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*Holmesimysis costata***

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San Jose State University, 1994

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EFFECTS OF PRODUCED WATER FROM OIL PRODUCTION PLATFORMS
ON GROWTH AND SURVIVAL OF THE MYSIDS,
MYSIDOPSIS INTII AND *HOLMESIMYSIS COSTATA*

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

By

Amy L. Wagner

August, 1994

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ABSTRACT

EFFECTS OF PRODUCED WATER FROM OIL PRODUCTION PLATFORMS ON GROWTH AND SURVIVAL OF THE MYSIDS, *MYSIDOPSIS INTII* AND *HOLMESIMYSIS COSTATA*

by Amy L. Wagner

Produced water is a potential source of pollution from offshore oil platforms since it is associated with oil formations and is extracted and discharged during oil production. The west coast mysids *Holmesimysis costata* and *Mysidopsis intii* were used to estimate the sub-lethal toxicity of five produced water samples from four southern California offshore oil production platforms. In three concurrent tests, the mysid species were comparable in sensitivity when subjected to the produced water samples tested. For all five samples, *Mysidopsis intii* growth and survival were significantly affected at produced water concentrations at or below 10%. In addition, the growth toxicity response was equally or more sensitive than the survival response. No temporal toxicity differences were observed for mysid survival from samples collected four months apart. Samples from three platforms in the Santa Barbara Channel were more toxic than samples from one platform over a different oil-bearing formation south of Los Angeles.

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INTRODUCTION

Produced Water

Water is trapped within petroleum-bearing formations and is brought to the surface during oil and gas production (Gilbert, 1983). This water is called "formation water" and represents a contaminant that must be separated from the petroleum products after being brought to the surface. During offshore oil production this waste water, now termed "produced water," is discharged into the sea surrounding the production platform. Produced water is the primary aqueous discharge from oil and gas production platforms and is discharged continually during production (Boesch and Rabalais, 1987). As offshore oil production continues at a formation, more produced water than oil is extracted and eventually discharged at sea (Read, 1978). When discharged, produced water can affect aquatic organisms surrounding offshore oil platforms.

Because formation water is associated with geological strata at high temperatures and pressures, it often contains elevated levels of metals, dissolved petroleum hydrocarbons, dissolved organic ions, and other organic compounds (Boesch and Rabalais, 1987). Formation water is isolated from free oil with primary treatment by mechanical gravity separation and with secondary treatment by air or gas flotation (Lysyj, 1982). However, this separation is not total, and dissolved inorganic and organic components can be discharged into the surface waters with the produced water (Lysyj, 1982). Produced water may also contain several chemicals added during the production process. These chemicals include biocides used to prevent hydrogen sulfide formation, corrosion inhibitors to reduce corrosion in a well, as well as detergents, metals, and solvents from oil platform deck drainage (Middleditch, 1981).

The potential environmental effects of produced water discharged from oil and gas production platforms or onshore facilities into the marine environment are commonly assessed on the basis of chemical analyses (Federal Register, 1993). Detection of chemical

contamination is only one step in assessing the potential consequences of produced water. Determining toxicity of produced water to marine organisms provides direct quantification of potential effects on aquatic life. Toxicity tests can assess the bioavailable portion, additive toxicity, as well as integrated effects of complex effluents (USEPA, 1991a). A study by Middleditch (1981) identified components in produced water that are not normally chemically analyzed, which also reinforces the need for biological testing of produced water to define toxic constituents in complex mixtures.

Concern over potential environmental effects of oil and gas production has prompted many researchers to conduct primarily acute lethality toxicity tests with various produced waters and their constituents on a wide variety of test organisms. Rose and Ward (1981) assessed 96-hour acute lethal toxicity of produced water from Texan oil platforms using the adult, subadult, and larval brown shrimp (*Penaeus aztecus*), adult and subadult white shrimp (*P. setiferus*), adult barnacles (*Balanus tintinnabulum*) and adult crested blenny (*Hypleurochilus geminatus*). Their results showed that the most sensitive organism was the larval brown shrimp, which concurs with studies showing that earlier life stages of organisms are generally less tolerant to pollutants (e.g., Connor, 1972; Neff and Anderson, 1981; Rose and Ward, 1981; Capuzzo, 1987). Produced water from southeastern U.S. offshore oil platforms between concentrations of 1.3-9.3% (volume of produced water in seawater) caused 50% mortality in the juvenile mysid, *Mysidopsis bahia*, after 96-hour exposure (Parrish et al., 1988). By comparison, the 96-hour LC50s of three produced water samples from the Gulf of Mexico to juvenile *M. bahia* ranged from 4.9% to 11.4% (Schiff, 1992). The range of 48-hour EC50s from three acute lethality toxicity tests conducted with produced water from a North Sea oil production platform using the juvenile copepod *Acartia tonsa* was 2.6-4.5% produced water (Girling, 1989).

The aforementioned studies lack data demonstrating sub-lethal chronic toxicity from low-level exposure to produced water discharges. Ecologically, chronic pollution from

long-term discharge of produced water is possibly more damaging than catastrophic oil spills (Daniels et al., 1990). The sub-lethal responses such as reduced growth and reproduction are significant in the consideration of the perpetuation of sensitive organisms in marine environments affected by produced water. In addition, sub-lethal effects studies may include exposure during a particularly sensitive life stage of the test organism. Such studies may provide better insight into the potential long-term effect of a pollutant due to the fact that larval and juvenile forms are considered the life stages most sensitive to discharges of wastes (e.g., Neff and Anderson, 1981; Rose and Ward, 1981; Capuzzo, 1987).

Several recent studies have investigated the sub-lethal effects of produced water upon early life stages of the west coast purple sea urchin *Strongylocentrotus purpuratus* (Krause et al., 1992), mussel, *Mytilus edulis* (Osenberg et al., 1992), and giant kelp, *Macrocystis pyrifera* (Reed and Lewis, 1992). These complementary field investigations in the Santa Barbara Channel reported effects upon marine organisms at distances far from the produced water outfall. Field toxicity tests in the vicinity of produced water discharges have indicated sub-lethal effects of these discharges on populations of the calanoid copepod *Acartia tonsa* (Girling, 1989) and drastic reductions in survival, settlement, and metamorphosis in the red abalone *Haliotis rufescens* (Raimondi and Schmitt, 1992).

Various studies have assessed the more subtle cytogenic and teratogenic effects of produced water. On a sub-cellular level, produced water has been found to cause chromosomal aberrations in sheepshead minnow (*Cyprinodon variegatus*) embryos exposed to low concentrations (2 and 4%) of produced water (Daniels and Means, 1989). Middaugh et al. (1988) also found that exposure of inland silverside embryos to 10 and 20% produced water generated statistically significant amounts of skeletal terata.

Test Organisms

Mysids, or opossum shrimp, have been widely used in toxicological studies

investigating the effects of pollutants (Nimmo and Hammaker, 1982). They are ideal test organisms due to their sensitivity to petroleum hydrocarbons (Anderson et al., 1974; Wells, 1982; Laughlin & Linden, 1983; Smith & Hargreaves, 1984; Carls & Korn, 1985; Neff et al., 1976) and metals (Reish and LeMay, 1986; Martin et al., 1989; Lussier et al., 1985). In addition, mysids are significant links in marine coastal food webs as planktonic predators (Fulton, 1982) and prey of fish and marine mammals (Singer, 1985; Love et al., 1991; Mauchline, 1980; Hobson and Chess, 1976; Wellington and Anderson, 1978). Two west coast mysid species were chosen for this study because of their presence in southern California reefs, kelp forests, and soft-bottom habitats (Coyer, 1983; Steve Bay, personal observation). An important criterion for selecting organisms for toxicity tests is the presence of indigenous species, since regional differences in environmental conditions exist. Also, the use of non-native species in toxicity tests risks their introduction into new areas.

