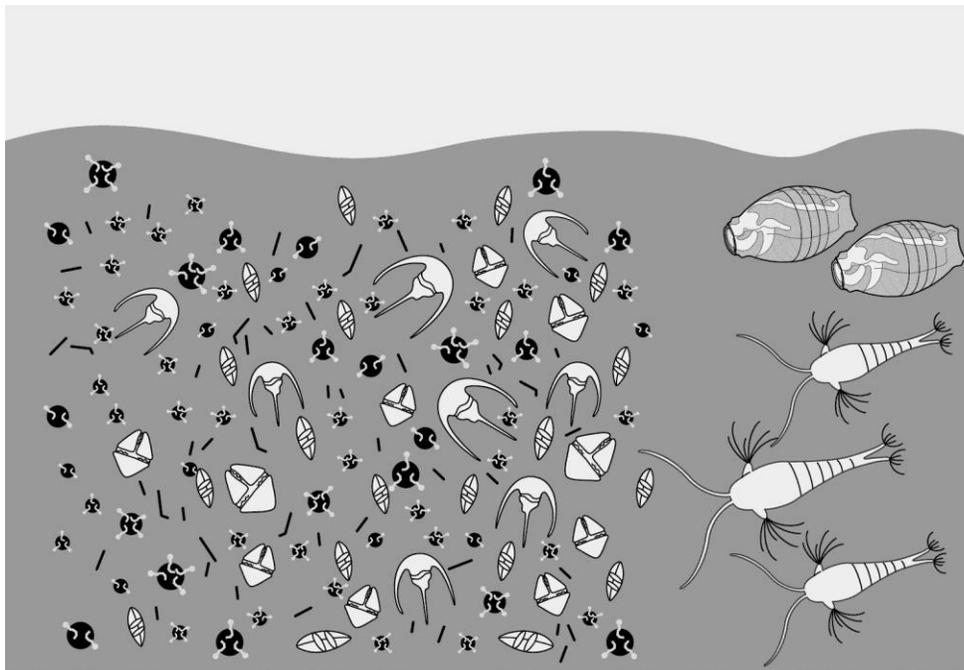
Application of dispersant onto oil



Oil slick disperses into droplets



Dispersed oil consumed by zooplankton feeding



plume of dispersed oil
mixes with shoals of phytoplankton

copepods and doliolids ingest the
phytoplankton and
dispersed oil droplets

(possible effects on reproduction, feeding and growth)

Figure 1. Mechanism for the formation of dispersed oil from an oil slick and ingestion of dispersed oil by zooplankton.

