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Planktonic Influence on the Toxicity of HEWAFS (High-Energy Water Attenuated Fractions)

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PLANKTONIC INFLUENCE ON THE TOXICITY OF HEWAFS (HIGH ENERGY WATER ATTENUATED FRACTIONS)

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ABSTRACT

The *Deep Water Horizon* oilrig explosion led to the release of $6.8 \pm 1.7 \times 10^8$ kg or 4.9 million barrels of petroleum hydrocarbons into the Gulf of Mexico. The spill persisted from the end of April to mid July 2010 affecting a significant portion of the Gulf coast. The effects of this spill are being studied in labs across the country, and this thesis investigated high-energy water accommodated fractions (HEWAFs) as a potential tool for use in this effort. Water accommodated fractions (WAFs) are mediums containing only the fraction of petroleum that remains in aqueous phase after a mixing energy has been removed, and after a period sufficient for phase separation. HEWAF was the exposure medium of choice because this type of WAF is created by vigorous mixing of seawater with crude oil, similar to what occurs in the environment. The effects of plankton (a zooplankton and phytoplankton species) on the toxicity of HEWAFs were also investigated because the spill occurred at a time of year when plankton populations are especially high in the Gulf of Mexico. Dr. Sarah Webb looked at how the HEWAFs and inclusion of plankton affected the test species, *Anchoa mitchilli*. The inclusion of plankton was also meant to more closely resemble the natural environment, and give a more accurate indication of oil spill dynamics in the Gulf ecosystem. The trials conducted throughout this experiment demonstrated that concentrations of aromatics and alkanes increased steadily with increases in HEWAF loadings. At high HEWAF loadings (33.3% HEWAF and above), alkane and aromatic concentrations in samples containing plankton were elevated; however, this effect dropped off in lower HEWAF concentrations (15% HEWAF and below). HEWAF shows potential as a tool in exposures simulating GoM conditions, but much more work needs to be done before the

results they yield are reliable. The species responsible for the planktonic effect seen in the high HEWAF loading studies was not determined in this study; however, the results do show that plankton might play a significant role in crude oil partitioning in the environment. This would make plankton important in exposures for determining real world oil toxicities.

CHAPTER 1. INTRODUCTION

Petroleum is a complex mixture of predominately hydrocarbon compounds. Most of these compounds are either straight chain (alkane) or ring (aromatic) structures [1]. The aromatic fraction contains polycyclic aromatic hydrocarbons (PAHs) which are chiefly responsible for the toxicity of oil; they are genotoxic, carcinogenic, and mutagenic [2]. Accurate ways to measure the toxicity of crude oil on the environment are important because roughly 3 million tons of oil enters the ocean each year, and rising demand is increasing the frequency of oil spills [3].

The *Deep Water Horizon* (DWH) oil spill occurred on April 20, 2010. It released an unprecedented 780,000 cubic meters of crude oil into the Gulf of Mexico (GoM) [4]. Over an 87 day period, the oil spilled from the Macondo 252 (MC252) well 1,500 meters below the water's surface. This long journey through the water column and the addition of dispersants created a viscous emulsion [5]. The DWH oil rig was located 76 kilometers southeast of Louisiana's coastline, and the emulsified crude oil traveled as far as 300 kilometers through GoM waters to oil 1,773 kilometers of shoreline from Louisiana to Florida [4].

Gulf fisheries are among the most productive in the world, and as a result, many research projects have been initiated to better understand the effects of this colossal spill. The water-accommodated fraction (WAF) is the accepted tool for evaluating oil toxicity on marine organisms [6]. They are mediums that contain only the water soluble fraction of petroleum, the portion responsible for most of oil's toxicity [2].

There are guidelines for WAF preparation that make them more reproducible [6]. Some of these guidelines, namely mixing velocity, are not realistic for recreating

environmental conditions. For this reason, this study used the Hemmer method for preparation of a High Energy Water Accommodated Fraction (HEWAF) [7]. The use of an MC252 surrogate crude oil and the HEWAF method should yielded results that are directly relatable to those created by the oiling event.

The GoM has a large, diverse plankton population [8]. Because of their position at the base of the aquatic food web, and phytoplankton's high lipid content, they are pertinent to oil exposure studies. They were included to more closely mimic natural conditions, and determine if their presence affects WAF toxicity. Their effect on HEWAF toxicity was evaluated by using Gas Chromatography coupled with Mass Spectrometry (GC/MS) to analyze the hydrocarbon concentrations of the alkane and aromatic fractions.

The experiments included the test species *Anchoa mitchilli* (Bay Anchovy). They are not recreationally or economically valuable, but they are an important food source for significant predatory fish and sea birds [9]. Bay Anchovy are one of the most abundant fish in the GoM, and are often a dominant species in stressed environments [10]. This made them a good candidate for toxicity testing. Dr. Sarah Webb, a post-doc at Louisiana University Marine Consortium (LUMCON), evaluated the effects (mortality and physical deformities) of the HEWAFs on embryonic and larval stages of the *Anchoa m*.

CHAPTER 2. REVIEW OF LITERATURE

2.1 Deep Water Horizon Oil Spill

The Deep Water Horizon oilrig exploded on April 20, 2010. Approximately 6.8 ± 1.7 x 10^8 kg or 4.9 million barrels of petroleum hydrocarbons were subsequently released from the Macondo Well over an 87-day period (until July 15, 2010) [11, 5]. Soluble and volatile hydrocarbons dissolved in the water column, and about 70,000 tons of larger petroleum hydrocarbons (larger than n-C_{15}) formed slicks, and oiled beaches [11]. The oil came on shore over an extended time, the majority within a 3-month period [5].

Although fingerprinting of the crude oil has been used to verify the Macondo well as the source, the character of the oil was different from other spills because it was released from the sea floor and rose through 1,500 meters of water [11, 5]. It was also treated with dispersants subsea and on the surface, and travelled 80-300 km in warm Gulf water to reach the shoreline. The oil that reached the Gulf Coast was a thick viscous emulsion (60% water). 1,773 km of shoreline has been documented as having been oiled, majority of which was in Louisiana (60.6%) [5]. This thesis aimed to determine how the toxicity of the oil released in the DWH spill (MC252) may have been affected by the presence of plankton.

2.2 Crude Oil Composition

Petroleum forms by breaking down large molecules of fats, oils, and waxes that make up kerogen with heat and pressure [1]. This process results in hundreds of compounds, which together are 83-87% carbon, 11-16% hydrogen, 0-4% oxygen plus nitrogen, and 0-4% sulfur. Most of the hydrocarbon compounds are made of 5 to 20 carbon atoms in either straight chain (alkane) or ring (aromatic) structures [1].

Petroleum is separated into 4 fractions: saturated, aromatic, resin, and asphaltene. The saturated fraction of crude oil consists of hydrocarbons with no double bonds, either alkanes (C_nH_{2n}+2) or cycloalkanes (C_nH_{2n}) [12]. Compounds in the aromatic fraction contain 2-13 benzene rings (C_6H_6) [13]. The other two fractions contain non-hydrocarbon polar compounds with trace amounts of nitrogen, sulfur, and oxygen. These two heavier fractions form complexes with heavy metals [12].

Because petroleum is made of thousands of hydrocarbon compounds and other substances with different physical and chemical properties, it is impossible to measure them all with one technique; so, this study focused on the light fractions, alkanes and aromatics [3]. Alkane and aromatic content was determined with GC/MS [1].

A high demand for petroleum has led to an increase in petroleum contamination in marine and estuarine environments. About 3 million tons of oil per year enters the ocean [3]. The effects of various spills can have drastically different effects due to differences in the environment, hydrocarbon composition, and concentration of the petroleum released [3]. This experiment tried to determine some of the unique interactions that occurred between MC252 oil and a local species, Bay Anchovy, in simulated natural conditions through the inclusion of plankton. This thesis focuses on the interaction between MC252 water-accommodated fraction toxicity and plankton.

MC252 is a Louisiana sweet crude. Sweet crude is a type of petroleum that contains less than 0.42% sulfur, anything above is referred to as sour crude. The oil that spilled from *Deep Water Horizon* is classified as light sweet crude, because a very large portion of it can be processed into gasoline, kerosene, and high-quality diesel [14]. The exposure experiments analyzed in this thesis used an MC252 surrogate because only

limited amounts of the actual oil were recovered from the DWH spill, and it is in high demand for experiments. In response to this need BP called for selection of an acceptable surrogate based on the similarity of toxicological and analytical properties [15]. Crude oil from the Gulf of Mexico platform, Marlin (36 miles northeast of Macondo), was chosen because it had the most similar geochemical properties and chemical composition, although BTEX is slightly lower in the surrogate as you can see in figure 1 below [15].