One crustacean commonly utilized in effluent toxicity tests is *Holmesimysis costata* (= *Acanthomysis sculpta*), a mysid found primarily in dense aggregations above and within giant kelp canopies along the west coast. Reish and LeMay (1986) found that *H. costata* was the most sensitive species compared to twenty other west coast species tested with ten toxicants. Laboratory tests have also shown three day-old juvenile *H. costata* to be very sensitive to copper, zinc, and sewage effluents when compared to other crustaceans (Martin et al., 1989). Tatem and Portzer (1985) found that, compared to the Gulf Coast mysid *Mysidopsis bahia*, *H. costata* was more sensitive to sodium dodecyl sulfate but less sensitive to mercury. Other toxicity tests effectively using *H. costata* have assessed the effects of heavy metals on feeding and survival (Asato and Reish, 1988), the effects of tributyltin on survival, growth, and reproduction (Davidson et al., 1986), and the acute effects of drilling muds from southern California oil platforms (Machuzak and Mikel, 1987).

Several factors contribute to difficulties in using *Holmesimysis costata* for marine toxicity tests. *H. costata* has a long life cycle (about 120 days, Anderson et al., 1990) compared to other marine mysids, which makes it time-consuming to rear in the laboratory. In addition, reliance on wild populations can prove unreliable due to the species' seasonal changes in abundance (Turpen, 1992; Coyer, 1983). Lastly, differences in sensitivity to toxicants throughout the year (Hunt et al., 1991) may be attributable to stresses in the natural environment.

Laboratory cultures of the west coast epibenthic mysid *Mysidopsis intii* from sample populations collected in the outer Los Angeles harbor have been successfully maintained for five years at the Hatfield Marine Science Center, Oregon State University. This species has proved to be equally or more sensitive to several toxicants as *M. bahia* (Shaffer *et al.*, 1991), the standard mysid used in east and Gulf coast toxicity tests. Laboratory cultures of this species are more desirable than wild-caught mysids since gravid females are available year-round, their life cycle is short (19-20 days) (Langdon, 1990), and laboratory cultures may produce less genetically and seasonally variable results than wild caught populations.

Objectives

The objectives of this study were 1) to determine the chronic toxicity of produced water from several offshore oil platforms upon two mysid species, 2) to compare the relative sensitivity of the mysids *Mysidopsis intii* and *Holmesimysis costata* to produced water samples, 3) to determine whether growth or survival is a more sensitive endpoint for estimating toxicity of produced water to mysids, 4) to observe temporal differences in toxicity of produced water from the same site, and 5) to identify chemical components in each produced water sample which may have contributed to mysid toxicity.

MATERIALS and METHODS

Produced Water Sample Collection

Sampling was conducted at oil platforms in an open coastal area in southern California located between the mainland and the Channel Islands National Marine Sanctuary, which emphasizes the study sites' proximity to protected habitats (Figure 1). Four of the twelve platforms that discharge produced water into the federal outer continental shelf of southern California were sampled.

Due to the relatively low temporal variability observed in toxicity data using produced water (Moffitt et al., 1992) and the logistical problems involved with collecting 24-hour composited samples at offshore oil platforms, grab sampling was chosen for collecting a "representative" sample of produced water. Samples of produced water discharges from the production platforms were collected in pre-cleaned 5-gallon cubitainers without headspace. The cubitainers were sealed, packed in ice chests, and transported immediately by helicopter to the mainland. Subsamples were preserved by acidification to pH 2 with nitric acid for metals and sulfuric acid for oil and grease and phenols. Subsamples for cyanide analysis were preserved with sodium hydroxide to pH 12.

The produced water was then shipped on ice via overnight air freight to the U.S. Environmental Protection Agency Environmental Research Laboratory in Newport, Oregon. All toxicity tests were performed at the Hatfield Marine Science Center, Oregon State University, Newport, Oregon. All samples were stored at 4°C in darkness in sealed containers without headspace until initiation of tests. Renewals were conducted with partial samples stored at 4°C with headspace. All toxicity tests were conducted within four weeks of collection. Schiff et al. (1992) found that storage of produced water samples in this manner over an eight day period caused only minor changes in volatile aromatic hydrocarbons and total organic carbon (TOC), a measure of sample oxidation. In addition, Montgomery et al. (1988) observed similar toxicity responses in mysids exposed to

produced water samples tested immediately and after being held at 4°C for 4 and 8 weeks. Gould and Dorris (1961) observed no significant change in toxicity of refinery effluents to fathead minnows after 30 day storage at room temperature in 5-gallon polyethylene bottles with no headspace.

Chemical Analyses

Subsamples of each produced water sample were chemically analyzed for metals, cyanide, oil and grease, and phenolics. All chemical analyses were performed by chemists at the U.S. EPA Region IX Laboratory in Las Vegas, Nevada. Mercury was measured by direct-injection atomic absorption spectroscopy with the semi-automated cold vapor technique according to EPA method 245.2 (USEPA, 1983). Chromium, nickel, and arsenic concentrations were measured by graphite furnace Zeeman atomic absorption spectroscopy following EPA method 200.12 (USEPA, 1992). Copper, zinc, cadmium, silver, and lead were analyzed by Inductively Coupled Plasma - Mass Spectrometry following EPA method 200.10 (USEPA, 1992). Cyanide was analyzed colorimetrically using EPA method 335.3 (USEPA, 1983). Oil and grease analyses, determining the suspended fraction of free oil, were analyzed gravimetrically by EPA method 413.1 (USEPA, 1983). Phenols were measured spectrophotometrically by EPA method 420.1 (USEPA, 1983).

Mysid Cultures, Collection, and Handling

Organisms used in all tests were 3 day-old juvenile *Holmesimysis costata* and *Mysidopsis intii* released on the same day. Three day-old mysids were chosen since younger (1-2 day-old) mysids suffer high mortality in controls and older mysids tend to be less sensitive to toxicants (Martin et al., 1989 and Shaffer et al., 1991). *M. intii* juveniles were obtained from broodstock females maintained at the Hatfield Marine Science Center,

Oregon State University. Gravid females with eyed juveniles in the brood pouch were placed in a shallow square 38 liter aquarium (juvenile harvest system). Mysids were fed 2 day-old, algae-enriched (with *Chaetocerus calcitrans* and *Isochrysis galbana*, C-strain) *Artemia salina* and the harpacticoid copepod *Tigriopus californicus* to increase the release of viable young (Kreeger et al., 1991). The juveniles were released at night and migrated across a 1-mm mesh toward a 1-hour light source at 2:00 A.M. and 7:00 A.M. to separate them from the cannibalistic adult females. The newly-released juveniles (0 days old) were then held for 3 days at 20°C in a 20 liter aquarium and fed newly-hatched *Artemia* daily.

Holmesimysis costata were collected using dip nets at the surface of giant kelp (*Macrocystis pyrifera*) in Monterey Bay. Gravid females with eyed juveniles visible in the brood pouch were selected from the collected population and sent by overnight mail in a cooler maintained between 12°C-15°C to the U.S. EPA Environmental Research Laboratory in Newport, Oregon. After adult females were acclimated for at least one day, *H. costata* juveniles were released at night in a juvenile harvest system and held according to methods from Anderson et al. (1990). Samples from both mysid species were identified using appropriate keys. Holmquist (1979, 1981) was consulted for identifying *H. costata* and Holmquist (1957) for *M. intii*.

Reference Toxicant Tests

To verify the sensitivity of the test organisms and the precision of the tests, a 7-day static renewal reference toxicant test using zinc (as zinc sulfate) was performed concurrently with several produced water toxicity tests when enough mysids were available. One reference toxicant test was conducted with *Holmesimysis costata* and three with *Mysidopsis intii*. Zinc was chosen as the reference toxicant because 1) it has been routinely used in toxicity tests with *H. costata* (Martin et al., 1989; Anderson et al., 1990; Reish and LeMay, 1986) and *M. intii* (Shaffer et al., 1991), 2) it is a measurable toxicant,

3) it is a common constituent in produced water (Menzie, 1982) and sewage effluents (Schiff et al., 1992), and 4) it is relatively safe to use in the laboratory.