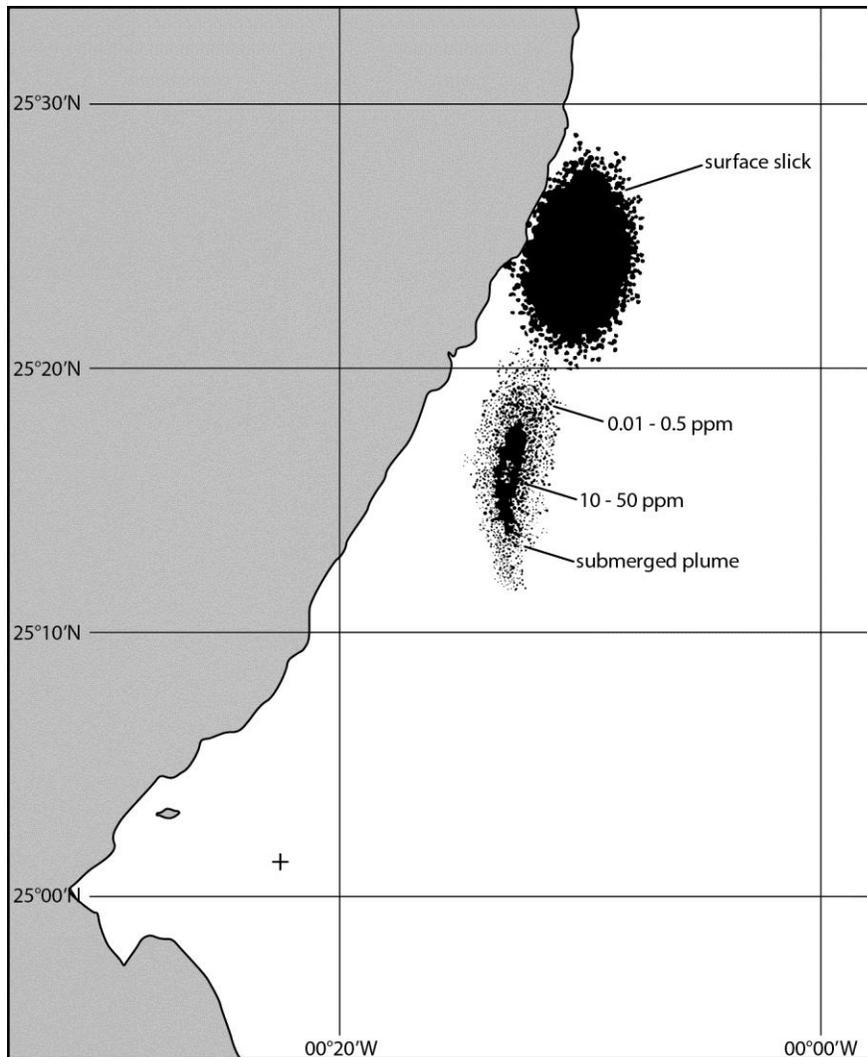


Figure 2. Predicted movement and hydrocarbon concentrations (ppm) of submerged plume of Alaskan North Slope crude (24 hours after application of dispersants to the surface oil slick with a 10 m/sec wind). Modified from *National Academy of Sciences*, (2005).

II. Studies on Zooplankton at Spill Sites

The National Academy of Sciences report (NAS, 2005) on the use of dispersants on oil spills, reports on the physical chemistry of dispersed oil droplets and the effects of dispersants on marine life, but does not address the uptake or effects of dispersed oil by zooplankton. There are several publications which report on the ingestion of oil droplets by zooplankton and effects on zooplankton populations after large spills. After the Ixtoc blowout in the Gulf of Mexico, where there was extensive use of dispersants, there was a four-fold decrease in zooplankton concentrations for 3 years after the spill compared with zooplankton

concentrations quantified in the same area a decade earlier (Guzman del Proo et al., 1986). Zooplankton collected from the Ixtoc spill site were found to have taken up dispersed oil suspended on fine sediments (Casey et al., 1980). In the *Tsesis* oil spill off Sweden there was a decline of the zooplankton biomass during the first 5 days after the spill. After this spill oil droplets were commonly observed within the gut as well as outside on the furca and feeding appendages of copepods (Johansson et al., 1980). A mesocosm study by Jung et al. (2012) found a greater decrease in zooplankton after treatment with dispersed oil than oil alone. After a Bunker C oil spill in Chedabucto Bay, Nova Scotia, Conover (1971) found copepods in the spill area with oil particulates. After the discharge from the Deepwater Horizon well Perry and co-workers reported the presence of oily appearing droplets in crab larvae collected off the coast of Louisiana (Perry, unpublished data; Fig. 3). Analysis of the megalopae with these oily droplets showed the presence of both petroleum hydrocarbons and dispersants (Perry, unpublished data). The depletion of ^{13}C in zooplankton collected from the area of the Gulf of Mexico, which received discharge of the Deepwater Horizon well, suggested the entrance of oil-derived carbon via dispersed oil droplets, into Gulf of Mexico zooplankton (Montoya et al., 2012; Graham et al., 2010). Multivariate statistical analysis of the polycyclic aromatic hydrocarbons (PAHs) from zooplankton collected from the northern Gulf of Mexico after the DWH spill showed a distribution of PAHs related to DWH oil (Mitra et al., 2012). Figure 1 diagrams formation of a plume after an oil slick is treated with dispersant followed by ingestion of dispersed oil droplets by herbivorous zooplankton [copepods and pelagic tunicates (doliolids)] while feeding on their phytoplankton food.



Fig. 3. Megalopa of blue crab collected off the Louisiana coast after the Deepwater Horizon blowout in the Gulf of Mexico. Note the numerous oil droplets. Photo courtesy of H. Perry at the University of Southern Mississippi.