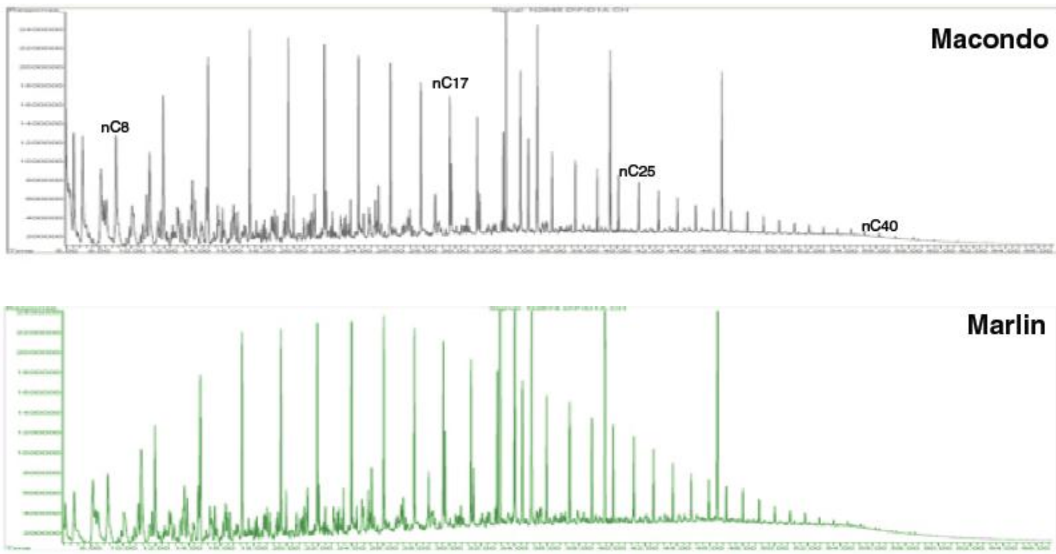


Figure 1. Chromatographic profiles of Macondo (MC252) oil, and its surrogate [15]

2.3 Weathering of Crude Oil

Physical, chemical, and biological processes act on crude oil that has been released into the environment. The low molecular weight fractions evaporate, water-soluble components dissolve, oil droplets mix with water, and photochemical oxidation and biodegradation take place [12]. Dispersion of oil droplets occurs by wave action, and water-in-oil emulsification takes place when petroleum contains polar components that act as emulsifiers. Compounds with boiling points below 250 °C evaporate; this includes n-alkanes with chain lengths less than C_{14}, and low molecular weight aromatics (2 or 3 rings) [12, 16]. Oil is considered “weathered” once evaporation has reduced the amount

of volatile compounds; this can be as large as a 40% decrease by volume. A sample of weathered crude oil MC252 was analyzed, and only aliphatic and cyclic hydrocarbons greater than n-C_{14} were found [14].

Small molecular weight saturates are readily biodegraded in marine environments by various strains of bacteria; 2 or 3 ring PAHs can break down to metabolite products over a period of weeks to months by microbial action [12, 17]. Polycyclic aromatic hydrocarbons containing 4 or more benzene rings are more resistant to biodegradation, making them environmentally persistent [12, 18]. Photo-oxidation is the dominant process for the breakdown of PAHs and other organics in water; they are sensitive to visible light, and highly susceptible to UV light. Anthracene, phenanthrene, and benz[a]anthracene are the most sensitive to photodegradation; but, chrysene, fluorene, pyrene, and benz[a]pyrene are relatively resistant. PAHs attached to particulate matter are more susceptible to photolysis than PAHs in solution [16]. They are also thermo-sensitive.

PAHs are non-polar, hydrophobic, and lypophilic; this enables them to dissolve well in organic solvents [16]. They don't ionize, and are only slightly soluble in water (which decreases with increasing number of rings); however, aromatics are much more soluble in water than alkanes of similar molecular weight [3, 13, 16]. Because PAHs are weakly soluble, they accumulate in sediments and aquatic organisms [13]. The hydrophobic nature of PAHs drives them to bind particles suspended in the water column (especially organic) [17, 13, 16]. Eventually, as these particles settle out, the PAHs reach the bottom sediment and accumulate [17, 16].

2.4 Crude Oil Toxicity

In crude oil, smaller molecules are typically more toxic than larger molecules in the same series, and aromatic compounds are usually more toxic than aliphatic compounds [12]. In fact, most toxic effects of crude oil are attributed to PAHs in the aromatic fraction [2]. PAH resistance to oxidation, reduction, and vaporization increases with increasing molecular weight, whereas aqueous solubility decreases. This causes a difference in PAH behavior, distribution in the environment, and effect on biological systems [17]. Water-soluble components of petroleum are toxic to marine organisms; so, it follows that the low molecular weight PAHs (2-3 ring structures like naphthalene and fluorene) have significant acute toxicity to aquatic organisms [12, 17].

Oil constituents can cause a host of problems in biological systems. PAHs are known to be carcinogenic, mutagenic, and genotoxic; although, PAH metabolites are the true culprits [17, 13]. In addition, both aliphatics (C_5-C_9) and aromatics (C_6-C_{10}) can cause central nervous system disturbances like disorientation, convulsion, paralysis, and death [19].

The chemical composition of each individual PAH determines its toxicity [13]. PAHs in the environment are inactive and unable to cause carcinogenesis. Once a PAH enters an organism, it is metabolized to increase its solubility and promote its excretion from the system (the PAH is oxidized by enzymes of the cytochrome p450 family, and hydroxylation is catalyzed by epoxide hydrolase) [13, 16]. The subsequent metabolite (an electrophilic epoxy diol) is more soluble, but has been activated to its toxic form (hydroxylation makes it more active towards DNA and proteins) [13]. It can now bind nucleophilic parts of macro particles and form adducts [16]. In the second phase, the

activated PAH is made even more water-soluble by various reactions like the conjugation of glutathione. This glutathione-PAH conjugate can no longer bind DNA or protein, the cells are protected, and the compound can be excreted [13].

Photo transformation can increase oil toxicity in water. In photo-degradation, solar radiation absorbed by PAHs is transferred to oxygen molecules, creating oxygen radicals that can damage DNA and cellular membranes [2]. This makes inclusion of light conditions that simulate solar radiation in intensity and spectrum a beneficial addition to WAF exposures.

PAHs can cause a variety of side-effects in early life-stage fish that have been chronically exposed as embryos. These include mortality, deformities, and edemas [20]. The acute toxicity is attributed to narcosis, with lipids as the site of action [21]. In aquatic organisms, narcosis is an anesthetic effect caused by hydrophobic chemicals partitioning into cell membranes and nervous tissue that results in disruption of CNS function [21].

Research indicates that PAHs are unlikely to act as narcotic agents in early life-stages of fish that have been chronically exposed as embryos [21]. Sub lethal effects caused by embryonic PAH exposures include edema of the yolk sac and pericardium, hemorrhaging, disruption of cardiac function, binding to the aryl hydrocarbon receptor, mutation and heritable changes in progeny, craniofacial and spinal deformities, neuronal cell death, anemia, reduced growth and impaired swimming [21]. This host of pathologies is known as blue sac disease (BSD) [20].

2.5 Bioconcentration

Bioconcentration of nonpolar organic pollutants like PAHs by aquatic organisms is characterized by a two compartment model that partitions between the water (dissolved) phase and the tissue phase (lipids of the organism) based on relative solubilities in the two phases [22]. The bioconcentration factor (BCF) is a partition coefficient that defines this partitioning phenomenon. In simple terms, it is the concentration of the pollutant in an organism divided by the soluble concentration in ambient water [23]. Bioconcentration through water phase is believed to play a larger role in determining PAH concentrations in fish tissue than biomagnification via the food web [22]. This makes the procedure used for creating water accommodated fractions in exposure experiments particularly important.

2.6 Water Accommodated Fractions (WAFs)

The accepted tool for evaluating oil toxicities is a water-accommodated fraction. Oil and its products are complex and variable, making them very hard to evaluate toxicologically. There is little consistency between investigation test prep methodologies, which makes cross-comparisons of data difficult [6]. With the understanding that only the fraction of oil that enters the water column has the potential to yield toxic effects on aquatic species, the Chemical Response to Oil Spills Ecological Effects Research Forum (CROSERF), has been working to standardize test media preparation [6].

The exposure medium for toxicity tests of low solubility compounds, like crude oil, should be prepared as a water accommodated fraction (WAF). "WAFs are mediums containing only the fraction of petroleum that remains in aqueous phase after a mixing

energy has been removed, and after a period sufficient for phase separation" [2]. WAFs are saturated with petroleum products, mainly hydrocarbons in a solution or a stable emulsion [2]. The consistency of WAF preparation depends on pretreatment of dilution water, mixing energy, and mixing duration [6].

The preferred matrix is natural seawater. Studies show that WAFs are most consistent if seawater is pre-sterilized and filtered; however, it is recognized that sterilization of large volumes is impractical. Filtration alone improves reproducibility of WAFs, although it does not eliminate bacteria [6]. This experiment used seawater collected from the Gulf of Mexico that had been double filtered, UV treated, and continuously aerated.

Mixing of WAFs can be performed in various ways including shaking, swirling, and rotating. The use of a magnetic stirrer on an electromagnetic stir plate was found to create the least variability in WAF production [6]. The stir rate was the maximum that could be achieved without creating a vortex. This minimized dispersions and emulsifications in the WAFs; however, is considered unrealistic because field conditions are rarely as calm. In the case of the DWH oil spill, as stated earlier, the oil that reached the Gulf Coast was a thick viscous emulsion (60% water). That is why for this experiment high-energy water accommodated fractions (HEWAFs) were chosen as the test mediums, because they more closely mimic what occurs in the environment. Much higher concentrations of oil-in-water can be obtained by violent mixing than by gentle stirring [3]. The oil was blended with seawater for 30 seconds on the high setting in a commercial blender, in accordance with the Hemmer method; which requires that an apparatus with stainless steel blades is operated at a speed that provides a 70% vortex [2].

The minimum mixing time must be assessed on an individual basis for each oil; however, mixing/settling durations beyond 24 hours are discouraged because of bacterial action [2]. In this study the mixing time was 30 seconds and the settling time was 1 hour. Durations should be selected based on the amount of time it takes for the aqueous phase to be saturated with the available soluble compounds [2].

The numerous water solubilities of oil constituents make serial dilutions of WAFs ineffective because partitioning of compounds into aqueous phase is not directly correlated to the oil:water ratio. Test treatments should be derived from a range of oil loadings instead [6]. In a study by Anderson, et al., the amount of petroleum hydrocarbon compounds in the aqueous phase increased linearly with the amount added. A Louisiana crude oil observed in the same study had more concentrated oil-in-water dispersions than the other crudes tested (81.2 ppm) [3].

Lastly, it is important to reduce the loss of volatiles to the environment in order to increase reproducibility [2]. In a study of water-soluble fractions and oil-in-water dispersions of crude oils in seawater, alkanes disappeared from the dispersions much quicker than aromatics. These experiments require that the static exposures be aerated, and it is important to note that aeration causes hydrocarbon concentrations to drop drastically in a short amount of time [3]. This loss can be minimized by sealing exposure vessels [2]. In this experiment, plastic wrap snuggly placed over the beaker mouths was used for this purpose.

