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Acute Toxicity of Oil and Bilge Cleaners to Larval American Oysters (*Crassostrea virginica*)

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Decline in the natural productivity of oyster beds (MCHUGH & WILLIAMS 1976) and increased demand have resulted in the development of intensive culture systems for oyster larvae. Although hatchery production is periodically highly successful, epizootics of disease can completely destroy hatchery production for extended periods. Vibriosis may be the most important cause of these epizootics and recently has been studied in detail (LEIBOVITZ 1978; LEIBOVITZ & ELSTON 1980; ELSTON & LEIBOVITZ 1980; ELSTON et al. 1980).

In the spring of 1979, there was heavy mortality among oyster larvae at an Oyster Bay, Long Island hatchery. Preliminary investigations could not implicate a bacteria; histologic examination of larvae pointed to the possible chemical contamination of the baywater used in larval culture (LEIBOVITZ, ELSTON, unpublished results). It was felt that bilge cleaners and No. 2 fuel oil were contaminating the baywater. Further preliminary investigations indicated a toxic effect on oyster larvae for bilge cleaners and No. 2 fuel oil (LEIBOVITZ, ELSTON, unpublished results).

The purpose of this study was to examine the toxicities to oyster larvae of three bilge cleaners and No. 2 fuel oil, as well as a detergent which is used to clean larval culture containers. The three bilge cleaners and the one detergent were tested alone and in combination with No. 2 fuel oil. No. 2 fuel oil was also tested alone. The toxicity of these substances to oyster larvae was determined in the laboratory under simulated hatchery conditions.

MATERIALS AND METHODS

The acute toxicities of No. 2 fuel oil, Boatlife Bilge Cleaner, Amway LOC, Sudbury's Bilge Cleaner, and

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Alcanox detergent[1] to oyster larvae Crassostrea virginica were evaluated in a static system. Each of the three bilge cleaners and Alcanox detergent were assayed alone and in combination with No. 2 fuel oil. Table 1 lists the reported composition of Amway LOC. The compositions of the other toxicants were unobtainable.

Table 1. Chemical composition of Amway LOC (provided by the manufacturer)

water	approximately 80%
primary alcohol alkoxylate	approximately 10%
n-alkyl diethanolamide	approximately 10%
dye	less than 1%
fragrance	less than 1%

All equipment in contact with the toxicants was glass, aluminum foil, or Teflon. These substances are inert to petroleum products. Prior to the experiment, all equipment was rinsed with acetone and then with pentane. The larvae were held in 3.8-L glass jars containing 3.0-L of the synthetic sea salt mixture Instant Ocean[2] at 21 parts per thousand. Larvae, approximately 120 um in size, were obtained for this study from the F. M. Flower Oyster Company at Bayville, New York. Two strains of algae, Woods Hole Tahitian Isochrysis sp. and Monochrysis sp., were fed. The stock cultures were supplied by the F. M. Flower Oyster Company. The two genera of algae were grown as sterile monocultures. Equal volumes of each were mixed together prior to feeding. The volume of algae added on a particular day was the same for all larval jars, and was such that by the next day, the larval jar was nearly completely cleared of algae.

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[1]American Petroleum Institute (API) No. 2 fuel oil was obtained from the Department of Biology, Texas A & M University, College Station, TX
Alcanox Inc., New York, NY 10003
Amway Corp., Ada, MI 49355
Boatlife Inc., Hicksville, NY 11801
Sudbury Laboratory Inc., Sudbury, MA 10776

[2]Aquarium Systems, Eastlake, OH 44904

Each bilge cleaner or Alcanox detergent was mixed alone or in combination with No. 2 fuel oil. One mL of each bilge cleaner or one g of Alcanox detergent with or without 0.2 mL of No. 2 fuel oil was mixed with 21‰ Instant Ocean on a rotary shaker for five min.

The water-soluble fraction (WSF) of API No. 2 fuel oil was prepared by a slightly modified method of ANDERSON et al. (1974). One part of No. 2 fuel oil was stirred over 100 parts of 21‰ Instant Ocean in a covered glass jar on a magnetic stirrer for 20 h. The height of the vortex created was one-third the height of the glass jar. After the stirring was stopped, the solution was allowed to equilibrate for 1 h. The WSF of No. 2 fuel oil was siphoned out and serially diluted with 21‰ Instant Ocean by 10-, 100-, and 1000-fold. The composition of the WSF of API No. 2 fuel oil is given in ANDERSON et al. (1974).

The total hydrocarbons of the WSF of No. 2 fuel oil were determined by infrared spectrophotometric analysis (BATELLE 1973). The samples and standards were scanned from 2500 to 3500 cm^{-1} on a Beckman IR-33 infrared spectrophotometer. Standards composed of 37.5% iso-octane, 37.5% cetane, and 25% benzene were mixed in 21‰ Instant Ocean at concentrations 1, 2.5, 5, and 10 ppm.