III. Laboratory Studies with Zooplankton and Dispersed Oil

Studies by Olsvik et al. (2011) and Hansen (2013) (www.copepoda.info) have noted uptake of dispersed oil by fish larvae and copepods collected from the North Sea. After copepods (*Calanus finmarchicus*) ingested of dispersed North Sea oil there was a reduction in feeding rates (Hansen et al., 2008) and similarly lobster (*Homarus americanus*) larvae exposed to a water soluble fraction and dispersions of Venezuelan crude oil showed reduced feeding rates (Wells and Sprague, 1976). There was reduced egg production by *Calanus finmarchicus* after exposure to the polycyclic aromatic hydrocarbon, pyrene (Hjorth and Nielsen, 2011). Thus, there is evidence, based on laboratory and field studies, that oil droplets can affect the feeding, growth and reproduction of crustacean zooplankton.

In addition to crustacean zooplankton, marine protozoans have been observed to ingest oil droplets (Andrews and Floodgate, 1974; Lanier and Light, 1978). Recent work by Lee et al. (2012) have shown that pelagic tunicates (salps and doliolids), which are abundant in continental shelf waters (Deibel and Paffenhöfer, 2009), can ingest and discharge dispersed oil droplets into their fecal pellets (Table 1; Fig. 5-8). Oil droplets formed in the absence of dispersants were unstable and not ingested by the doliolids. Over a 24 hr exposure period there was a gradual accumulation of oil droplets by the doliolids (Table 1). The collection of phytoplankton by doliolids involves fine mucous filters which retain a variety of small particles, including oil droplets. Ingestion of dispersed oil droplets by pelagic tunicates, which are found in all of the world's oceans, is a likely occurrence after oil spills.

Fecal pellets from doliolids exposed to dispersed oil showed an abundance of oil droplets as well as undigested diatoms (Fig 8). The importance of doliolid fecal pellets in carrying oil after a spill into the deep benthos is illustrated by following calculation based on literatures values of doliolid concentrations during a bloom (Deibel and Paffenhöfer, 2009), fecal production rates (Köster et al., 2011) and fecal pellet hydrocarbon concentrations.

$$3.2 \text{ doliolids/L} \times 144 \text{ fecal pellets/doliolid-day} \times 0.0005 \mu\text{g oil/fecal pellet} = 0.2 \mu\text{g oil/L-day or } 200 \mu\text{g/m}^3 \text{ - day}$$

The sinking velocity of fecal pellets from doliolids has been reported to range from 59 to 405 m/day (Deibel, 1990), so our calculations suggest that a substantial amount of the dispersed oil after a spill could be carried to the benthos via doliolid fecal pellets.



Fig. 4. Phorozoid form of doliolid (top) and Gonozooid cluster (lower)

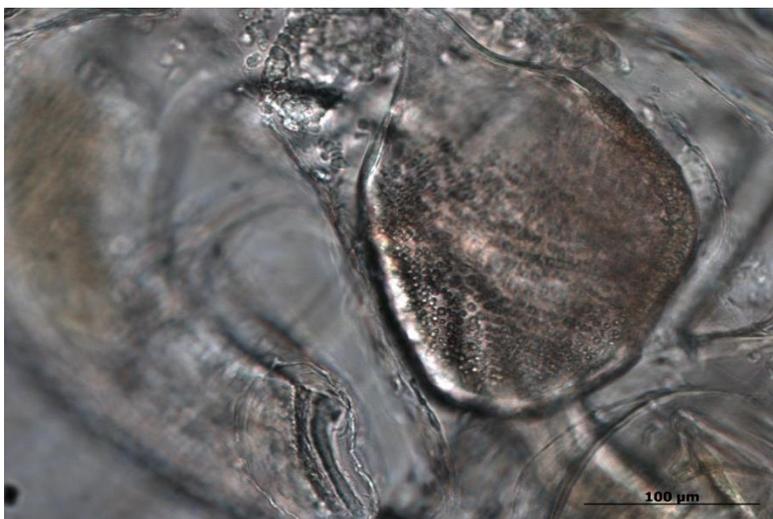


Fig. 5. Doliolid stomach full of dispersed oil droplets after ingestion of dispersed oil (12,000 droplets/ml)