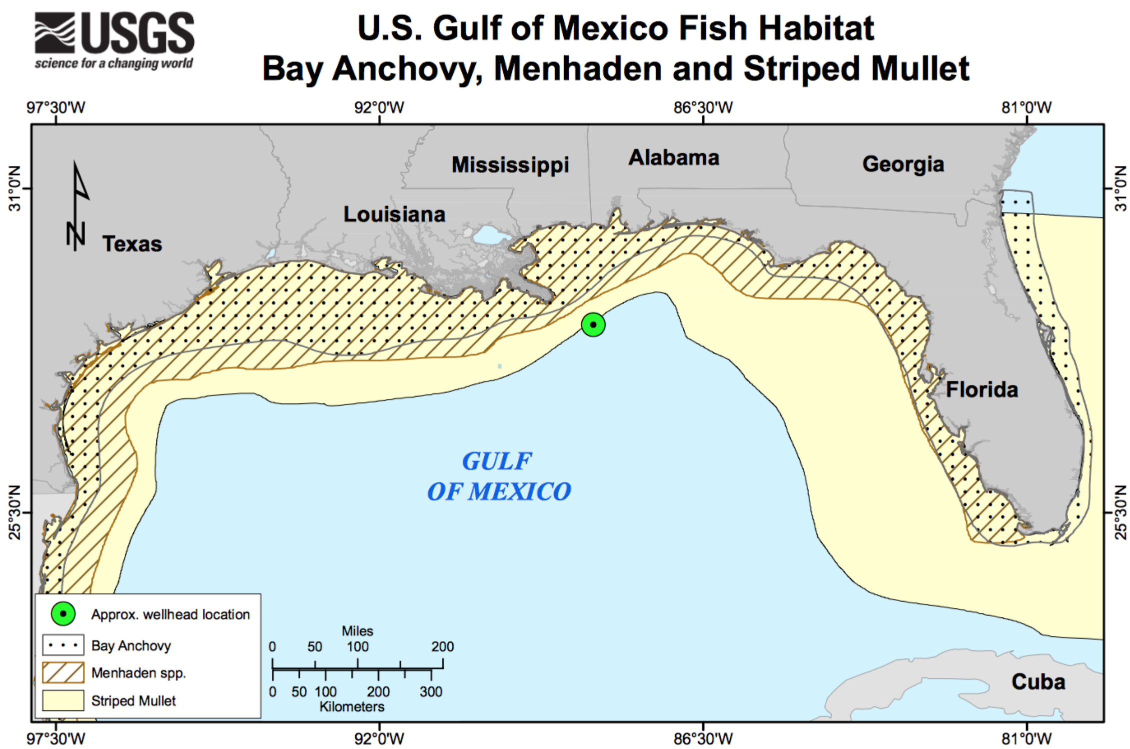
WAFs are commonly used in early life stage marine toxicity tests, as they were in this study. The exposure protocol used in this experiment was a modified version of the static acute toxicity test method followed by U.S. EPA (U.S. Test Method 821/R-02-012)

[24]. The most significant deviation from the EPA protocol was the addition of plankton (phytoplankton and zooplankton species) as a food source for the Bay Anchovy larvae in an effort to recreate natural conditions. Total hydrocarbon analysis is the yardstick used to determine if WAFs in various experiments are comparable. This is achieved by verification of concentrations with GC/MS, the tool used in this experiment [6].

2.7 *Anchoa mitchilli* (Bay Anchovy)

Forage (prey) fish are small, pelagic fish preyed upon by larger fish and seabirds. They mainly feed on zooplankton, and some phytoplankton, making them omnivorous [9]. They are the link that transfers energy created by primary consumers to higher trophic levels. Forage fish school in large numbers, and swim in synchronized grids that are advantageous to filter feeders [9]. Anchovy are typically oily fish found in abundance, although there are few known species.

Bay Anchovy is a small (10-14 cm long), slender forage fish [25]. It is one of the most common fish inhabiting coastal areas; their range extends from Massachusetts to Yucatan, Mexico [25]. Surveys have shown that they are one of the most abundant fish in the northern Gulf of Mexico, the peak abundance occurring from spring through early winter [10, 26]. They are euryhaline, but prefer lower salinities; they can be found anywhere from freshwater to salinities of 45 ppt [27]. The Bay Anchovy is often the dominant species in stressed environments because they can survive in water with low oxygen [27, 28]. For these reasons, *Anchoa m.* was chosen as the test species for determining the effect DHW horizon might have had on embryonic and larval stages of fish in the Gulf of Mexico. Their habitat is displayed in figure 2 below.



This map depicts the extent of Bay Anchovy (*Anchoa hepsetus*), Menhaden (*Brevoortia spp.*), and Striped Mullet (*Mugil cephalus*) habitat in relation to the BP Deepwater Horizon oil wellhead.

Figure 2. Bay Anchovy, Menhaden, and Striped Mullet habitats in relation to the DWH oil well head [29]

Bay Anchovy spawn in fairly shallow water (less than 20 meters deep) almost year round [30, 31]. Their eggs are about 0.75 mm long, but the size decreases as salinity increases [30]. The eggs hatch within 24 hours of spawning [32]. The larvae are about 1.8 to 2.0 mm long, and the yolk sac is absorbed within 15-18 hours after hatching [33, 34].

They don't have recreational or commercial value, but they do play an important role in coastal ecosystems. *Anchoa mitchilli* are filter feeders that use gil-rakers to strain zooplankton out of water for food [35]. Their abundance and small size make them an important food source for sea birds and economically significant predatory fish, including sea trout, flounder, and striped bass [10].

2.8 *Brachionus plicatilis* (Rotifers/Zooplankton)

Rotifers are mostly found in freshwater environments, but can be found in saltwater as well. They make up a significant portion of the zooplankton class, and are an important foodsource for small fish (like Bay Anchovy), copepods, bryozoan, comb jellies, jellyfish, and starfish. They eat particulate organic detritus, dead bacteria, algae, and protozoans [36].

Brachionus plicatilis (L strain) was the rotifer species chosen for this study, and is the euryhaline rotifer most frequently used to feed fish larvae in aquaculture [37]. *Brachionus p*. is 200-350 mm in length and constantly drifts. Their small size and soft outer bodies make them highly digestible, and their slow movement and permanent suspension in the water column makes them easily available to larvae [38].

Rotifers themselves are not especially nutritious, but act as “nutrient carriers”. They pass along the nutrition of their food source to fish larvae. They are planktonic filter feeders; beating cilia around the rotifer’s head (corona) circulate water, food, and nutrients toward the mouth [39]. When the cilia detect a food source (usually phytoplankton in the 3-12 mm range), it pulls its mouth opening towards it, surrounds and crushes it. This whole process is repeated several times within seconds to meet its high-energy demand [40].

In aquaculture, *Nannochloropsis* is usually chosen as the algal species to feed rotifers because it is high in lipids, protein, and calories. One rotifer can eat 115,000 *Nannochloropsis* cells a day [41]. Other nutrient sources for rotifer cultures include yeast, bacteria, and emulsified oil; as long as a particle’s size is appropriate, rotifers can

consume it [39]. Rotifers have a voracious appetite and graze continuously; they can starve within 2-4 hours of depleting their food source [37].

Herbivorous zooplankton is primarily dependent on phytoplankton. In the Gulf of Mexico, a strong correlation between phytoplankton and zooplankton biomass was found for neritic (0.54) and oceanic (0.67) zones. Near the northwestern coast of Cuba, 90% of the phytoplankton produced daily were consumed by zooplankton according to Kondratieva's calculations [8].

2.9 *Nannochloropsis oculata* (Phytoplankton)

Phytoplankton are autotrophic, photosynthesizing organisms found in the euphotic zone of almost all bodies of water [42]. They synthesize organic compounds from carbon dioxide and energy harvested from the sun, making them the primary producers at the base of the aquatic food web. Phytoplankton is essential to aquaculture and mariculture as foodstock for rotifers, which are used as food for other organisms [43]. There are over 5,000 species of marine phytoplankton alone [44].

Microalgae reproduce rapidly and have a high oil content. They can double their biomass three times a day, and can produce 100 times more oil per hectare than a terrestrial plant. The naturally occurring lipid content of microalgae can be as much as 67% of their dry weight [45].

Nannochloropsis oculata is a unicellular green algae with non-motile, spherical cells 2-5 μm in diameter [46]. It is distributed in oceans worldwide, mainly in marine environments, but can also thrive in fresh and brackish water [47]. The six known *Nannochloropsis* species are able to accumulate high levels of polyunsaturated fatty acids, and under some conditions (nitrogen limitation) can accumulate up to 60-70% of

their biomass as lipids [48]. This makes them a particularly energy-rich food source for fish larvae and rotifers. They are also used as a food additive in human nutrition, and have recently gained attention as a potential source of biofuel [49].

Algal oils can be similar to other vegetable oils, or can be composed primarily of hydrocarbons, depending on the species [50]. A study extracted the lipid content of naturally occurring *Nannochloropsis oculata*, and found that it yielded 15% ± 0.3% based on dry weight. This lipid fraction contained fatty chains 14 to 20 carbons long [50].

Plankton thrive in high nutrient environments. Because of upwellings and runoff from the Mississippi River, that supply phosphates and nutrients to plankton populations, the northwestern Gulf of Mexico, Bank of Campeche, and west Florida Shelf have high plankton productions. The species composition and concentration of these phytoplankton communities changes spatially due to cyclonic and anti-cyclonic horizontal circulations [8]. In the Gulf of Mexico, phytoplankton abundance and biomass maximums were observed in October, June, and April; the highest in June at 23 mg/m^3. As show in figure 3 on the following page, the Gulf of Mexico is a very productive water body, averaging 47 mg/m^3 (96 mg/m^3 in the neritic zone and 10.4 mg/m^3 in the oceanic zone) [8].