The toxicity tests consisted of three replicates, with eight to ten juvenile mysids in each replicate, exposed to each of five zinc concentrations: 0, 10, 18, 32, 56, and 100 $\mu\text{g/L}$ for *Holmesimysis costata* and 0, 32, 56, 100, 180, and 320 $\mu\text{g/L}$ for *Mysidopsis intii*. To maintain dissolved oxygen and pH at acceptable levels, test solutions were renewed after 48 and 96 hours. One liter plastic polypropylene beakers were used with the reference toxicant tests to avoid the sorption or leaching of trace metals that can occur with glass.

Produced Water Toxicity Tests

To determine sub-lethal and lethal effects of produced water to the mysids, comparative 7-day static renewal toxicity tests comparing *Mysidopsis intii* and *Holmesimysis costata* were conducted on each of the samples using body length and survival as final endpoints. The mysids were exposed to concentrations of produced water at levels comparable to those found near the discharges. Tests generally followed the methods specified by the USEPA (in prep, 1994) for *M. intii* and the State Water Resources Control Board (Anderson et al., 1990) for *H. costata*.

To determine effluent toxicity, mysids were exposed to a range of different produced water concentrations predetermined from range finder tests. One range finder test was conducted with *Mysidopsis intii* using each of the produced water samples except the second Platform 2 sample. The range finder tests were 7 days in duration and concentrations ranged from 0.056-32% produced water. The same concentrations were used for each species in each definitive toxicity test; however, concentrations were different for every produced water sample. Each experiment included four effluent treatments between 1 and 10% produced water and a control. The concentrations for each platform were as follows: 0.18, 3.2, 5.6, 7.0% for Platform 1; 1.8, 5.6, 7.0, 10% for Platform

2A; 3.2, 5.6, 7.0, 10% for Platform 2B; 0.56, 1.0, 1.8, 3.2% for Platform 3; and 0.18, 0.56, 1.8, 3.2% for Platform 4. Produced water and 0.7 μm -filtered seawater were dispensed into each test beaker to produce a 1 liter produced water concentration.

Table 1 summarizes test conditions for the reference toxicant and produced water tests. Large three liter test containers were chosen because of their high surface area-to-volume ratio, thus minimizing the need for aeration of the produced water. Three replicates were used per treatment, with eight or ten 3 day-old mysids randomly distributed among the three replicate containers. In conformity with the protocols, tests with *H. costata* were conducted in a 12°C room and *M. intii* in a 20°C room. The salinity of all treatments was $34 \pm 2\text{‰}$. No salinity adjustments were necessary since salinities of produced water samples were similar to those of dilution water. All Platform 3 test containers were aerated due to the nearly anaerobic conditions in the sample (dissolved oxygen levels were far below 65% saturation). All tests were conducted with a 16 h light:8 h dark photoperiod under ambient laboratory illumination in the temperature-controlled rooms. Mysids were fed daily with 75 newly-hatched *Artemia salina* nauplii per mysid to prevent cannibalism and ensure growth (Cripe et al., 1989). Test solutions were renewed after 48 and 96 hours by siphoning off 80% of the liquid and replacement with fresh test solutions. Dissolved oxygen (D.O.), temperature, pH, and salinity were measured before and after renewals in one randomly selected test container per concentration. The number of living mysids in each concentration was recorded before renewals and at the end of the experiment. Death was determined by gently prodding an inactive mysid with a glass rod and checking for movement.

Test Termination

Mortality was determined by the number of test organisms absent or inactive at the end of a 7-day period. All dead mysids were removed from the test containers daily and

missing mysids were presumed to have died and decomposed. Mortality due to cannibalism was considered unlikely due to ample brine shrimp available for mysid feeding. At the end of each experiment, surviving mysids from each replicate were placed in small sample vials in 10 milliliters of test solution and chilled to relax the animals. This procedure was designed to prevent abdominal curling during fixation. To achieve appropriate relaxation, different temperatures were required for the two species. *Mysidopsis intii* were refrigerated at 4°C for 4 hours and *Holmesimysis costata* were chilled at -20°C and removed prior to ice formation (~1 hour). The mysids were then fixed with a 5% buffered formalin solution, and the body length of each individual was measured using a video image analysis system. The total body length was defined as the distance between the anterior end of the eye to the dorsal posterior end of the uropod measured as the total distance of four lines along the lateral surface of the mysid (Figure 2).

Data Interpretation

All toxicity data were analyzed using the database program, Toxicity Information Management System (TOXIS) 2.2 (EcoAnalysis, 1993) and the statistical macro of Excel 4.0.

Point estimates

One measure of toxicity was the 7-day LC₅₀, or median lethal concentration, defined as the produced water or zinc concentration that is lethal to 50% of the test population within 7 days. Data were first tested for heterogeneity using the chi-square test (Zar, 1984). The 7-day LC₅₀s and 95% confidence intervals were estimated by using Probit analysis (Finney, 1971), the trimmed Spearman-Kärber method (Hamilton et al., 1977), or linear interpolation (USEPA, 1988a). Abbott's correction (Finney, 1971) was applied on control survival when necessary.

Growth toxicity data are expressed as the inhibition concentration (IC_p). The IC_5 is a value determined by linear interpolation estimating the concentration that causes a five percent decrease in growth relative to control organisms. The 7-day IC_{5s} and 95% confidence intervals were calculated using the TOXIS linear interpolation program based on the bootstrapping program developed by Battelle Columbus in cooperation with the U.S. EPA Environmental Research Laboratory, Duluth. Only an IC_5 could be calculated in this study since most tests showed only a 5% mean reduction in mean mysid length in treatments. The growth endpoint does not measure the change in mysid length but instead combines the initial length of mysids with growth during the test. Both *Mysidopsis intii* and *Holmesimysis costata* increase in size 1.5-2 millimeters from days 3 to 10 (Shaffer et al., 1991; Turpen, 1992).

Hypothesis testing

The data were first tested for normal distribution and homogeneity of variances using Shapiro-Wilk's test and Bartlett's test (Zar, 1984). If the assumptions of normality and/or homogeneity were not met, analysis by the robust Super-Dunnett procedure was used (EcoAnalysis, 1993). Where critical values and probabilities are not available, this procedure uses a simulation approach similar to that followed by Dunnett (1964) and Gaines and Rice (1990). The mortality data, expressed as binomial percentages, were arc-sine square root transformed before analysis (Zar, 1984).

For each test, the Lowest Observable Effect Concentrations (LOECs) for produced water toxicity were determined for growth and survival using a one-way ANOVA followed by Dunnett's multiple range comparison test (Zar, 1984) at a confidence level of 95%. Dunnett's test compares mean responses at each produced water concentration with the control mean and identifies those concentrations where responses are significantly different

from the controls. The LOEC is defined as the lowest concentration of the produced water tested in which survival or growth effects are statistically significantly different from the control (USEPA, 1988b). Thus, the lower the LOEC value, the more toxic the produced water.

Other statistical manipulations

To determine reference toxicity test precision, the coefficient of variation among LC₅₀s was calculated. To compare differences in toxicity between two sampling dates, a multiple-comparison ANOVA was conducted to determine significant differences between the variables of sampling time and concentration. Correlations between tests using different mysid species were assessed qualitatively by comparing LOECs and quantitatively by linear regression analysis of log-transformed point estimate values. Significant linear relationships were defined by $p \leq 0.05$ using Spearman's rank correlation (Zar, 1984).

RESULTS

Table 2 shows the depth of produced water discharges, the geological formations being tapped at each platform, and the average produced water volume discharged monthly from the platforms sampled for this study. Since all sites are located offshore in federal waters, discharges are in fairly deep water. Discharges vary in volume depending upon production capabilities and formations. Two of the platforms (2 and 4) are high discharge rate sites. Platforms 1 and 4 produce oil and water from the Repetto formation, Platform 3 farther offshore produces from the Monterey formation, and Platform 2 farther south produces from the Puente formation (Table 2).