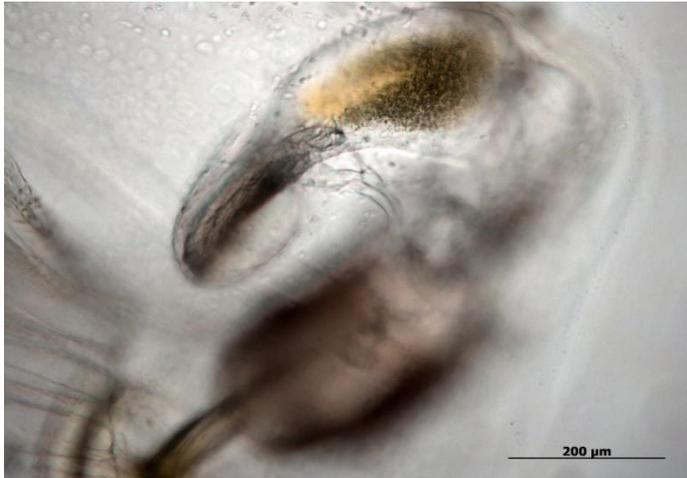


Fig. 6. Doliolid fecal pellet full of oil droplets that has formed within the gut and ready to be released.

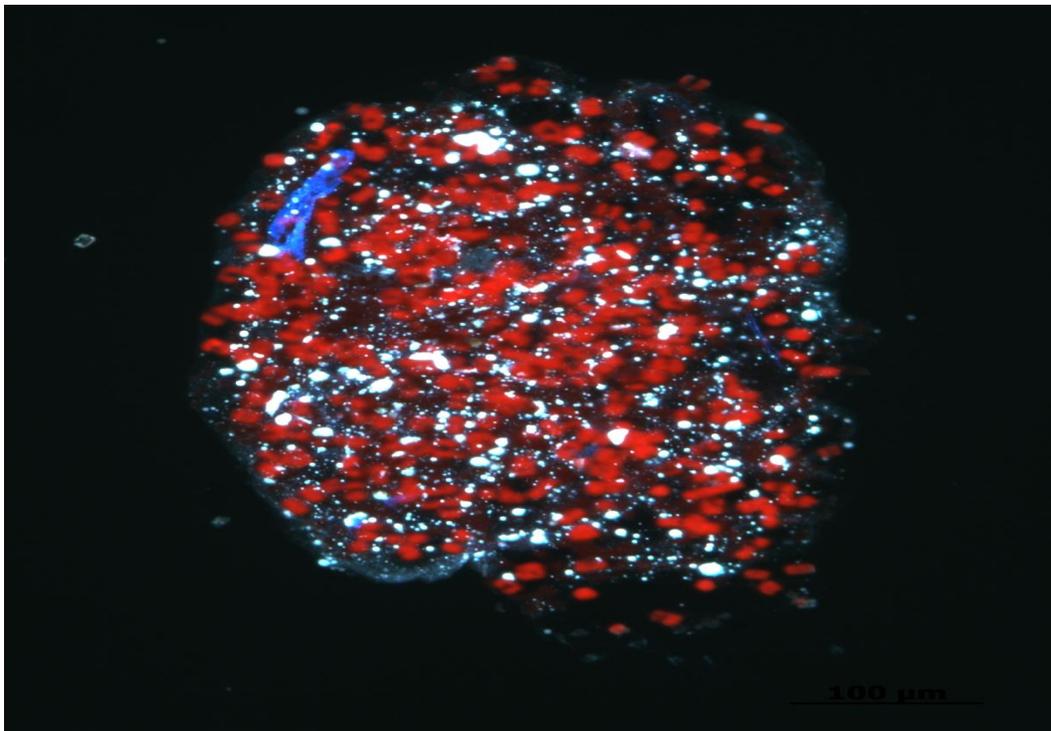


Fig. 7. Epifluorescent microphotograph of a DAPI stained fecal pellet produced by a doliolid which had ingested dispersed oil droplets. The strong red autofluorescence is from the diatoms and the numerous light-blue fluorescence is from dispersed oil droplets .