Zernova and Krylov found planktonic flora to be more diverse in the Gulf than the Caribbean Sea and the Straits of Florida. In a separate study, Krylov concluded that the distribution of phytoplankton in the southern Gulf of Mexico is mosaic, creating a homogenous phytoplankton community [8].

Traditional aquatic oil exposures don't include phytoplankton. Because phytoplankton is found in abundance in the Gulf of Mexico as a crucial part of the ecosystem, they are pertinent to oil exposure studies. Their high lipid content suggests

that their role in post oil spill dynamics may be significant. This study selected *Nannochloropsis o.* as the species to represent GoM phytoplankton communities because its use in aquaculture is well established.

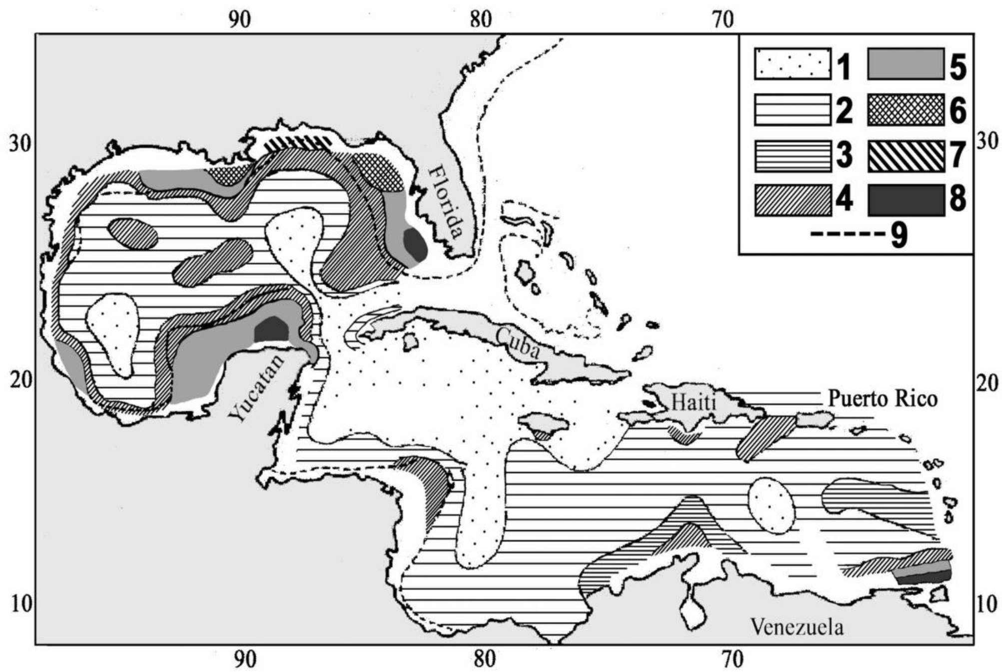


Figure 3. Average distribution of plankton in the upper 100-m layer of the Gulf of Mexico (mg/m^3) and the Caribbean Sea. 1: 30-100, 2: 50-150, 3: 100-200, 4: 100-300, 5: 200-600, 6: 200-1,000, 7: 100-3,000, 8: 300-1,000, 9: shelf margin [8]

CHAPTER 3. MATERIALS AND METHODS

3.1 Exposure Set-Up and Preparation

The oil exposures analyzed in this thesis were conducted in Cocodrie, Louisiana, at Louisiana University Marine Consortium, by Dr. Sarah Webb and Mr. Bill Childress.

As shown in figure 4, the exposure systems consisted of 2-liter glass beakers containing stock seawater (25ppt) and the desired amount of High Energy Water Attenuated Fraction (HEWAF). The beakers were wrapped in opaque black plastic to minimize light contamination and were placed in a warm water bath to maintain a temperature of 27 °C throughout the 24-hour exposures. Beakers represented the various treatment groups.

The experiments were designed to determine the effect of plankton on HEWAF toxicity. The experiments used *Nannochloropsis oculata* as a phytoplankton species and *Brachionus plicatilis* (rotifers) as a zooplankton species. *Anchoa mitchilli* (Bay Anchovy) were included as a test species in all but one of the experiments, in which Dr. Webb determined the morphological effects of the HEWAFs.

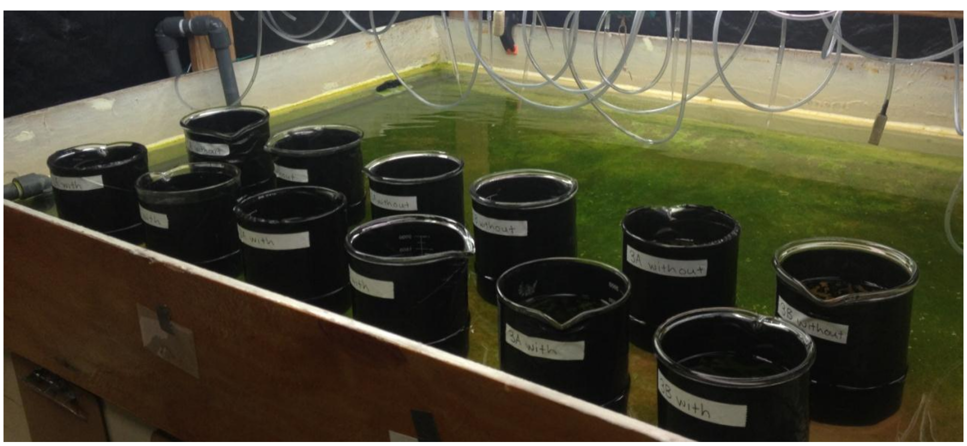


Figure 4. Experimental set-up for WAF Exposures

3.1.1 Stock Seawater

The seawater used in the trials was collected offshore in the Gulf of Mexico using the research vessel *Pelican*. The seawater was adjusted to 25 ppt salinity using filtered fresh water. It was UV-treated, double-filtered, and continuously aerated prior to use. Dissolved oxygen, salinity, and pH of stock water were tested prior to experiments.

3.1.2 Plankton Cultures

The test treatments contained either: zooplankton and phytoplankton, phytoplankton alone, zooplankton alone, or no plankton. *Nannochloropsis oculata*, the phytoplankton, was cultured in polyethylene bags (16 L capacity). Micro Algae Grow was added to each bag at 0.3 mL/L. The zooplankton, *Brachionus plicatilis* (rotifers), was cultured in 2-liter beakers; a 50% volume change occurred daily, and fresh *N. oculata* was added as a food source. The plankton cultures are pictured in figure 5. The plankton cultures were on a 24:0-h lighting schedule.

Figure 5. Cultures of *Nannochloropsis oculata* on left, and *Brachionus plicatilis* on right

3.1.3 Aquaculture of *Anchoa mitchilli*

Between the months of April and June of 2013 and 2014, adult *Anchoa mitchilli* were collected from Little Cocodrie Bayou in Cocodrie, Louisiana. They were

maintained at LUMCON in a re-circulating fiberglass tank fitted with a biofilter as shown in figure 6. Spawning was induced by maintaining the water temperature at 27.0 ±0.5 °C and the salinity at 25 ppt. A light:dark cycle of 17:7-h was used. The fish were fed with *Brachionus plicatilis* and dry food.

Figure 6. Aquaculture of *Anchoa mitchilli*

3.1.4 HEWAF Preparation

High-energy water accommodated fractions (HEWAFs) were made by utilizing the same method as Hemmer, et al. [7]. Surrogate Macondo Oil (MC252) was added to stock seawater (25 ppt) at a ratio of 3 mL/L (3 g/L). The oil/seawater mixture was blended on high for 30 seconds in a Waring Commercial CB15 blender. The resulting mixture was poured into a 2-liter separatory funnel, where it sat and settled for one hour before collection. Dr. Sarah Webb can be seen preparing the HEWAF stock in figure 7 below. The water fraction was bled off and used for stock concentrations to create the dilutions in the exposures. There were 8 different HEWAF treatments used throughout the experiments as seen in table 1 on the next page. The percent concentrations were based on the last 400 mLs added to each beaker.

Table 1. HEWAF treatments and their corresponding loading volumes

Treatment Group	Loading Volume (mLs/2L)
0% HEWAF (Control)	0 mLs
7.5% HEWAF	30 mLs
15% HEWAF	60 mLs
30% HEWAF	120 mLs
33.3% HEWAF	133.2 mLs
60% HEWAF	240 mLs
66.6% HEWAF	266.4 mLs
100% HEWAF	400 mLs

Figure 7. HEWAF Preparation

3.1.5 Exposure Protocol

Stock seawater, rotifers, phytoplankton, and Bay Anchovy larvae were collected and placed in the water bath to be warmed before introduction to the exposure system. It was necessary that all components were at 27 °C and 25 ppt salinity. First, 1600 mLs of the stock seawater was placed in each 2-liter beaker. Next, the plankton was added to the treatments that called for it. *Nanochloropsis* was added to the beakers to reach a final concentration of 5 NTU in 2000 mLs, and *Brachionus* was added for a final count of 5/mL in 2000 mLs. At this point, dissolved oxygen, salinity, and pH of the water were tested before larvae were added. Sixty-five *Anchoa* larvae (10-12 hours post-spawn) were placed in each beaker. Varying amounts of HEWAF were added to the beakers

before stock seawater was used to bring the final volume of the beakers to 2 liters. Adding the components in this manner minimized impact on the larvae. Finally, an aeration tube was placed in each beaker, and cellophane was wrapped tightly over the mouth of each beaker to prevent contamination and evaporation.

The water bath maintained the 27 °C temperature throughout the 24-hour exposures, and a light:dark cycle of 24:0 hours was used. Over the course of the exposure, the Bay Anchovy hatched. At the termination of the experiment, water quality measurements were taken again, and water samples (both filtered and unfiltered) were saved for analysis in 1-liter TraceClean™ amber glass bottles manufactured by VWR (Radnor, PA). The PYREX® glass beakers (Tewksbury, MA) and Sterlitech glass fiber membrane filters (1.2 micron) (Kent, WA) used in trial 4 were also saved.