Water conditions were measured daily. Dissolved oxygen was 8.1±1.1 mg/L. The temperature was 22.5± 0.5°C. Salinity was 21‰. pH was 7.7±0.1. Ammonia-nitrogen was at levels undetectable by the Nezzlerization method.

The bioassay lasted 96 h. All test solutions were run at the same time. All larvae tested were offspring of the same parents. No replicate test solutions were run.

The density of larvae in the water column was determined daily. Ten spatially separate one mL samples were taken from each jar with a one-mL pipet. Five samples were drawn about four cm below the top of the water surface. Five samples were drawn about four cm above the bottom.

Average density counts were calculated from the ten one-mL counts for each jar on each day. The average density counts were transformed by isotonic regression (BARLOW et al. 1972). LC_{50} values were calculated by maximum liklihood from the transformed average density counts to fit a logistic funciton for

each toxicant on each day (FINNEY 1971).

Samples of larvae were taken at the start of the experiment and each day prior to the culture medium change. Live larvae were examined daily with interference contrast microscopy. Larvae were also taken daily for histological examination. The larvae were anesthetized with diethyl ether, fixed in 70% ethanol, decalcified with sodium EDTA and embedded in the plastic medium JB-4[3].

RESULTS

The total hydrocarbon concentration of the WSF of No. 2 fuel oil was determined by infrared spectrophotometry to be 8.65 ppm. The absorbance peak was at 2930 cm^{-1}.

The 24 h LC_{50} value for No. 2 fuel oil is 1.7 ppm (Table 2). No additional mortality occurred with the daily renewal of the No. 2 fuel oil, since the LC_{50} value for the next three days remained between 0.21 and 1.9 ppm.

Table 2. LC_{50} values for bilge cleaners alone and combined with No. 2 fuel oil for days one to four in ppm

Toxicant	Day One	Two	Three	Four
Alcanox detergent	NSM[a]	NSM	NSM	NSM
Boatlife Bilge Cleaner	NSM	NSM	NSM	NSM
Amway LOC	1.8	1.8	11.4	8.7
Sudbury's Bilge Cleaner	1.9	1.6	0.2	1.8
No. 2 fuel oil	1.7	1.7	0.2	1.9
Alcanox detergent & No. 2 fuel oil	NSM	NSM	56.9	4.0
Boatlife Bilge Cleaner & No. 2 fuel oil	47.7	10.6	4.1	4.6
Amway LOC & No. 2 fuel oil	1.8	1.8	0.6	1.6
Sudbury's Bilge Cleaner & No. 2 fuel oil	2.4	2.8	1.7	1.7

[a]NSM - no significant mortality

[3]Polysciences Inc., Warrington, PA 18970

Alcanox detergent and Boatlife Bilge Cleaner at the concentrations tested did not cause significant larval mortality. Addition of No. 2 fuel oil to each initially did not cause significant mortality, however within three to four days, heavy mortality of the larvae occurred. The LC_{50} value fell to 4.0 ppm for Alcanox detergent and No. 2 fuel oil on day four, and to 4.1 ppm for Boatlife Bilge Cleaner and No. 2 fuel oil on day three.

The 24-h LC_{50} value was 1.8 ppm for Amway LOC alone and in combination with No. 2 fuel oil. The larval density for Amway LOC alone varied somewhat on days three and four, creating anomalous variations in the 72-h and 96-h LC_{50} values. No additional mortality occurred from day two to four for Amway LOC and No. 2 fuel oil, as seen in the LC_{50} value which remained between 0.6 and 1.8 ppm.

The 24-h LC_{50} value was 1.9 ppm for Sudbury's Bilge Cleaner alone and 2.4 ppm when in combination with No. 2 fuel oil. Little or no additional mortality was evidenced in following days. The LC_{50} value for days two to four for Sudbury's Bilge Cleaner alone was between 0.2 and 1.8 ppm. When combined with No. 2 fuel oil and LC_{50} value for days two to four was between 1.7 and 2.8 ppm.

Wet specimens of oyster larvae were observed daily with an interference contrast microscope. In jars containing No. 2 fuel oil alone, or in combination with the bilge cleaners (but not the bilge cleaners alone, nor Alcanox detergent alone or in combination with No. 2 fuel oil), oil droplets were seen in the mantle cavity and the digestive system (especially the digestive diverticula) of larvae and were associated with heavy larval mortality. The oil droplets were transluscent and refractile, and their edges were sharply delineable. Larger droplets were seen in the digestive diverticula of all the larvae, including the controls. These larger droplets were presumably stored lipids. Well defined negative images (oils and lipids are solubolized by the fixation process) were found in the digestive gland, stomach wall and style sac wall of all larval samples. Their source may have been any of the following: stored lipids, bilge cleaner droplets and/or No. 2 fuel oil.