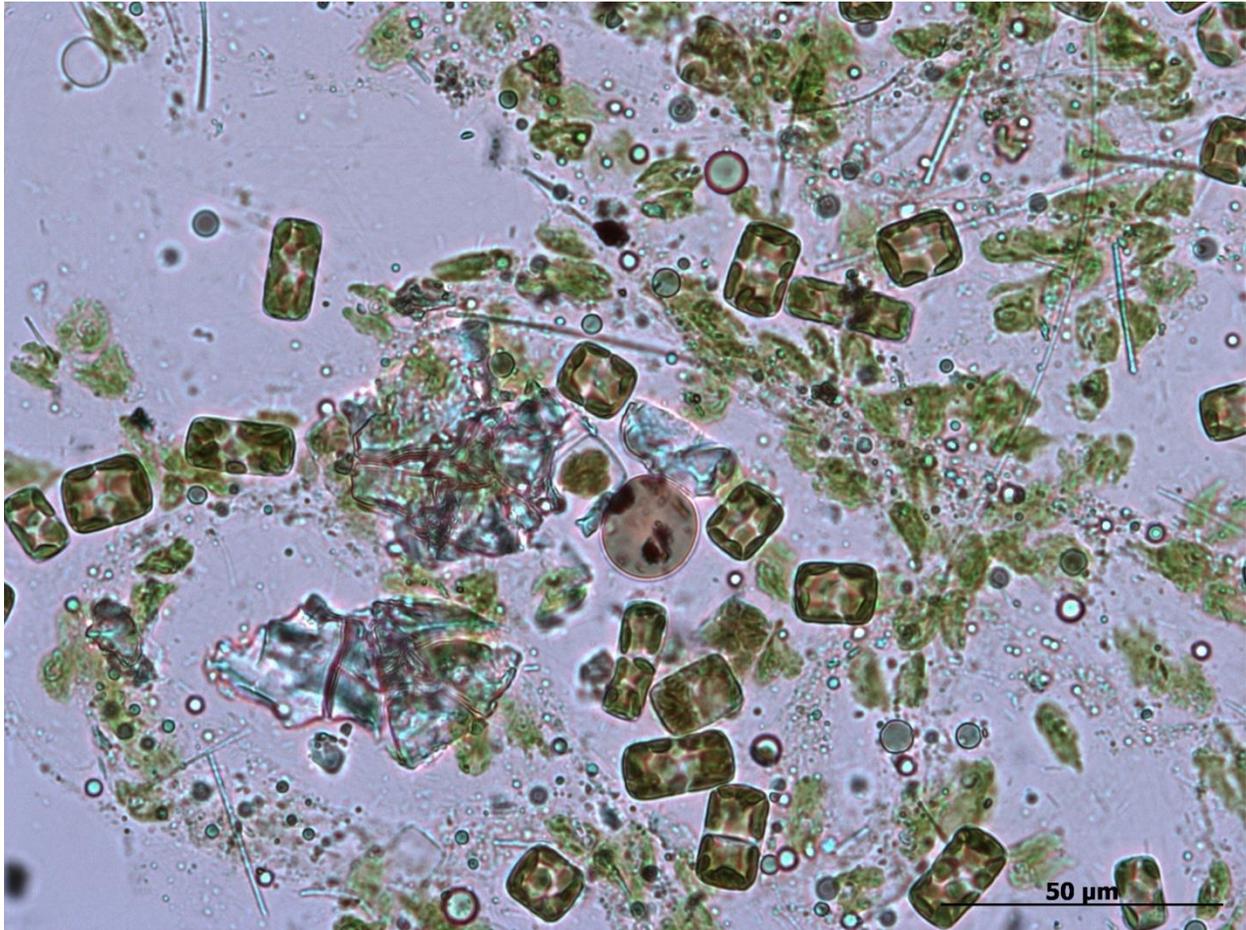


Fig. 8. Fecal pellet from doliolid exposed for 12 hours to suspension of dispersed oil droplets (17,000 droplets/ml) and mixture of phytoplankton (diatoms and flagellates). Note the many oil droplets throughout the pellet.

Table 1 . Oil Droplet Ingestion by the doliolid, *Dolioletta gegenbauri* (Lee et al., 2012)

Exposure Concentrations: Group A – 17,000 droplets/ml (7.1 μg of oil/ml);

Group B – 1200 droplets/ml (0.6 μg of oil/ml).