Chemical Analyses

The chemical characteristics from each sample are summarized in Table 3. Several metal values fell outside of permit limits set for produced water dischargers in southern California (Federal Register, 1983), particularly in samples from Platforms 3 and 4. Excessive concentrations of silver, chromium, and cyanide were found in the Platform 3 sample. The Platform 4 sample contained the highest levels of metals, particularly zinc, nickel, mercury, and total chromium. None of the organic constituents analyzed exceeded permit limits; the highest concentration of phenols was 200 $\mu\text{g/liter}$ in the Platform 1 sample.

The physical parameters of all samples were reasonably consistent. Salinities of produced water samples were between 24-35 ‰. All samples were characterized by low dissolved oxygen concentrations (1.0 - 4.3 mg/L). The samples were fairly neutral, with pH (7.03-7.28) slightly lower than that for seawater (ca. 7.8-8.0).

Water Quality

Ranges of water quality measurements from each test are shown in Table 4. All

water quality parameters were within acceptable limits for the tests. The measurements showed relatively consistent salinity (32-35 ‰) and temperature ($12 \pm 2^\circ\text{C}$ and $20 \pm 2^\circ\text{C}$). Changes in pH were small, presumably due to the buffering capability of the dilution seawater. However, all samples showed a slight increase in pH with increasing concentration, possibly due to un-ionized ammonia (NH_3) in the produced water. Dissolved oxygen concentrations were 85% of saturation or higher. As concentrations of produced water from Platform 3 increased, the dissolved oxygen concentrations decreased, presumably due to the microbial activity in samples. With the exception of the Platform 3 test, *Holmesimysis costata* survival in the seawater controls was $\geq 80\%$, thus meeting the test acceptability criteria.

Reference Toxicant Tests

Three reference toxicant tests using zinc sulfate were conducted with *Mysidopsis intii* (Figures 3 and 4) and one test with *Holmesimysis costata* (Figure 5). The tests with *M. intii* indicated consistent sensitivity between batches of test organisms with similar dose response curves and identical LOECs ($320 \mu\text{g/L}$) for both survival and growth (Figures 3 and 4). In terms of variability, the survival endpoint had better precision with a coefficient of variation of 8.97% compared to the growth endpoint which had a larger value of 31.35%. The only reference toxicant test conducted with *H. costata* indicated that this species is more sensitive to zinc than *M. intii*. The mean length and survival of exposed *H. costata* were significantly lower than the control at $100 \mu\text{g}$ zinc/liter (Figure 5).

Produced Water Toxicity Tests

The point estimate results (LC_{50} s and IC_{50} s) from three comparative tests with the two mysid species indicate that the relative toxicities of produced water were measured similarly by both mysid tests (Figures 6 and 7). The point estimates from *Mysidopsis intii*

and *Holmesimysis costata* chronic tests were significantly correlated with each other for survival ($r^2 \geq 0.91$) and growth ($r^2 \geq 0.99$). The mysids did not differ in their growth sensitivities to produced water samples as seen by the similarity in point estimates and overlap of confidence limits. The lethal concentrations (LC₃₀s or LC₅₀s) of the two mysid species to produced water from Platforms 1, 3, and 4 samples shown in Figure 6 differ by less than a factor of two. Comparisons of the growth inhibition concentrations for the two mysid species are shown in Figure 7. The growth responses of the two mysid species were similar, with *H. costata* appearing to be slightly more sensitive in most instances, although not enough so to be statistically significant.

In Table 5, the effects on survival and growth are expressed as the LOEC, the lowest concentration of the produced water that is significantly different from the control. The hypothesis test results are quite different from the point estimate comparisons since a greater difference in LOECs existed between the two mysid species. The survival and growth LOECs for *Holmesimysis costata* in the Platform 1 produced water sample were lower than both LOECs for *Mysidopsis intii*. However, two of three tests using *Mysidopsis intii* had lower LOECs than *Holmesimysis costata* tests, indicating higher toxicity to *M. intii*. Most values for *Holmesimysis costata* are shown as "> [value]" because the highest concentration tested produced no statistically measurable effect.

Results are not shown for tests with *Holmesimysis costata* for Platform 3 because the test protocol acceptance criteria for control organism survival ($\geq 80\%$) was not met. These samples had a strong odor of hydrogen sulfide and low dissolved oxygen (1 mg/L in the original sample), probably due to biodegradation by micro-organisms. Thus, all test containers were aerated including a control treatment to maintain consistency in all treatments for quality control. There was a significant difference in controls with and without aeration (t test, $p = 0.05$), indicating that the aeration was the likely cause of

excessive mortality in this test. This comparison indicates that *H. costata* may be sensitive to the physical effects of aeration. On the other hand, *M. intii* tests showed no significant mortality in controls with aeration.

The produced water toxicity was not consistent between different platforms. Based on nominal concentrations of produced water, the 7-day LC₅₀ values for this series of tests ranged between 2.83 and >10% produced water (Figure 6). The 7-day IC₅ values among sites were between 0.93 and 8.05% produced water concentration (Figure 7). Survival and growth LOECs ranged from 0.18% to > 7% produced water from the four platforms (Table 5).

Samples of produced water from Platforms 1, 3 and 4 caused the greatest adverse effects on growth and survival of *Mysidopsis intii*. These produced water samples were approximately 2-3 times more toxic to *Mysidopsis intii* than the samples from Platform 2 (Table 5). The growth LOECs for Platforms 1, 3, and 4 were 3.2% (Dunnett's test, $p < 0.05$), while the growth LOECs of both Platform 2 samples were 7% produced water. In addition, the survival LOECs for Platform 2 samples (10% produced water) were higher than survival LOECs from all other platform samples, indicating less toxicity. Point estimates demonstrated the same toxicity relationships as LOECs, with LC₃₀ and IC₅ values for Platform 2 much higher (less toxicity) than values for all other platforms (Figures 6 and 7). Comparisons of point estimate values from Platforms 1, 3, and 4 differ by no more than a factor of 2.

A similar trend in produced water toxicity between sources was seen with tests using *Holmesimysis costata*. Among the three tests using *H. costata*, the samples from Platform 1 and 4 were the most toxic while the sample from Platform 2 was the least harmful. This is illustrated clearly by the point estimates (Figures 6 and 7), in which the shorter bars indicate greater toxicity. In addition, the point estimates from Platforms 1 and 4 closely match each other, indicating a similar toxic response by *H. costata*.

Based on estimates of produced water discharge and toxicity at each platform, the toxic units discharged per day were calculated to estimate the magnitude of toxic effects surrounding each platform (Table 6). Toxic units (TUs) are measures of toxicity in 100% effluent determined by dividing 100 by the effect concentration (%). The toxic units clarify the level of toxicity since higher toxic units equate to higher toxicity. Platforms 1, 3, and 4 discharged the highest chronic and acute toxic units per day. Platform 4 appeared to discharge over three times more toxic units per day than the other platforms.

The toxicity of Platform 2 samples collected at different times were very similar. This similarity was shown by identical survival and growth LOECs, 10% and 7% produced water, respectively (Table 5). No significant difference between time of sampling and different samples of produced water existed with the survival endpoint (ANOVA, $F_{2,5}$, $p < 0.05$). After normalization of the growth data (Zar, 1984), there was no significant difference in the growth endpoint between sampling dates ($F_{2,5}$, $p < 0.05$).

Qualitative comparisons between toxic effects of the two samples are shown in Figures 8 and 9. A similar survival dose-response was seen for both tests (Figure 8). Survival decreased as concentrations of produced water were increased, and mysid survival was significantly lower than the control at 10% produced water for both samples. For growth, the effects of produced water from the fall sampling followed a pattern similar to those effects from the spring sampling (Figure 9), in which 7% and 10% produced water concentrations caused significant differences in mysid length as compared to the controls.