DISCUSSION

All of the substances tested except Alcanox detergent alone and Boatlife Bilge Cleaner alone

produced significant larval mortality (based on LC_{50} values) at concentrations between about one and five ppm (Table 2). Significant larval mortality was delayed two to three days for Alcanox detergent with No. 2 fuel oil, and Boatlife Bilge Cleaner with No. 2 fuel oil. All of the other toxicants and combinations of toxicants produced significant larval mortality within twenty-four hours, although there was an anomalous increase in larval density on days three and four for Amway LOC alone. The spatial distribution of the larvae generally was not uniform, the variability increased with larval mortality, and thus it may have caused the unusual larval densities for Amway LOC alone.

BYRNE & CALDER (1977) tested the toxicity of the WSFs of six different oils for clam (Mercenaria sp.) embryos ('embryo' here includes both the fertilized egg and trochophore stages) and larvae. The LC_{50} value after two and six days was 1.3 ppm for API No. 2 fuel oil, which compares favorably to the four day LC_{50} value of 1.9 ppm for oyster larvae from this study. BYRNE & CALDER (1977) also found growth reduction accompanied mortality and surviving larvae were smaller than controls.

The larvae were initially morbid (as denoted by slowed or stopped swimming) at concentrations of toxicants in which acute death did not occur. The velum generally continued to function when the larvae were stationary, although it was often clogged with algae and oil droplets. RENZONI (1973, 1975) found that the swimming speed of the larvae was reduced and irregular, and that the velum was constantly extended, at the two highest concentrations tested (1000 ppm, 100 ppm, volume added to water). In addition, the intoxicated but normally swimming larvae were smaller than the controls and sometimes lacked a shell. Not mentioned by RENZONI (1973, 1975), but observed in this study, was the association of oil droplets in the body cavity and sometimes the digestive system of larvae with heavy mortality in jars containing No. 2 fuel oil.

ANDERSON et al. (1974) found that gentle aeration of an oil in water dispersion of No. 2 fuel oil for 24 h resulted in a 90% decrease in the total hydrocarbon content. Thus, a large part of the WSF of No. 2 fuel oil added daily to the larval jars was probably lost by the end of the day. The LC_{50}'s found in this study thus may be high estimators of harmful concentrations of the substances tested.

The importance of the results of this study is

tied to relating significant lethal levels of toxicant to levels found and documented in the marine environment. The hydrocarbon content of the surface layer of seawater (upper few meters) in the open ocean appears to be about a few ppb (MALINS 1977; NAS 1975). Concentrations orders of magnitude higher can be found in areas of acute and chronic petroleum input. Dispersants were used on a 65,000 barrel spill from an offshore production platform, producing primarily an oil in water dispersion and concentrations around the platform from 1 to 70 ppm (MCAULIFFE et al. 1975). Samples of water were taken at the time of the Eleni V oil spill in May, 1978 (BLACKMAN & LAW 1980). Concentrations of fuel oil in harbors and pools ranged from 1.2 to 3.3 mg/L and 0.37 to 0.56 mg/L in inshore areas. TANACREDI (1977) found that total extractable hydrocarbons in Jamaica Bay, New York (which forms part of the waters adjacent to NYC) ranged from 0.50 to 5.10 mg/L and that waste crankcase oil was the probable source of the hydrocarbons. These concentrations of petroleum hydrocarbons are comparable to concentrations that this study showed to cause significant acute mortality to larvae and were found in areas similar to those in which oysters spawn and grow. Therefore, it is felt that both chronic and acute inputs of oil into the inshore environment are potential threats to oyster stocks.

Areas that are less polluted than Jamaica Bay may cause sublethal effects, or in combination with secondary effects, may cause significant mortalities. A preliminary trial with the same procedure as this study produced mortality trends similar to this study, but with larval mortality occurring more rapidly. High numbers of spiral bacteria were attached to oyster larvae in jars containing larvae that were experiencing significant mortality. The bacteria may have been acting secondarily on sublethally affected larvae to produce overall heightened mortality. The high larval morality that occurred in 1979 and served to initiate this study may have been due to oil and/or bilge cleaners in association with a secondary bacterial infection, especially since bacterial numbers tend to rise to high levels in the confines of a hatchery.

In summary, in enclosed natural waters where oysters generally spawn successfully, and in oyster hatcheries, water levels of petroleum hydrocarbons may reach levels that were found in this study to cause significant larval mortality.

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