Group	Exposure Time (hrs)	Droplets/doliolid (droplets ± S.D., n=3)	Oil concentration (μg/doliolid ± S.D; n=3)
A	4	800 ± 350	0.6 ± 0.2
	8	1500 ± 710	0.8 ± 0.5
	12	2300 ± 420	1.1 ± 0.7
	24	5300 ± 1200	2.3 ± 1.2
B	12	450 ± 130	0.3 ± 0.1

IV. Toxicity Studies with Dispersed Oil

Anderson et al. (1985) presented an interesting approach for the study of the toxicity of dispersed oil to marine animals where dispersions were combined with toxicity data to generate a relative effective toxicity (RET) value.

$$\text{RET} = \text{DOR}_{90} \times 10^4 / \text{LC}_{50}$$

DOR_{90} = Dispersant-to-oil ratio that gives dispersion value of 90% (in the MNS test as described by Mackay and Szeto, 1980)

LC_{50} = concentration of dispersant required to cause 50% mortality in the test organisms after 96 hours of exposure.

A range of LC_{50} values were found for various dispersants with one group having values between 1 and 10 ppm while a second group was between 15 and 32 ppm and a third group was classified as non-toxic because the values were greater than 200 ppm. The LC_{50} of a DOR of 1:20 of Corexit 9527:Prudhoe crude oil was 4 ppm while the LC_{50} of Corexit 9527 alone was 40 ppm. The tests were carried out on an estuarine mysid shrimp, but there are no reports of this test on open ocean zooplankton. Particularly useful would be a study of toxicity combined with ingestion of dispersed oil so that the concentration of oil droplets within the zooplankton could be linked to observed toxicity.

One of the few studies on open ocean zooplankton (*Calanus finmarchicus*) was carried out by Hansen et al. (2012) who found that chemically dispersed (using the dispersant Dasic) oil was slightly more toxic to this copepod than the naturally dispersed oil.

V. Possible Effects of Dispersed Oil on Prince William Sound Zooplankton

von Westerhagen (1988) summarized the literature on the effects of water soluble fractions of oil to fish larvae, but not the effects of dispersed oil. Shahunthala et al. (2004) noted that there was enhanced polycyclic aromatic hydrocarbon uptake by fish exposed to dispersed oil, formed from mixing Corexit 9500 with crude oil, compared with the same concentration of water soluble fraction of crude oil. The effects of dispersed oil on herring larvae in Prince William Sound (PWS) is of special interest since Pacific herring (*Clupea pallasii*) are demersal spawners that lay their eggs on floating kelp in PWS. While there does not appear to be any published papers on the effects of dispersed oil on herring larvae, there is literature describing the effects on Pacific herring larvae of laboratory exposure to water soluble fraction of oil and relates these abnormalities

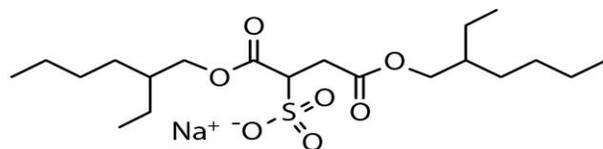
to those observed in herring larvae collected in PWS after the *Exxon Valdez* spill (Kocan et al., 1996; Marty et al., 1997; Norcross et al., 1996). Linden (1974) reported that concentrations as low as 1 ppm of the dispersant, Finasol SC, induced abnormalities in Baltic herring (*Clupea harengus*). A recommended future study on this topic would be to expose herring larvae to dispersed oil along with food particles to determine if ingestion of dispersed oil droplet occurs while feeding and the effects of ingested dispersed oil droplets growth and development of the larvae.

The application of dispersants after an oil spill in the coast of Alaska and Prince William Sound would lead to the formation of dispersed oil droplets which would be taken up by copepods and other zooplankton. It can be assumed that dispersed oil effects on marine zooplankton, as discussed above, has implications for the resident species of zooplankton in Prince William Sound and the Gulf of Alaska if they ingest dispersed oil. An earlier study by Carls et al. (2005) showed that only small amounts of petroleum hydrocarbons from the ballast waters from tankers bound for Port Valdez, AK were taken up by *Neocalanus plumchrus*. However, dispersed oil would likely be accumulated to a greater extent than ballast water hydrocarbon. Three species of large calanoid copepods, *Neocalanus flemingeri*, *Neocalanus plumchrus* and *Neocalanus cristatus*, dominate the spring biomass of mesozooplankton in the coastal Gulf of Alaska (Miller, 1993; Coyle and Pinchuk, 2003; Liu et al., 2007) and are important food for herring. It would be predicted that reproduction and growth of herring would be effected after ingesting oil via their copepod food containing dispersed oil. *Neocalanus* sp. accumulate large amounts of reserve lipid (Lee et al., 2006) and these lipid rich copepods are important food for herring populations since these fish also need to accumulate lipid reserves. R. Campbell at the Prince William Sound Science Center is carrying out studies on the zooplankton in the Prince William Sound and their importance for herring. Hansen et al. (2008) found that lipid-poor *Calanus finmarchicus* in the North Atlantic showed more bioaccumulation of dispersed oil than lipid-rich copepods. Accumulation of dispersed oil effected reproduction of these copepods. Thus, there is a close relationship between lipid and oil accumulation in cold water copepods. Hjorth and Nielsen (2011) have discussed the exposure of copepods off Greenland to oil and its effects on their reproduction. Lipid buildup by Gulf of Alaska copepods are likely to play an important role in the uptake and effects of dispersed oil on copepods, which in turn effects the health of the herring population.