Growth endpoints were consistently equivalent or more sensitive indicators of toxicity than survival endpoints. In all tests with *Mysidopsis intii*, growth LOECs were the same or lower than survival LOECs (Table 5). For samples from Platforms 1 and 2, growth effects in mysids were significant at concentrations that had no significant mortality. This implies that low levels of produced water contamination could have a significant effect on growth, but no direct effect on short-term survival of juvenile mysids.

Comparing the growth and survival dose-response graphs for *M. intii* illustrates differences in the two endpoints (Figures 10-12). The growth of *Mysidopsis intii* was reduced at a much lower concentration than survival for samples from Platform 1, as seen by a gradual downward slope in Figure 10. The survival and growth curves are similar for both samples from Platform 2. The LOECs were equal for survival and growth for Platform 3 at 3.2% produced water. However, the slope of the curve for survival shows a much steeper decline, indicating a more dramatic effect on survival than growth (Figure 11). The survival and growth curves are nearly identical for Platform 4 (Figure 12), indicating that growth and survival were fairly equal in sensitivity for this sample with significantly different responses from the control at 3.2% produced water.

DISCUSSION

Toxicity Range

Produced water significantly reduced the growth and survival of juvenile *Mysidopsis intii* at concentrations $\leq 10\%$ (represented by LOEC values) with all five samples tested. In addition, all calculable LC₅₀ and IC₅ values for both mysid species tests were less than 10% produced water. These results are consistent with the findings of Neff (1987), who determined that 37% of the produced water samples tested showed acute toxicities with LC₅₀s between 1 and 10%. In addition, similar results have been observed using the copepod *Acartia tonsa* (Girling, 1989) and the Gulf Coast mysid *Mysidopsis bahia* (Moffitt et al., 1992). Neff (1987) classified this level of toxicity as intermediate.

Most Toxic Samples

The samples of produced water from Platforms 1, 3, and 4 were substantially more toxic than the sample from Platform 2. These more toxic samples of produced water originate in an area clustered in the Santa Barbara Channel (Figure 1). These combined platforms discharge less produced water than Platform 2 (Table 2), which was shown to have lower toxicity produced water. However, the toxic units discharged per day from Platforms 1, 3, and 4 exceeded the toxic units discharged per day from Platform 2. It is very likely that the Repetto and Monterey formations from which these three platforms produce oil consist of a different chemical makeup from that of the Puente Formation farther south in the Los Angeles Basin and from which Platform 2 produces oil and water (Figure 1 and Table 2). This can be seen by a higher proportion of most pollutants in produced water from Platforms 1, 3, and 4 (Table 3). The toxic units discharged per day from Platform 4 were at least three times higher than all other platforms, which corresponds to the highest number and concentrations of metals found in the produced water sample.

Comparing Species

The results from comparative tests using point estimates demonstrate that the produced water had similar effects on both mysid species. All toxicity endpoints agreed within a factor of two and the point estimate endpoints correlated significantly. Point estimates may be better estimates of toxicity than hypothesis test estimates. Because hypothesis testing is dependent upon the range of concentrations chosen in each toxicity test, the true LOEC value may lie in between the concentrations chosen (USEPA, 1991a). Interlaboratory studies with seven-day *Ceriodaphnia dubia* survival and reproduction tests and fathead minnow larval survival and growth tests have demonstrated that endpoints determined by point estimation are significantly less variable than those estimated by hypothesis testing (DeGraeve et al. 1988; NCASI, 1990).

Conclusions drawn from test results expressed by hypothesis testing (Table 5) are fairly different from point estimate comparisons (Figures 6 and 7). As an example, hypothesis testing for Platform 4 showed that the test using *Mysidopsis intii* was more sensitive than the test using *Holmesimysis costata*, while point estimates indicated similar results for both species. Ranking sensitivity of the mysids to produced water samples is difficult since statistical sampling differed for both tests. The precision of hypothesis testing relies upon the variability of the data (Zar, 1984). It is possible that growth effects appeared greater for *M. intii* than for *H. costata* with hypothesis testing because of greater precision. Improved precision could have been due to better control performance, possibly due to the fact that *M. intii* were not stressed during shipping like *H. costata*. Another factor that improved precision in the tests with Platforms 1 and 2 samples with *M. intii* was a larger number of organisms as well as treatment and control replicates due to the quantity of juveniles available for testing. Both of these factors could lower variability among treatments and produce more sensitive statistical significance. Since the power of the ANOVA was lower for *H. costata* tests due to a smaller sample size, there was more of a

chance to commit a Type II error in which toxicity was not detected when it was present (Zar, 1984).

Toxicity testing endpoints are used to determine not only relative toxicity differences between pollutants but also to compare the sensitivities of standard test species. The inherent difference in test species, life stages, and duration of tests makes it difficult to compare toxicity data (Reish, 1988). When species from similar taxonomic groups are tested using the same test conditions and endpoints, comparative sensitivities are more valuable (Hemmer et al., 1992). Both survival and reproductive responses of two congeneric mysids, *Mysidopsis bahia* and *M. bigelowi*, to cadmium were identical in paired tests (Gentile et al., 1982). In comparisons between standard test species and locally representative species, *Mysidopsis bahia* and *Neomysis americana* were equally sensitive to sodium lauryl sulfate, cadmium, and Lannate (Roberts et al., 1982). Hemmer et al. (1992) attributed such similar sensitivities to toxicants in atherinid fishes to the close phylogenetic relationship between the test species. Thus, it appears that comparative species sensitivity to toxicants can be determined based on toxicity data if the test conditions, endpoints, and taxonomic group are similar.

Several factors must be considered when choosing either mysid toxicity test method for produced water toxicity tests. The sensitivity of *Holmesimysis costata* to the physical effects of aeration and inconsistent supplies of gravid females in the wild (Turpen, 1992; Coyer, 1983; this study) are factors to deliberate when using this mysid species. It must also be considered that test temperature differences may impact the toxicity of a sample. Since mysid respiratory exchange occurs through the inner surface of the carapace (Pearse et al., 1987), intake of toxic materials might occur faster in *Mysidopsis intii* at the higher test temperature (20°C vs. 12°C). Many studies have shown that higher temperatures increases toxicity of pollutants. Bryant et al. (1984) found that toxicity of chromium to three estuarine invertebrates increased as temperature increased. Increased temperature has

been linked with increased toxicity of chromium, nickel, and zinc in the mysid *Praunus flexuosus* (McLusky and Hagerman, 1987). Jones (1975) observed higher mortalities in marine isopods exposed to cadmium, zinc, and lead at higher temperatures. Since most produced water discharge pipes are located near the ocean floor, *M. intii* may also be a more representative indicator of toxicity since it is an epibenthic dweller. In addition, the result of a 7-day test may approximate the result of a life-cycle test more for *M. intii* than for *H. costata* since it represents at least one third of the mysid's life cycle. The short life cycle of *M. intii* also makes it amenable to life cycle toxicity tests with a reproductive endpoint. Tests using either mysid species are useful in determining the toxicity of produced water depending upon the circumstances.

The sensitivity of invertebrate species, particularly crustaceans, to produced water has been well documented. Hall et al. (1978) and Buikema et al. (1981) showed that crustaceans were more sensitive than other invertebrates and vertebrates to an "artificial reference mixture" similar in composition to produced water. This difference is presumably due to the smaller size of invertebrates and thus greater surface to volume ratio, as well as the vulnerability of crustaceans to toxicants between molts. Moffitt et al. (1992) concluded that the mysid *Mysidopsis bahia* was more sensitive to produced water samples than the sheepshead minnow *Cyprinodon variegatus*. Rose and Ward (1981) found that larval brown shrimp were the most sensitive organisms to produced water when compared to subadult and adult brown and white shrimp, adult crested blenny, and adult barnacles.

Growth and Survival Endpoints

Growth was consistently an equal or more sensitive measurement of toxicity than survival when comparing endpoints from produced water tests. This finding indicates that concentrations of produced water that are not high enough to cause mortality in mysids can inhibit growth. Results from zinc toxicity tests performed at Hatfield Marine Science

Center with *Mysidopsis intii* (USEPA, 1991b) and the Marine Pollution Studies Laboratory with *Holmesimysis costata* (Hunt et al., 1991) support this trend. In reference toxicity tests, mysids consistently showed reduced growth at concentrations where survival was not affected.