3.2 Exposure Trials

3.2.1 Trial 1: Zooplankton/Phytoplankton Test

Trial 1 contained no Bay Anchovy and was designed to determine if plankton had an effect on the exposure system. The high HEWAF percentages were chosen in this experiment and evenly spaced so that differences would be easily distinguishable between treatment groups. Two beakers with plankton, and two without plankton were run for each treatment group. A 1000 mL water sample was collected from each beaker at the end of the experiment, none of which were filtered. Table 2 below lists the water samples collected at the end of the trial.

Table 2. Water samples collected in trial 1

HEWAF Treatments	HEWAF only	HEWAF w/plankton	T_0 Samples (w/out plankton)
0%	2 Replicates	2 Replicates	2 Replicates
33.3%	2 Replicates	2 Replicates	2 Replicates
66.6%	2 Replicates	2 Replicates	2 Replicates
100%	2 Replicates	2 Replicates	2 Replicates

3.2.2 Trial 2: Phytoplankton Test

Trial 2 included Bay Anchovy as the test species, and lower HEWAF concentrations. The second trial was set up identically to the first except phytoplankton (*Nannochloropsis oculata*) was added at 5 NTUs, without rotifers to determine the effect of planktonic organisms on the first experiment. At the end of the experiment, the Bay Anchovy were collected, and both filtered and unfiltered water (500 mLs of each) was collected for GC/MS analysis from each beaker. Table 3 below shows the water samples collected at the end of the trial.

Table 3. Water samples collected in trial 2

HEWAF Treatments	HEWAF w/*Nanno* (filtered)	HEWAF w/*Nanno* (unfiltered)	HEWAF w/out *Nanno* (filtered)	HEWAF w/out *Nanno* (unfiltered)
0%	1 sample	1 sample	1 sample	1 sample
7.5%	1 sample	1 sample	1 sample	1 sample
15%	1 sample	1 sample	1 sample	1 sample

3.2.3 Trial 3: Rotifers Test

In the third trial, 65 Bay Anchovy embryos and *Brachionus plicatilis* (5/mL) were added to each 2-liter beaker. Phytoplankton was not included in trial 3 so that the contribution of rotifers to the planktonic effect seen in trial 1 could be isolated. Filtered and unfiltered water from each beaker was saved for GC/MS analysis at the conclusion of the trial (500 mLs of each). The HEWAF concentrations were chosen based on the LD50 of Bay Anchovy larvae. Table 4 below lists the water samples collected in this trial.

Table 4. Water samples collected in trial 3

HEWAF Treatments	HEWAF w/rotifers (filtered)	HEWAF w/rotifers (unfiltered)	HEWAF w/out rotifers (filtered)	HEWAF w/out rotifers (unfiltered)
0%	1 sample	1 sample	1 sample	1 sample
7.5%	1 sample	1 sample	1 sample	1 sample
15%	1 sample	1 sample	1 sample	1 sample

3.2.4 Trial 4: Filter and Glassware Test

Trial four was designed to repeat trial one with lower HEWAF concentrations. It included the test species Bay Anchovy (sixty-five embryos/beaker). The experiment was conducted to determine if oil was being lost to part of the exposure system (glassware) and being made unavailable to the test organisms. It was also used to determine if filtering the water samples post exposure had a significant impact on GC/MS analysis; and to get a rough estimate of how much oil the plankton might be sequestering.

At the beginning of the experiment, unfiltered 1000 mL samples were collected from the 7.5% HEWAF and 15% HEWAF treatments (without plankton) to determine the initial concentrations of oil constituents introduced to the exposure system. At the conclusion of the experiment, 1000 mLs of water (filtered and unfiltered) was collected from each beaker. Filters and glassware used were also saved for analysis. Table 5 lists the water samples collected, table 6 lists the filters samples collected, and table 7 the beakers collected.

Table 5. Water samples taken in trial 4

HEWAF Treatments	HEWAF with plankton (filtered)	HEWAF with plankton (unfiltered)	HEWAF w/out plankton (filtered)	HEWAF w/out plankton (unfiltered)	T_0 w/out plankton (unfiltered)
0%	2 replicates	2 replicates	2 replicates	2 replicates	1 sample
7.5%	2 replicates	2 replicates	2 replicates	2 replicates	1 sample
15%	2 replicates	2 replicates	2 replicates	2 replicates	1 sample

Table 6. Filter samples taken in trial 4

HEWAF Treatments	Filter from HEWAF w/ plankton	Filter from HEWAF w/out plankton
0%	2 replicates	2 replicates
7.5%	2 replicates	2 replicates
15%	2 replicates	2 replicates

Table 7. Glass beaker samples taken in trial 4

HEWAF Treatments	Glass Beaker from HEWAF w/ plankton	Glass Beaker from HEWAF w/out plankton
0%	2 replicates	2 replicates
7.5%	2 replicates	2 replicates
15%	2 replicates	2 replicates

3.2.5 Trial 5: Mid-Concentration Test

Trial 5 included phytoplankton, rotifers, and Bay Anchovy at the established concentrations. At the beginning of the experiment, a 1000 mL unfiltered sample of the control and 60% HEWAF (without plankton) was collected. At the conclusion of the experiment, filtered and unfiltered water samples from each beaker were collected (1000 mLs each). This experiment was conducted to bridge the information collected from trial one (high, unfiltered HEWAF concentrations) and the subsequent trials (lower filtered/unfiltered HEWAF concentrations). Table 8 lists the water samples collected in this trial.

Table 8. Water samples collected in Trial 5

HEWAF Treatments	HEWAF with plankton (filtered)	HEWAF with plankton (unfiltered)	HEWAF w/out plankton (filtered)	HEWAF w/out plankton (unfiltered)	T_0 HEWAF w/out plankton (unfiltered)
0%	1 sample	1 sample	1 sample	1 sample	1 sample
15%	1 sample	1 sample	1 sample	1 sample	
30%	1 sample	1 sample	1 sample	1 sample	
60%	1 sample	1 sample	1 sample	1 sample	1 sample

3.3 Extractions

The beakers from trial 4 were extracted to determine if the glass was sequestering hydrocarbons. In trial 1, water samples with plankton contained higher levels of hydrocarbons; trial 4 beaker extractions were meant to eliminate the possibility that oil

compounds were binding the glass in the absence of plankton. The filters were extracted in an attempt to estimate the amount of hydrocarbons sequestered by the plankton.

3.3.1 Aqueous Extractions

The aqueous extractions were performed using modified EPA method 3510C: Separatory Funnel Liquid-Liquid Extraction [51]. This method is applicable to the isolation and concentration of water-insoluble and water-soluble organics for analysis in chromatography work.

The 1000 mL water samples were brought to ambient temperature from a frozen state, and then poured into 2-liter separatory funnels that were washed and solvent rinsed. Approximately 50 mLs of dichloromethane was used to rinse the amber sample bottles. After the 50 mLs of dichloromethane (Sigma-Aldrich, St. Louis, MO) was added to the sample bottle, it was capped and shaken to collect the organic constituents that remained on the inner glass wall. The bottle was intermittently vented by loosening the cap. The dichloromethane (DCM) rinse was then poured into the separatory funnel with the original water sample. A 1 mL of surrogate spiking standard was added to each funnel. The funnels were capped and vigorously shaken for 2-3 minutes; they were vented periodically into a hood by inverting them and turning the stopcock to relieve the pressure. The funnels were then placed in ring stands inside a hood to settle and phase separate. Figure 8 below depicts one of the liquid extractions conducted in this thesis.

The organic layer was allowed to separate from the water phase for approximately 30 minutes. The solvent extract was collected in a flat-bottom Florence flask fitted with a powder funnel lined with glass microfiber filter (Whatman, Marlborough, MA) and paper filters (Fisher Scientific, Waltham, MA). The filter also contained Fisher Scientific brand

granular anhydrous sodium sulfate (10-60 mesh) to remove water from the sample extract. The process was repeated a second time with another 50 mL DCM rinse. Water blanks consisting of DI water were extracted with every run.

Figure 8. Liquid-liquid extraction of water samples

3.3.2 Filter Extraction

The filters from trial 4 were placed in plastic weigh boats and dried at 40 °C in an Isotemp® Muffle Furnance (Fisher Scientific). They were weighed on a Scout™ scale (UHAUS®, Parsippany, NJ) and placed in a Heritage Series™, Sunbeam blender (Boca Raton, FL) with an equal amount of 40-60 μm RediSep C-18 silica from Teledyne Isco, Inc. (Lincoln, NE) to be ground [52]. The resulting mixture was placed in a 50 mL glass beaker with 1 gram of sodium sulfate.

The mixture was spiked with 1 mL of surrogate spiking standard and sonicated in a Branson 2210 sonicator (Danbury, CT) for 20 minutes in 50 mLs of DCM. The same funnel/filter system used in the aqueous extractions was fixed to an air tight conical flask, which was attached to a vacuum manifold. The slurry created by the filter and silica was added to the funnel, and the 50 mL beaker was rinsed with 50 mLs of DCM three times

and poured into the filter as well. The solvent extracts were transferred from the conical flasks to Florence flasks. An unused filter was also extracted as a method blank.

3.3.3 Glassware Extraction

The empty 2-L beakers used in trial 4 were brought to room temperature, and thoroughly rinsed with 100 mLs of DCM before being spiked with 1 mL of surrogate standard. The mixture was then poured through the funnel/filter apparatus into a Florence flask. The beakers were solvent rinsed with 100 mLs of DCM three more times, and the rinses were poured into the funnels/flasks as well.