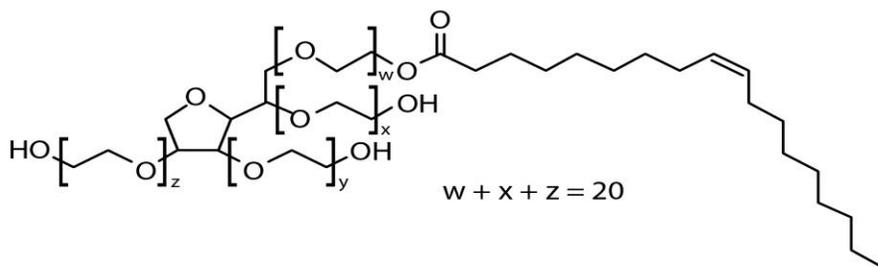
VI. Surfactants in Dispersants and Possible Effects on Zooplankton

Dispersants used on oil spills are composed of surface-active agents (surfactants), solvents and additives. Solvents, such as glycols, are used to dissolve the surfactants since many of them are highly viscous liquids. By decreasing their

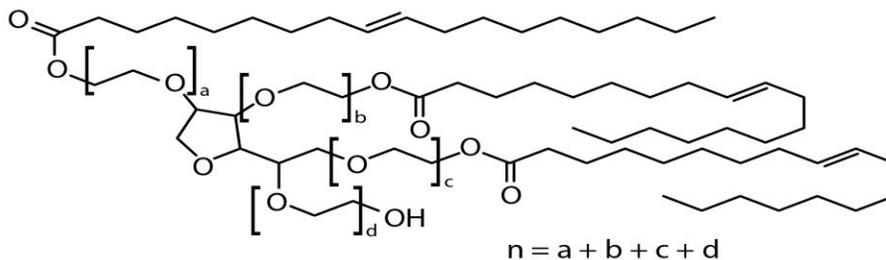
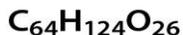
viscosity the dispersant can diffuse into the viscous oil slick. The additives help in the dissolution of the dispersant into the oil slick and increase the stability of the dispersed oil. The typical dispersant used for oil spills contain several surfactants since the effectiveness of a dispersant depends on the nature of the oil, the mixing energy available and the temperature. The toxicity of dispersants is generally ascribed to their various surfactants. While non-ionic surfactants are the major components of most commercial dispersants, many also contain ionic surfactants, such as sulfosuccinates, which facilitates the dispersion of oil into seawater. Fig. 9 shows the structures of the principal surfactants (sulfosuccinate, sorbitan esters) in Corexit 9500 which was the primary dispersant used on the oil from the Deepwater Horizon. In general, the ionic surfactants have a higher toxicity than the nonionic surfactants. However, there is a lack of literature on the effects of both ionic and non-ionic surfactants on marine zooplankton. Two reports since the Deepwater Horizon spill have suggested that the Corexit 9500 has greater impact on plankton than previously assumed (Ortmann et al, 2012; Martinez and Snell, 2013). The work by Martinez and Snell (2013) found that the effects of Corexit 9500 and oil were equally toxic to marine rotifers (*Brachionus* sp.) but when combined the dispersed oil was 50 times more toxic to the rotifers than the oil alone. Work by EPA (referred to in the Martinez and Snell website news) using estuarine shrimp and silversides found that dispersant when mixed with oil was no more toxic than oil alone. Thus, there is clearly a need for more studies on the effects on zooplankton with dispersant and dispersant combined with oil.