Long-term toxic effects on growth are significant since they may affect a population's ability to reproduce and perpetuate their species. In fact, Jensen (1958) established that the number of eggs produced by female mysids was directly related to body length. Lussier et al. (1985) found that life cycle tests with *Mysidopsis bahia* indicated that survival and growth are usually as sensitive to heavy metals as is reproduction. Thus, it is reasonable to assume that the produced water concentrations that reduce survival and growth of mysids in this study would also have impaired their reproduction. Reference toxicity tests in this study indicated that survival of *M. intii* was less variable than growth. In addition, Breteler et al. (1982) found that growth measurements were less variable than reproduction in determining chronic effects of silver and endosulfan on *M. bahia*. Therefore, it appears favorable to assess chronic toxicity by observing effects on both survival and growth of mysids due to the lower variability in these endpoints.

Reduction in mysid mean length could be due to several factors. One of these factors may be decreased feeding during exposure to toxicants. Mobility in some mysids was impaired due to visible oil droplets clinging to the appendages or difficulties with equilibrium. This was particularly apparent on both mysid species in the two highest concentrations of produced water from Platforms 2 and 4. This lack of mobility may have reduced the mysids' ability to locate and capture prey. Asato (1988) observed significant reductions in feeding of *Holmesimysis costata* during acute exposure to several metals. Furthermore, Cripe et al. (1989) observed a reduction in the swimming stamina of *Mysidopsis bahia* exposed to organophosphate insecticides. Loss of equilibrium, decreased growth, and reduced feeding activity were all noted prior to death in toxicity tests

exposing *M. bahia* to pesticides (Nimmo et al., 1981).

Another possible factor contributing to reduced growth during exposure to produced water may be interference in the molting process. Gentile et al. (1982) attributed morphological aberrations and mortality in mysids to cadmium inhibiting calcification during molting. Neff et al. (1976) observed inhibited molting in crab (*Rithropanopeus harrisi*) larvae exposed to high concentrations of oil solutions. Furthermore, Mecklenburg et al. (1976) found that high concentrations of the water soluble fraction of oil caused reduced molting success in crab and shrimp species.

Temporal Differences in Produced Water Toxicity

The similar concentration responses seen with two separate samples of produced water from Platform 2 indicated that the temporal variability in the produced water from that site was probably low. There was no statistically significant difference between the dose-response effects upon *Mysidopsis intii* survival and growth for samples collected 4 months apart from the same site. Although this comparison only involves one site and two sampling dates, other studies support this temporal consistency in produced water samples through chemical and toxicological investigations. Moffitt et al. (1992) found no statistically significant differences between toxicity test results with mysids and sheepshead minnows from three samples collected at two week intervals from the each of eight platforms over a two month period. Boesch and Rabalais (1987) reported only subtle temporal variation in chemical constituents from the same formation. This consistency in produced water composition and toxicity is useful in temporal monitoring since large differences in produced water over time can be identified.

Reference Toxicant Tests

The effects of zinc on both mysid species was fairly consistent with previous

studies. Hunt et al. (1991) found the range of survival LOECs for *Holmesimysis costata* between 32-100 $\mu\text{g/L}$ and growth LOECs between 10 and $\geq 32 \mu\text{g/L}$ in eight zinc toxicity tests. This is fairly comparable with the LOEC of 100 $\mu\text{g/L}$ zinc determined for both survival and growth in this study. However, growth of *H. costata* in the present reference toxicant tests was impacted slightly less than mysid growth measured in some previous studies. This difference is shown by LOEC values several concentrations higher than those previously reported. These disparities may be due to differences in methods of growth measurements, seasonal sensitivity of mysids, dilution water, and test container size.

Growth LOECs for *Mysidopsis intii* have been reported as $\geq 180 \mu\text{g/L}$ zinc (Hunt et al., 1991) and survival LOECs $\geq 100 \mu\text{g/L}$ zinc (USEPA, 1991b) and $\geq 180 \mu\text{g/L}$ zinc (Hunt et al., 1991). In this study, the survival and growth LOECs for *M. intii* exposed to zinc were consistently 320 $\mu\text{g/L}$. Since zinc concentrations were not high enough to produce toxic effects in many previous studies, it is difficult to compare LOEC values among studies. One study showed a low growth LOEC of 50 $\mu\text{g/L}$ zinc (Langdon et al., 1989) for a 7 day test; however, this study was conducted with newly released juvenile *M. intii* which are probably more sensitive than 3 day-olds used in this study. Further studies (USEPA, 1991b) indicated that growth of 4 day-old *M. intii* were not adversely affected by zinc exposures of 50 $\mu\text{g/L}$. However, growth of juveniles exposed to zinc throughout their brood pouch developmental period and their first 4 days post-release were significantly affected at 50 $\mu\text{g/L}$ zinc.

The reference toxicity test results are consistent with other interlaboratory tests comparing both mysid species' sensitivity to zinc. In trials between the two mysid species, Hunt et al. (1991) found tests with *Holmesimysis costata* more sensitive. *Mysidopsis intii* survival had a LOEC of $>180 \mu\text{g/L}$ zinc, while *H. costata* was more sensitive with a survival LOEC of 56 $\mu\text{g/L}$ zinc. Growth effects were less conclusive with *M. intii* LOEC $>180 \mu\text{g/L}$ zinc and *H. costata* growth LOEC $>100 \mu\text{g/L}$ zinc. Similarly,

in the first comparative 7-day chronic tests conducted with both mysid species (USEPA, 1991b), *H. costata* had a survival LOEC of 32 $\mu\text{g/L}$ zinc and growth LOEC of $>100 \mu\text{g/L}$ zinc. The survival and growth LOEC for *M. intii* was $>100 \mu\text{g/L}$ zinc. Although the growth LOEC values for both mysids were not conclusive since no significant difference between treatments and controls was observed, the lower LOEC values of 32 and 56 $\mu\text{g/L}$ zinc for *H. costata* survival supports the conclusion of Martin et al. (1989) that it is more sensitive to zinc than other crustaceans recorded in the literature.

Relation to Field Studies

Most field studies assessing the chronic biological impacts from offshore produced water discharges have highlighted effects on benthic communities rather than on the planktonic organisms (Neff, 1987; Neff et al., 1992; Payne et al., 1987; Spies, 1987; Mancini and Stilwell, 1992; Rabalais et al., 1992). This focus on benthic communities is primarily because dilution in the water column (especially in high energy coastal systems) is thought to eliminate any toxic effects of discharges. Moreover, accumulation of contaminants in the sediments is more likely to affect benthic communities.

However, field studies investigating the effects of produced water have shown widespread water column impacts. Raimondi and Schmitt (1992) demonstrated effects on young larval red abalone (*Haliotis rufescens*) survival, settlement, and swimming behavior when out planted at a distances of up to 100 meters from a produced water outfall in Santa Barbara Channel. When the produced water outfall was not operating, no effects on larval abalone survival and settlement could be detected at any distance. These field studies were supplemented with laboratory studies to confirm that effects on abalone in the field were caused by a produced water discharge. Direct exposure to produced water in concentrations as low as 0.01% in laboratory tests resulted in depressions in abalone settlement and cessation in larval swimming behavior at 10% produced water

concentrations. Quantitatively, a predicted value of toxicity based on field dilution at certain distances from the outfall was remarkably similar to the laboratory experiment settlement results. Furthermore, Osenberg et al. (1992) demonstrated that mussels suspended closest to a produced water discharge in Santa Barbara Channel grew slower and had less tissue than mussels farther from the outfall.