3.3.4 Sample Concentration

The eluents in the Florence flasks were rotary evaporated to a volume between 1 and 5 mLs using either a BUCHI R-114 or BUCHI RE111 rotavapor. The extracts were then transferred to graduated test tubes. If required the samples were further concentrated using a nitrogen evaporator (N-EVAP^{TM} 111).

One mL of each sample was added to an Agilent Technologies (Santa Clara, California) 2 mL amber vial, and 10 μL of internal standard was added before the sample was capped and deemed ready to be analyzed by the GC/MS.

3.3.5 Surrogate Spiking Standard

A surrogate-spiking standard typically has similar chemical composition to the analytes being studied. By adding a surrogate at the beginning of the sample preparation process, the surrogate undergoes similar changes to the target analytes, which eliminates variation after calibration curves are determined [53]. Calibration curves establish the relationship between the relative responses of the target analytes to the amount of surrogate.

A 1 mL aliquot of a surrogate-spiking standard was added at the beginning of every extraction to determine method efficiency based on the percent recovery of the standard. The optimal recovery range was from 70-120%. 5α-androstane was used to establish how well alkanes were recovered, and phenanthrene-d_{10} was used for aromatics. Both compounds were bought from AccuStandard Inc (New Haven, CT).

The surrogate spiking standard was prepared by adding 1.0 mL of the 5α-androstane (dissolved in DCM at 10 mg/mL) to 500 mL of DCM in a volumetric flask. 10.0 mg of powdered phenanthrene-d_{10} was weighed out and added to the volumetric flask. After the phenanthrene-d_{10} dissolved, the standard was stored at 32 °C and aliquoted as needed. The final concentration was 20 µg/mL.

3.4 Sample Analysis

3.4.1 Internal Standard

Internal standards consist of compounds with similar characteristics to the target analytes (i.e. volatility, molecular weight, molecular structure). Normally, multiple internal standards are chosen that elute near the beginning, middle, and end of the analytical window [54]. This accounts for volatility differences. They can also be chosen based on chemical functionality.

A 10 uL aliquot of internal standard was added to each sample before it was capped and placed on the GC/MS instrument as a way to determine the validity of the instrument. It contained four compounds of varying molecular weights (AccuStandard Inc.). Napthalene-d_8 was the lightest compound added, and the molecular weights increased from acenapthene-$d_{10,}$ to chrysene-$d_{12,}$ to perylene-d_{12}.

The internal standard was prepared by adding 1 mL each of naphthalene-d_8 (4.0 mg/mL in DCM), acenapthene-d_{10} (4.0 mg/mL in DCM), chrysene-d_{12} (4.0 mg/mL in DCM), and perylene-d_{12} (4.0 mg/mL in DCM) to a 5 mL amber vial. The final volume of the standard mix was 1000 μg/mL.

3.4.2 Calibration Standard

The LSU RCAT laboratory maintained a 5-point calibration curve. It used a prepared oil analysis standard purchased from Absolute Standards. A mid-range calibration standard was analyzed with every batch of samples to ensure the relative response factor was within 20% of the average relative response factor calculated in the 5-point curve (use of any calibration standard is acceptable). The response factor can be calculated using the following equation:

$$RF = ((Ax))(Cis))/((Ais)(Cx))$$

where A_x is the area of the compound, C_x is the concentration of the compound, A_{is} is the area of the internal standard, and C_{is} is the concentration of the internal standard.

The calibration standard consisted of surrogate spiking standard and an oil analysis standard. The surrogate-spiking standard for the calibration standards was made by combining 3.0 mLs of DCM, 1.0 mL of 5-α androstane (1000 μg/mL in DCM), and 1.0 mL of phenanthrene-d_{10} (1000 μg/mL in DCM), in an 8 mL vial. The final concentration of the surrogate spiking standard was 200 μg/mL. Oil analysis standard contained 44 compounds, and had a concentration of 100 μg/mL in hexane and DCM (9:1; v/v) [53]. The oil analysis standard and surrogate spiking standard were combined to create 0.5 ppm, 1.0 ppm, 5.0 ppm, 10.0 ppm, and 25.0 ppm standards, which made up the 5-point calibration standard curve.

3.4.3 Reference Oil Standard

The reference oil used in this analysis was Macondo 252 (MC252). It was collected from the Deep Water Horizon oilrig. The standard was prepared by extracting 1 gram of oil in 40 mL of solvent. MC252 reference oil was analyzed in every GC/MS run as a QA/QC measure.

3.4.4 GC/MS

The GC/MS work was performed using a modified EPA protocol SW-846 8270; analysis of semi-volatile organic compounds [55]. The instrument analyzed the samples to identify 71 target compounds, listed in appendix C. These included polycyclic aromatic hydrocarbons (PAHs) and straight chain alkanes found in petroleum.

Gas chromatography separates and analyzes volatile substances in the gas phase. Samples dissolved in solvents are vaporized and separated between a stationary and mobile phase [54]. The mobile phase is a chemically inert gas that carries the sample through a heated column, and the stationary phase is usually a liquid or an inert support. The GC/MS analysis in this project used an Agilent 6890 GC system configured with a 5% diphenyl/95% dimethyl polysiloxane (stationary phase) high-resolution capillary column (30 meter, 0.25 mm ID, 0.25 micron film). It was fitted with an Agilent 7693 auto sampler, and was directly interfaced with an Agilent 5973 mass spectrometer detector system.

Sample extracts were injected by a 10 µl micro syringe through a rubber septum into the sample port where they were vaporized and introduced to the column. The vaporized sample extract was carried by Ultra High Purity Helium (mobile phase) through the column. Helium is often the inert gas of choice in GC/MS because it has a

large range of flow rates; the flow rates were optimized to provide the necessary degree of separation, especially between n-C_{17} and pristane (near baseline resolved), and n-C_{18} and phytane (baseline resolved).

GC Column ovens maintain temperatures within tenths of a degree. Temperature programming methods increase the temperature in steps throughout the separation process. This is best for samples with broad boiling point ranges. Low temperatures resolve the low boiling components, and increasing temperatures resolve the less volatile components [54]. Temperature programming separations occur at the rate of 5-7 °C/minute.

In this study, injection occurred at 280 °C. When the sample left the vaporization chamber and entered the column, the column was at 60 °C. After 3 minutes, the temperature was increased to 280 °C at a rate of 5 °C/minute and held for 3 minutes. The oven was then heated to 300 °C at a rate of 1.5 °C/minute and held for 2 minutes. Each sample had total run time of 65.33 minutes.

In GC/MS, the sample passes through a transfer line into the mass spectrometer's inlet from the end of the GC column. The sample is ionized and fragmented by an electron-impact ion source. After the sample is ionized, it is held in the ion trap mass analyzer by electric and magnetic fields (in this case a hollow ring electrode with two grounded end-cap electrodes). The ion fragments are sorted according to mass to charge ratio (m/z) by applying variable radio-frequencies; as the frequency increases, ions of stable m/z value are ejected in order of mass [54]. Emitted ions strike an electron multiplier, which converts the detected ions into an electrical signal, response which is converted to a chromatogram.

The mass spectrometer (detector) interface used in this study was held at a temperature of 300 °C. The source and quad temperatures were maintained at 230 °C and 150 °C. It was operated in selective ion-monitoring (SIM) mode to detect trace amounts of the target compounds. The selected ions were scanned at a rate greater than 1.4 scans/sec with a dwell time of 60 milli-seconds.

3.4.5 Chemstation™

Chemstation™ Software was used to process the spectral data with an analysis method developed by LSU-RCAT. Custom reports that contained the raw integration data were created using this method and exported to a spreadsheet for quantitative analysis. A macro print-out of the ion chromatography was also generated and used to compare to the source oil fingerprint. Integrations were adjusted/re-integrated as needed.

The analyte concentrations were calculated in the spreadsheet using the internal standard method, which used the mean relative response factors calculated from the 5-point calibration curve. The concentration of analytes in a sample can be calculated using the following equation:

$$Concentration\left(\frac{ng}{mg}\,or\,\frac{ng}{mL}\right) = (A_x \times I_s \times V_t \times 1000)/(A_{is} \times RRF \times V_i \times M\ or\ V)$$

where A_x is the area of the analyte, I_s is the concentration of internal standard injected (ng), V_t is the final volume of the total extract (mL), DF is the dilution factor, A_{is} is the area of internal standard, RRF is the mean relative response factor, V_i is the volume injected, M is the mass if a solid (mg), and V is the volume if a liquid (mL). The calibration curve only contained the parent hydrocarbons because alkylated standards are unavailable; so, the homologues are calculated using the response factor of the parent compound. This makes the homologue concentrations only semi-quantitative [53].

3.5 Statistical Analysis of Data

An analysis of variance (ANOVA) in conjunction with a Tukey test was performed on the alkane and aromatic concentrations of the water, filter, and beaker samples collected in trials 1 and 4. The results were used to determine if plankton, HEWAF concentrations, and the interaction between plankton and HEWAF concentrations had an effect on the alkane and aromatic concentrations of the samples. A type I error rate of 0.05 (α) was used. A p-value greater than 0.05 results in acceptance of the null hypothesis; that the alkane/aromatic concentrations were not affected by the effect (plankton, HEWAF concentration, plankton/HEWAF interaction). The ANOVA and Tukey analyses looked at the variability between sample groups and within sample groups, and compared all possible combinations of means to determine statistical differences. The Tukey test provided a standard error for each effect (HEWAF concentration, plankton, etc.) and assigned treatments to letter groups. Groups that share letters are not statistically different from each other. For example treatments in group A are statistically different from treatments in group B, but are not statistically different from treatments in groups A or AB. All statistical analyses were performed using SAS (see Appendix A for data and tables). The analyses were conducted separately for alkanes and aromatics, and ANOVA/Tukey analyses of log-transformed concentrations were used when necessary to better distinguish statistical differences between treatments.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Trial 1: Evaluation of the Contributions of Plankton to HEWAF Toxicity

Trial one was conducted to determine if plankton (phytoplankton and zooplankton combined) had an effect on the petroleum hydrocarbon concentrations in the water exposures at various HEWAF concentrations (0%, 33.3%, 66.6% and 100% HEWAF).