Dioctyl sodium sulfosuccinate



Polyoxyethylene sorbitan monooleate (Tween 80)



Polyoxyethylene sorbitan trioleate (Tween 85)



Figure 9. Structure of principal surfactants in the dispersant Corexit 9500.

VII. Ongoing Studies with Open Ocean Zooplankton and Dispersed Oil

Much of the prior work with dispersants and dispersed oil with zooplankton has used estuarine species, primarily crustaceans. Estuarine species are adapted to deal with highly variable conditions (e.g. salinity, temperature, particulate matter) and are less affected by organic contaminants than open ocean species. Thus, it is important to carry out work with dispersed oil on open ocean zooplankton. There are several groups who focus on the effects of dispersed oil on open ocean zooplankton. These include the researchers at SINTEF in Trondheim, Norway

(Hansen et al., 2012; Nordtug et al., 2011a,b; Olsvik et al., 2011) who carry out studies on the effects of chemically dispersed and natural dispersed oil on cod (*Gadus morhua*) larvae and a copepod (*Calanus finmarchicus*). Our own group at the Skidaway Institute of Oceanography in Savannah, GA continue studies on the ingestion and effects of dispersed oil on open ocean pelagic tunicates (primarily doliolids) (Lee et al., 2012). As noted earlier R. Campbell at the Prince William Sound Science Center is carrying out studies on open ocean zooplankton in the Prince William Sound and the importance of these species to herring. All of these studies should help in understanding and predicting the effects of dispersants and dispersed oil on zooplankton and fish larvae populations in open ocean areas.

VIII. Summary

1. A subsurface plume of dispersed oil at relatively high concentrations (up to 200 ppm) can form after application of dispersant to a large oil spill.
2. Zooplankton have been shown to ingest dispersed oil both in the field and laboratory.
3. Studies carried out after oil spills treated with dispersants have demonstrated effects on the growth and reproduction of zooplankton.

IX. Gaps and Priorities in Future Research

There are number of major gaps in research focusing on the dispersant and oil effects on marine zooplankton. Until recently most studies with dispersants and dispersed oil have used estuarine or nearshore zooplankton. As noted earlier, inshore zooplankton face a highly variable environment in contrast to the relatively stable environment faced by open ocean zooplankton. Laboratory work with nearshore zooplankton suggest toxicity of dispersants in the range of 10-50 mg/L. However, relatively few studies have been carried out on open ocean zooplankton. Listed below are suggested studies using open ocean zooplankton exposed to dispersants and dispersed oil.

1. Besides toxicity studies, more sublethal research is required to determine the effects of dispersants and dispersed oil on the reproduction and growth of open ocean zooplankton, particularly fish larvae, that have previously shown to be quite sensitive to low concentrations of dispersed oil. The priority in such research would be to focus on growth and reproduction to determine how dispersants and dispersed oil effect zooplankton populations. Work over the

past decade had demonstrated how the effects of pollutants on certain growth and reproduction parameters, e.g. molting rates in crustaceans, can be extrapolated to determine effects on zooplankton populations.

2. There has been much speculation about the effects of dispersant on zooplankton after the extensive application of Corexit 9500 after the Deepwater Horizon spill (Ortman et al., 2012; Martinez and Snell, 2012; <http://news.discovery.com/earth-dispersants-wildlife.html>). While there was good evidence that oil from the Deepwater Horizon spill entered the zooplankton food web, more follow up studies are necessary to demonstrate that zooplankton in the Gulf of Mexico were effected as a result of the ingestion of dispersants and dispersed oil.

3. Another aspect of dispersant effects that needs to be addressed in open ocean zooplankton are the lethal and sublethal effects of the non-ionic surfactants versus ionic surfactants present in commercial dispersants. This would be helpful in the design of future dispersants for use in treatment of future oil spills.

4. Perhaps the highest priority for the PWS region is to determine the effects of dispersants and dispersed oil on important zooplankton groups, particularly herring larvae, in Prince William Sound. The cold water zooplankton found in Prince William may respond quite differently after exposure to dispersants and dispersed oil than most work which has used warmer water zooplankton.

X. Acknowledgments

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