Other mesocosm studies have shown significant toxicity due to produced water discharges. Water column mesocosm studies in the North Sea (Hay et al., 1988; Gamble et al., 1987) found that crustacean larvae were more sensitive than fish larvae to typical field concentrations of produced water. Large plastic enclosures were placed in the field with diluted produced water to simulate effects approximately 0.5-1 km away from the discharge. In treated enclosures, significant mortality and reduced growth occurred in the early larval stages of the copepod *Acartia tonsa* but not among herring or cod larvae. Research conducted on freshwater produced water discharges have also suggested that laboratory toxicity tests can be predictive of instream effects. Mount et al. (1992) found that laboratory toxicity tests with *Ceriodaphnia dubia* correlated with effects of the produced water upon instream effects on a benthic community.

The above field toxicity studies that assessed produced water effects on communities where covarying factors existed support laboratory toxicity studies that demonstrate significant effects on marine organisms at certain concentrations. Although a quantitative linkage between field and laboratory studies is difficult, these studies revealed that field responses were most likely caused by exposure to produced water. These studies also suggest that persistence of contaminants in the water column from produced water may cause effects over a larger distance than originally thought.

Chemical Analyses

The physical characteristics in all produced water samples was consistent with prior

characterization of produced waters in southern California. High biological oxygen demand (BOD), a measurement of dissolved oxygen consumed by microbes, is characteristic of produced water in the Santa Barbara Channel (USEPA, 1985). The salinity of the produced water samples was similar to that found by Piltz (1983), who reported that California produced water samples approximate normal seawater salinity. The samples had neutral to slightly acidic pH, which is typical for most produced waters (Boesch and Rabalais, 1987).

Oil and grease analysis determines the amount of oil content in a sample, including hydrocarbons (Simms et al., 1992). Since mechanical filtration does not remove all oil droplets, petroleum hydrocarbons have been suspected as the major toxic components in produced water (Boesch and Rabalais, 1987; Menzie, 1982). However, additional manipulations have decreased the potential toxic material in produced water. Evaporation, aeration, or holding has reduced the concentration of volatile organics and reduced the toxicity of the produced water over time (Grahls-Nielsen, 1987; Moffitt et al., 1992; Fucik, 1992). In addition, Lysyj (1982) showed that gravity separation of produced water from crude oil prior to discharge reduced suspended oil and volatile aliphatic hydrocarbons. Some studies have demonstrated that toxicity of produced water is not associated with petroleum hydrocarbons. Brown et al. (1992) found that base-soluble polar organic fractions co-extracted with petroleum hydrocarbons from produced water with Freon did not cause significant toxicity to *Mysidopsis bahia*. In another toxicity identification evaluation of produced water, Higashi et al. (1992) found that organic fractions including hydrocarbons were not responsible for toxicity to mussel (*Mytilus californianus*) embryos. In addition, Schiff et al. (1992) demonstrated that concentration changes in the volatile aromatic hydrocarbons (benzene, ethylbenzene, toluene, and xylenes) in produced water had no substantial effect on results of mysid (*M. bahia*) toxicity tests. In support of these findings, the three platforms where produced water caused the highest toxicity (Platforms

1, 3, and 4) in this study did not contain excessive levels of oil and grease. Thus, the low levels of oil and grease were probably not substantial enough to cause toxic effects on the mysids.

Phenolic compounds were detected in all samples at concentrations consistent with other produced water studies (Lysyj, 1982; Neff et al., 1988). Phenols are present in crude oil and are highly soluble in water, which explains their persistence in produced water (Grahl-Nielsen, 1987). Lysyj (1982) determined that although aliphatic hydrocarbons were reduced with gravity separation of produced water, phenols still persisted in significant amounts. Phenols may be more toxic than aromatic hydrocarbons because they are more water soluble and persistent in the aqueous phase (Grahl-Nielsen, 1987). Many chlorophenolic biocides may be added during the treatment process, and they are known to cause significant toxicity as measured by grass shrimp (*Palaemonetes pugio*) limb regeneration (Rao et al., 1978). Exposure of marine crustaceans to various levels of phenols have revealed similar lethal effects. The 96-hour LC₅₀ of phenol was 5.8 µg/L to the grass shrimp *Palaemonetes pugio* (Tatem et al., 1978) and 5.0 µg/L to the mysid *Mysidopsis bahia* (Buikema et al., 1981). The produced water from Platform 1 had the highest phenolic content (200 µg/L), which may have been the primary source of toxicity in this sample. The lower test temperature of 12°C would theoretically maintain a persistence of volatile phenols, which may explain why the toxicity of this sample was greater to *Holmesimysis costata*.

Although hydrogen sulfide was not measured, the sample from Platform 3 had a distinct smell of the compound, which indicates that a measurable amount was present. Dissolved hydrogen sulfide is a common component in produced water due to its persistence in oil formations (Boesch and Rabalais, 1987). In addition to some high levels of metals, hydrogen sulfide may have contributed to the toxicity of this sample to *Mysidopsis intii*. Calanoid copepods have exhibited decreased growth to levels as low as

68 $\mu\text{g/L}$ hydrogen sulfide (Vargo and Sastry, 1977). White sea urchins (*Lytechinus pictus*) from southern California exposed for 49 days to 15.5 mg/L hydrogen sulfide showed increased mortality, lower gonad wet weight, and decreased growth of test diameter compared to controls (Thompson et al., 1991). Sauer et al. (1992) and Fucik (1992) attributed the toxicity of some produced water samples to hydrogen sulfide based upon toxicity after fractionation.

Some metals were detected at concentrations that exceeded both ambient seawater levels and permit limitations for the discharges. These concentrations are consistent with other studies showing that significant concentrations of metals are discharged from oil-producing platforms (Stephenson, 1992; Lysyj, 1982; Boesch and Rabalais, 1987). Lysyj (1982) reported that lead, chromium, nickel, and zinc comprised the majority of the toxicants present in produced water discharges. Menzie (1982) reported that cadmium, chromium, manganese, and barium levels in produced water from Buccaneer Field, Texas, exceeded seawater concentrations by factors of 10-100. Elevated levels of metals have also been found in the sediments surrounding many offshore oil platforms near produced water discharges (Boesch and Rabalais, 1987; Rabalais et al., 1991). Higashi et al. (1992) reported that the non-volatile components (primarily barium and strontium) in produced water from a southern California produced water discharge caused most of the toxicity in mussel embryo tests.

In this study, produced water from Platforms 3 and 4 displayed the most toxic effects upon *Mysidopsis intii*, and these samples also showed consistently higher values of metals than any of the other samples. High levels of silver, chromium, and cyanide were found in the Platform 3 sample. The Platform 4 sample contained the highest levels of metals, particularly zinc, nickel, mercury, and total chromium. These data suggest that certain heavy metals may contribute to the toxicity of the produced waters tested. Although the concentration of these metals was diluted for toxicity test concentrations, the cumulative

or synergistic effects of several metals may have caused toxicity. Negilski et al. (1981) demonstrated that the interactive effects of zinc, cadmium, and copper on mortality of the shrimp *Callinassa australiensis* was always greater than effects observed for each metal singly.

The toxicity of metals to mysids has been well documented. Reish and LeMay (1986) found that *H. costata* was the most sensitive of several invertebrates tested to zinc, cadmium, and copper. Martin et al. (1989) also reported lower LC₅₀ and NOEC values for *H. costata* exposure to zinc sulfate than previously reported for a marine crustacean. Nimmo et al. (1978) determined that *Mysidopsis bahia* was more sensitive to cadmium than other crustaceans tested.

Chemical fractionation of produced water followed by toxicity tests has been conducted by Fucik (1992) and Higashi et al. (1992) to attempt to isolate the constituents responsible for toxicity. These studies demonstrate that synergistic interactions between several toxicants may cause toxicity, which confounds attempts to isolate single toxicants in produced water. They also demonstrated that some toxicity is likely caused by or contributed to by the many constituents present in produced water that are not routinely measured in chemical analyses. Thus, the chemical make-up of produced water discharges is complex, and simple generalizations linking their chemistry and biological effects cannot be made.