4.1.1 Total Alkane Concentrations

Plankton had a significant effect on the total alkane concentrations in trial 1 water samples, the results are shown in figure 9 below. The statistical analysis yielded an F-statistic of 17.02 and a p-value of 0.0033. Treatments containing plankton (group A) contained significantly higher alkane concentrations than treatments without plankton (group B).

Phytoplankton have an extremely high oil content, up to 67% of their dry weight can be attributed to lipids [45]. *Nannochloropsis* in particular can accumulate high levels of polyunsaturated fatty acids [48]. They reproduce very rapidly, doubling their biomass up to three times per day [45]. All of these things considered, the increase in alkane concentrations between samples with and without plankton could potentially be attributed to phytoplankton. When determining the source of hydrocarbons, the carbon preference index (CPI) is used for alkanes. Biogenic sources of alkanes favor odd numbered n-alkanes. The CPIs of crude oil are usually around 1, biogenic sources are above 1. The equation for determining CPIs is below [56]. Based on a rough estimate, biogenic alkanes were responsible for a large portion of the alkane concentration.

$$CPI = (sum\ of\ odd\ (n) alkanes)/(sum\ of\ even\ (n) alkanes)$$

Rotifers are extremely voracious zooplankton. They can consume 115,000 *Nannochloropsis* cells a day [41]. This gives them the potential for controlling phytoplankton populations in the exposure system. In addition, they can eat almost anything in the appropriate size range, including emulsified oil [39]. Due to rotifers eating phytoplankton and potentially oil in the HEWAFs, their presence in the exposures could sequester alkanes making them unavailable for weathering processes, and retaining them in the beakers. Because the biomass of zooplankton is much smaller in comparison to phytoplankton, most of the plankton biomass is attributed to phytoplankton, making any planktonic effect observed likely due to phytoplankton [23]. Even so, the capability of rotifers to consume large amounts of food could cause some effect.

HEWAF concentration had a significant effect on total alkane concentrations as well. This fixed effect had an F-statistic of 5.75 and a p-value of 0.0214. 100% HEWAF treatments were included in group A, 66.6% HEWAF and 33.3% HEWAF treatments were included in group AB, and the 0% HEWAF treatments were included in group B. 100% HEWAF was significantly different from 0% HEWAF treatments, but not from the other two treatment groups (33.3% HEWAF and 66.6% HEWAF). The 66.6% HEWAF and 33.3% HEWAF treatments were not significantly different from each other, or the other two treatment groups, 0% and 100% HEWAF. This analysis placed HEWAF treatments with large differences in total alkane concentration estimates together; for this reason, analysis was conducted on the log-normalized values of the total alkane concentrations from each sample to distinguish the differences between groups better.

The HEWAF concentration effect was still significant in the log-normalized analysis (F-statistic of 42.85, p-value of <0.0001). The 100% HEWAF and 66.6%

HEWAF treatments were placed in group A, and 33.3% HEWAF and the 0% HEWAF treatments were separated into group B. This indicated that total alkane concentrations in the 100% HEWAF and 66.6% HEWAF treatments were not significantly different from each other, but were significantly different than in the 33.3% HEWAF and 0% HEWAF treatments. In addition, the 33.3% HEWAF treatment was not significantly higher than the control.

The HEWAF concentrations were based on different loading volumes, 0 mLs for 0%, 132 mLs for 33.3%, etc. High-energy water accommodated fractions were chosen for this experiment because of their similarity to natural phenomenon; however, this method most likely has a high occurrence of oil emulsions. Because oil emersions are not part of the water-oil solution, HEWAFs are not always homogenous, and small differences in loadings by volume may lead to large or almost not existent differences in actual HEWAF hydrocarbon concentration. This is likely the reason why there were no significant differences between each HEWAF treatment.

The HEWAF concentration/plankton interaction effect was significant. The analysis yielded an F-statistic of 4.5 and a p-value of 0.0395. The same problem was encountered for this effect as the previous (groups contained treatments with very different alkane concentration estimates); so, log-normalized analysis was used to distinguish groups. The HEWAF concentration/plankton interaction effect was significant in log-normalized analysis as well; the F-statistic was 21.39 and the p-value was 0.0004. Group A contained the 100% HEWAF treatment group with plankton; group AB: both 66.6% HEWAF with plankton and 33.3% HEWAF with plankton; group BC: 100% HEWAF without plankton; group CD: 66.6% HEWAF without plankton;

group DE: both 0% HEWAF without plankton and 0% HEWAF with plankton; and group E: 33.3% HEWAF without plankton.

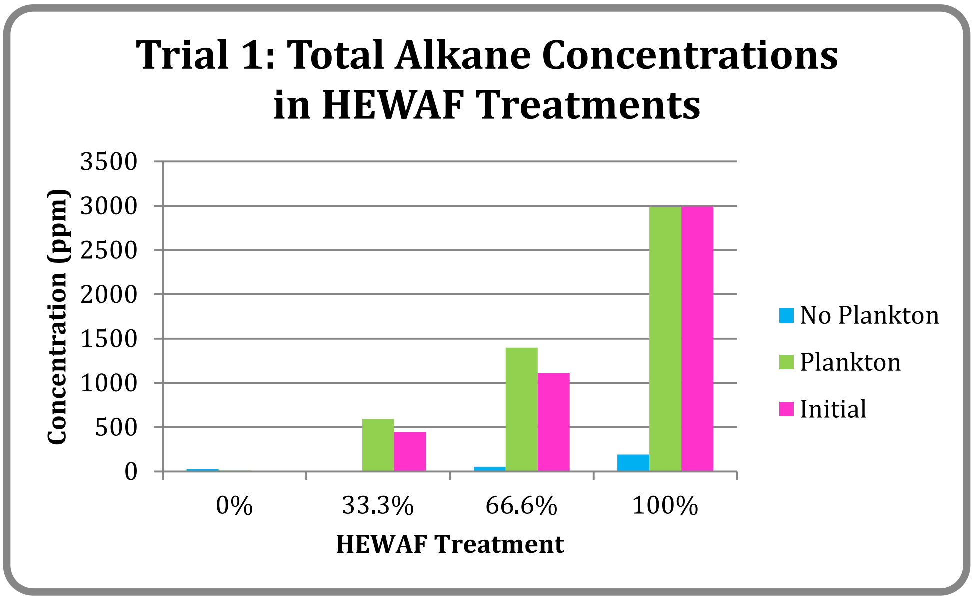


Figure 9. Trial 1: Alkane Concentration by HEWAF Treatment

When the interaction of HEWAF concentration and plankton was taken into consideration (Figure 9), the higher HEWAF concentrations with plankton contained the most alkanes. Identical HEWAF concentrations had statistically higher alkane concentrations in treatments containing plankton. In some cases, there were more alkanes in the samples taken post experiment (with plankton) than in the initial samples (without plankton) taken at the beginning of the experiment. The difference was within the standard error; however, this bolsters the odds of the phytoplankton contributing a biogenic portion to the alkane concentrations.

4.1.2 Aromatic Concentrations

Plankton had a significant effect on the aromatic concentrations in water samples from trial 1. Analysis yielded an F-statistic of 24.16 and a p-value of 0.0012. Samples containing plankton (group A) had significantly higher concentrations of aromatics than samples without (group B).

Aquatic organisms can bioconcentrate organic pollutants. The pollutants partition between the organisms and the environment based on the relative solubility in each [23]. The hydrophobic nature of phytoplankton gives them the tendency to associate with dissolved petroleum hydrocarbons. The high lipid content of the phytoplankton in trial 1 potentially caused a shift in the two-part partition model towards the organism, meaning the phytoplankton was able to absorb a significant amount of oil before reaching equilibrium with the water. As a result, more aromatics remained in the beakers, unavailable for volatilization, etc. Aromatic compounds are not found naturally in plankton, so the aromatics detected in the samples were sourced from the HEWAFs. PAHs also preferentially bind particulate matter, especially organic matter, and could have used both plankton species as a substrate for resisting vaporization and other weathering effects [16]. Rotifer consumption of oil or phytoplankton that has sequestered oil could also have caused an increase in aromatic retention.

The HEWAF concentration had a significant effect on the aromatic concentration (F-statistic 14.32, p-value 0.0014). This analysis was not effective for distinguishing significant difference between HEWAF treatments groups; it showed that the 100% HEWAF treatment (group A) had higher aromatic concentrations than the other three treatment groups (group B), but that the other groups were not significantly different from each other. Analysis of the log-normalized data agreed that HEWAF concentration had a significant effect on aromatic concentration (F-statistic 101.38, p-value <0.0001); and that the 100% HEWAF concentration treatment belonged to group A, 66.6% HEWAF treatment to group AB, 33.3% HEWAF treatment to group B, and 0% HEWAF treatment to group C. This analysis showed that 0% HEWAF treatments contained

statistically lower aromatic concentrations than the other 3 treatments, and there were statistically higher concentrations in 100% HEWAF treatments than 33.3% HEWAF treatments.

Aromatics are much more water-soluble than alkanes of similar molecular weight [16]. For this reason, it was expected that HEWAF loading would be a more effective way of obtaining distinct aromatic hydrocarbon levels than alkanes. Because the aromatic portion of oil is better solubilized, it should be more uniformly distributed throughout the water/oil mixture, and larger HEWAF loadings should correspond to larger aromatic concentrations. This was not the case. Aromatics were no more uniformly distributed throughout HEWAFs than alkanes.