Conclusions

In summary, the results of this study show that growth and survival of *Mysidopsis intii* were significantly decreased at or below concentrations of 10% produced water. The two west coast mysid species were comparable in sensitivity when concurrently exposed to three produced water samples. The growth inhibition of *M. intii* by day 7 was an equal or more sensitive indicator of sublethal toxicity than survival. The toxicity effects of produced

water samples from Platform 2 collected 4 months apart were the same. Lastly, samples from three platforms in the Santa Barbara Channel containing high metal concentrations were more toxic than samples from one platform over a different oil-bearing formation south of Los Angeles.

LITERATURE CITED

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Table 1. Test conditions for *Holmesimysis costata* and *Mysidopsis intii* toxicity tests.

TEST DURATION	7 days
TEST TYPE	static renewal
ENDPOINTS	survival and growth
TEST VOLUME	1 liter
# CONCENTRATIONS	4-5 + control
# REPLICATES	2-3
# MYSIDS/REPLICATE	8-10
AGE OF MYSIDS	3 days
FEEDING REGIME	75 <i>Artemia</i> /mysid/day
PHOTOPERIOD	16L:8D
SALINITY	34 ± 2 ‰
DISSOLVED OXYGEN	≥ 65% saturation
TEMPERATURE	20 ± 2 °C <i>Mysidopsis intii</i> 12 ± 2 °C <i>Holmesimysis costata</i>

Table 2. Comparison of produced water discharge volumes, formations, and depth of discharges from all sites.

Site	Formation	Discharge (bbls/month)	Discharge Depth (m)
Platform 1	Repetto	42,000	59
Platform 2	Puente	835,000	49
Platform 3	Monterey	46,049	97
Platform 4	Repetto	150,000	46

Table 3. Comparison of chemical constituents by study areas. All values are based on a single set of analyses from one sample and are expressed in $\mu\text{g/L}$ except where noted. Shaded values exceeded permit limits (Federal Register, 1983). a = compound not analyzed, * = compound not detected.

Parameter	Detection Limit	4/27/92 Platform 1	5/6/92 Platform 2	9/8/92 Platform 2	4/19/92 Platform 3	5/20/92 Platform 4
Oil & Grease (mg/L)	4.00	15.19	30.55	8.00	15.00	4.25
Phenols	4.00	200.00	93.00	20.00	87.00	74.00
Arsenic	2.00	*	*	2.20	*	30.00
Cadmium	0.04	*	*	1.10	*	37.00
Chromium	1.00	10.00	*	4.20	30.00	37.00
Copper	0.02	28.00	*	2.00	45.00	74.00
Cyanide	5.00	20.00	*	12.40	90.00	74.00
Lead	0.07	*	*	1.80	7.00	74.00
Mercury	0.02	0.20	*	0.30	*	38.00
Nickel	1.80	230.00	*	18.00	140.00	2700.00
Silver	2.00	30.00	*	2.70	100.00	*
Zinc	5.00	110.00	110.00	a	160.00	369.00

Table 4. Ranges of water quality parameters measured during toxicity tests with *Holmesimysis costata* and *Mysidopsis intii*. Each range reflects measurements from all test concentrations. PW = Produced Water, D.O. = Dissolved Oxygen.

Toxicant	Species	Date	Temperature °C	D.O. mg/L	pH	Salinity ppt
Zinc	<i>M. intii</i>	5/5/92	19.4-21.2	6.9-7.8	7.89-8.17	32-34
Zinc	<i>M. intii</i>	5/31/92	20.2-21.3	6.8-7.4	7.71-7.787	35
Zinc	<i>M. intii</i>	6/9/92	19.0-22.0	7.2-7.8	7.81-7.99	32-34
Zinc	<i>H. costata</i>	5/5/92	11.2-13.4	8.6-9.5	7.34-8.26	32-35
PW 1	<i>H. costata</i>	5/31/92	11.1-12.0	7.6-9.9	7.5-7.92	35
PW 2A	<i>H. costata</i>	6/9/92	11.5-12.3	8.5-9.6	7.46-7.71	32-34
PW 4	<i>H. costata</i>	6/2/92	11.7-12.2	7.4-10.0	7.49-7.95	34-35
PW 1	<i>M. intii</i>	5/31/92	19.0-21.3	7.2-7.5	7.62-8.19	34-35
PW 2A	<i>M. intii</i>	6/9/92	19.3-21.2	7.1-8.5	7.57-7.88	32-35
PW 2B	<i>M. intii</i>	9/20/92	19.9-22.5	7.4-9.2	7.64-8.17	34-35
PW 3	<i>M. intii</i>	5/5/92	18.1-20.9	6.8-8.2	7.81-8.27	34-35
PW 4	<i>M. intii</i>	6/2/92	19.0-20.9	7.1-8.0	7.70-8.21	34-35

Table 5. Seven day survival and growth Lowest Observed Effect Concentrations (LOECs) calculated for each produced water toxicity test with the mysids *Mysidopsis intii* and *Holmesimysis costata*. All values are expressed as concentration in % of the original produced water sample. Values reported as ">" signify no LOEC could be determined with the concentrations tested.

	SURVIVAL LOEC	GROWTH LOEC
PLATFORM 1		
<i>Mysidopsis intii</i>	7.0	3.2
<i>Holmesimysis costata</i>	3.2	0.18
PLATFORM 2A 6/9/92		
<i>Mysidopsis intii</i>	10.0	7.0
<i>Holmesimysis costata</i>	>7.0	>7.0
PLATFORM 2B 9/20/92		
<i>Mysidopsis intii</i>	10.0	7.0
PLATFORM 3		
<i>Mysidopsis intii</i>	3.2	3.2
PLATFORM 4		
<i>Mysidopsis intii</i>	3.2	3.2
<i>Holmesimysis costata</i>	>3.2	>3.2

Table 6. Chronic and acute toxic units discharged per day for *Mysidopsis intii* at each platform. TUa = acute toxic units = 100/LC50. TUc = chronic toxic units = 100/NOEC. TU/day = TU/liter * liters produced water discharged/day. Each platform is ranked from highest to lowest toxic units discharged per day. - calculation was not possible since no LC50 could be determined.

Site	TUa/day	Rank	TUc/day	Rank
Platform 1	3,591,810	#3	13,250,000	#3
Platform 2	-	-	4,259,610	#4
Platform 3	8,833,218	#2	14,528,525	#2
Platform 4	30,102,106	#1	47,325,214	#1

Figure 1. Site map showing general location of produced water discharge sites sampled in southern California.

Figure 2. Juvenile mysid illustration with location of straight lines used to measure total length with video image analysis system. Total length was measured from the tip of the eye to the posterior end of the uropod, excluding the setae.

Figure 3. Reference toxicant tests showing survival effects on *Mysis intii*. Lowest Observed Effects Concentrations (LOECs) are marked with an x.

Figure 4. Reference toxicant tests showing mean length effects on *Mysidopsis intii*. Lowest Observed Effects Concentrations are marked with an x.

Figure 5. Reference toxicant tests showing growth and survival effects on *Holmesimysis costata*.

Figure 6. 7-day survival LC_{50} values (% produced water) for *Holmesimysis costata* and *Mysidopsis intii*. Vertical bars represent 95% confidence intervals. *Due to low mortality, no lethal median concentration (LC_{50}) could be estimated, so an LC_{30} concentration was calculated for comparison. Values without confidence intervals are best estimates based on the data.

Figure 7. 7-day growth IC_5 values (% produced water) for *Holmesimysis costata* and *Mysidopsis intii*. Vertical bars represent 95% confidence intervals.

Figure 8. Survival response of *Mysidopsis intii* to produced water from both Platform 2 samples.

Figure 9. Growth response of *Mysidopsis intii* to produced water from both Platform 2 samples.

Figure 10. Dose-dependent growth and survival rate decrease of *Mysidopsis intii* due to produced water concentrations from Platform 1.

Figure 11. Dose-response for growth and survival of *Mysidopsis intii* exposed to produced water from Platform 3.

Figure 12. Mean concentration-response curves for *Mysidopsis intii* 7-day survival and growth for Platform 4 produced water.