The HEWAF concentration/plankton interaction effect (Figure 10) on aromatic concentrations was statistically significant (F-statistic 6.39, p-value 0.0161). 100% HEWAF concentration with plankton belonged to group A; 66.6% HEWAF with plankton belonged to group AB; and 33.3% HEWAF with plankton, 100% HEWAF without plankton, 66.6% HEWAF without plankton, 33.3% HEWAF without plankton, 0% HEWAF without plankton, and 0% HEWAF with plankton belonged to group B. Again, analysis of log-normalized data was used to give a clearer picture of differences between treatment groups. This analysis accepted the null hypothesis of no effect (F-statistic of 3.31, p-value of <0.0781); however, it better separated treatments into statistically different groups. Group A contained 100% HEWAF with plankton treatments. Group AB contained 66.6% HEWAF with plankton, 33.3% HEWAF with plankton, and 100% HEWAF without plankton treatments. Group BC contained 66.6% HEWAF without plankton treatment. Group C contained the 33.3% HEWAF without

plankton treatment. Group D contained the 0% HEWAF with and without plankton treatments.

100% HEWAF, 66.6% HEWAF, and 33.3% HEWAF (all with plankton) contained statistically similar aromatic concentrations. They had the three highest concentrations of all of the treatment groups, although not statistically higher than 100% HEWAF and 66.6% HEWAF without plankton. The presence of plankton appeared to give exposure beakers the ability to retain higher levels of aromatics, probably due to particulate binding.

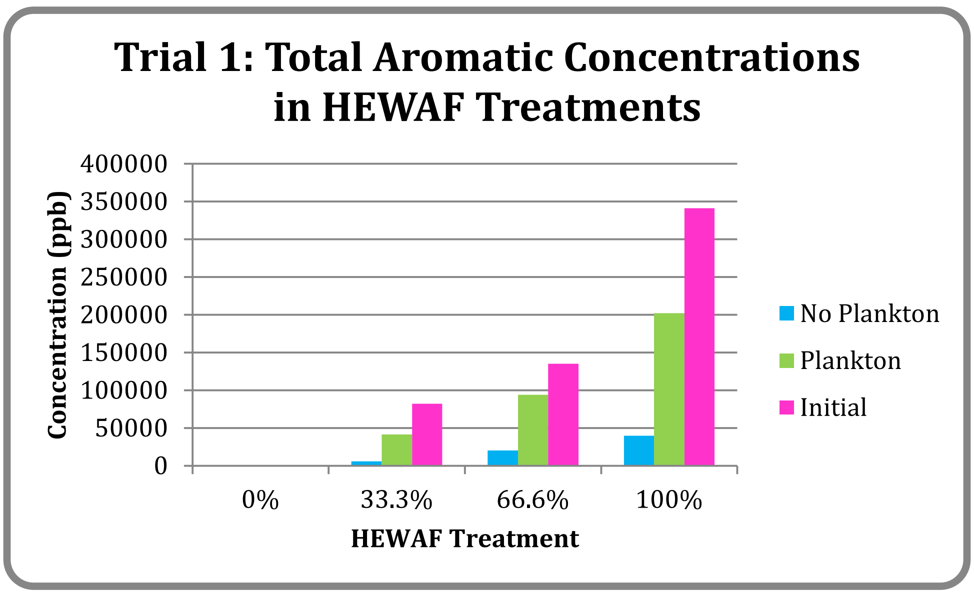


Figure 10. Trial 1: Aromatic Concentration by HEWAF Treatment

4.2 Trial 2: Evaluation of the Contribution of Phytoplankton to HEWAF Toxicity

Trial 2 was conducted to determine if the effects that plankton had on alkane and aromatic concentrations in trial 1 were due to phytoplankton. Half of each beaker's water was filtered at the end of the trial to remove the phytoplankton biomass, and the filtered and unfiltered waters were extracted separately to roughly estimate how much of the alkane and aromatic concentrations the phytoplankton might be sequestering. The HEWAF concentrations in this experiment, trial 3, and trial 4 were based on the LD_{50} for

Bay Anchovy (0%, 7.5%, and 15%). Trial 2 was not run in replicate, so no conclusions could be drawn from the data, which will be reported as trends.

4.2.1 Alkane Concentrations

In the 0% HEWAF concentration group, the filter removed 2.91 ppm (15%) of the total alkane concentration from the sample without phytoplankton and 32.16 ppm (86%) of the total alkane concentration from the sample containing phytoplankton. The total alkane concentration was higher in the unfiltered sample containing phytoplankton than the unfiltered sample without phytoplankton. The level of alkanes in the control suggests there may have been a contamination issue, since they are comparable to the 7.5% HEWAF samples. The high alkane level in the sample containing phytoplankton could also be due to a biogenic contribution. The stock water used in the exposures was from the Gulf of Mexico, which could have contributed some background levels.

The 7.5% HEWAF sample with phytoplankton had a higher alkane concentration than the sample without. Filtering removed 16.96 ppm (90%) of the total alkanes from the sample without phytoplankton, and 7.11 ppm (25%) from the sample with phytoplankton.

The 15% HEWAF sample with phytoplankton had a lower alkane concentration than the sample without, which goes against the trend in trial 1. The filter removed 42.15 ppm (63%) of the total alkane concentration in the sample without phytoplankton, and 39.05 ppm (66%) in the sample containing phytoplankton.

As shown by Figure 11 below, overall, there was no visible trend in alkane concentrations related to HEWAF loading or the addition of phytoplankton, and there

was no trend in the amount of alkane removed by filtration in treatments with and without phytoplankton.

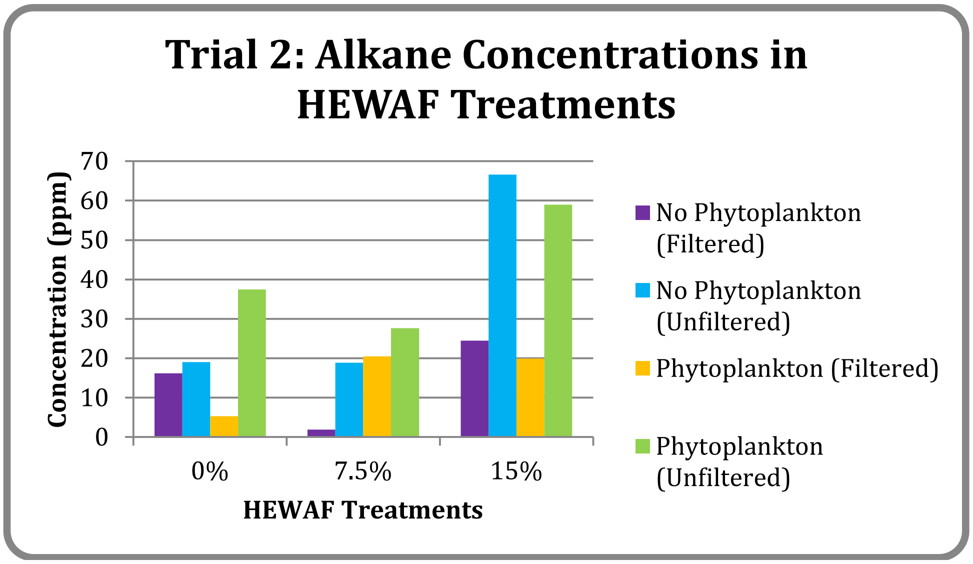


Figure 11. Trial 2: Alkane Concentration by HEWAF Treatment

4.2.2 Aromatic Concentrations

There was a very small amount of aromatics in the 0% HEWAF samples. In the 7.5% HEWAF treatment, the aromatic concentration was higher in the sample without phytoplankton than with. 9774 ppb (69%) of the aromatics were removed by filtration from the sample without phytoplankton, and 4399 ppb (64%) from the sample with phytoplankton. In the 15% HEWAF concentration group, the aromatic concentration was higher in the sample without phytoplankton than 15% HEWAF with phytoplankton. Filtration removed 27418 (82%) ppm of the aromatics from the sample without phytoplankton, and 8677 ppm (66%) from the sample with phytoplankton.

As Figure 12 below displays, there was an increasing trend in aromatic concentration that corresponded to an increase in HEWAF concentration, but there was no trend in the amount of aromatics removed by filtration. Also, aromatic concentrations were higher in the samples without phytoplankton.

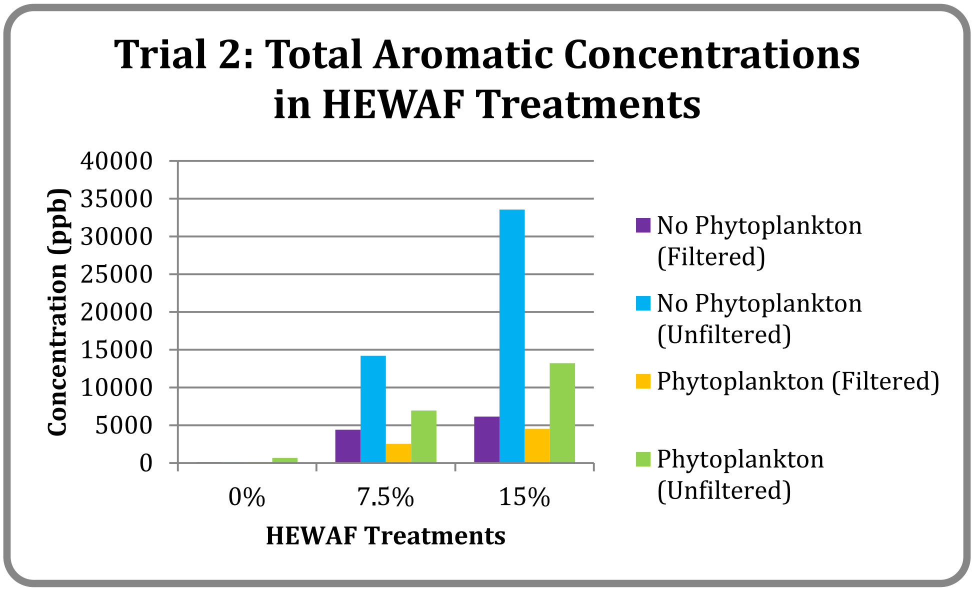


Figure 12. Trial 2: Aromatic Concentration by HEWAF Treatment

4.2.3 Mortality

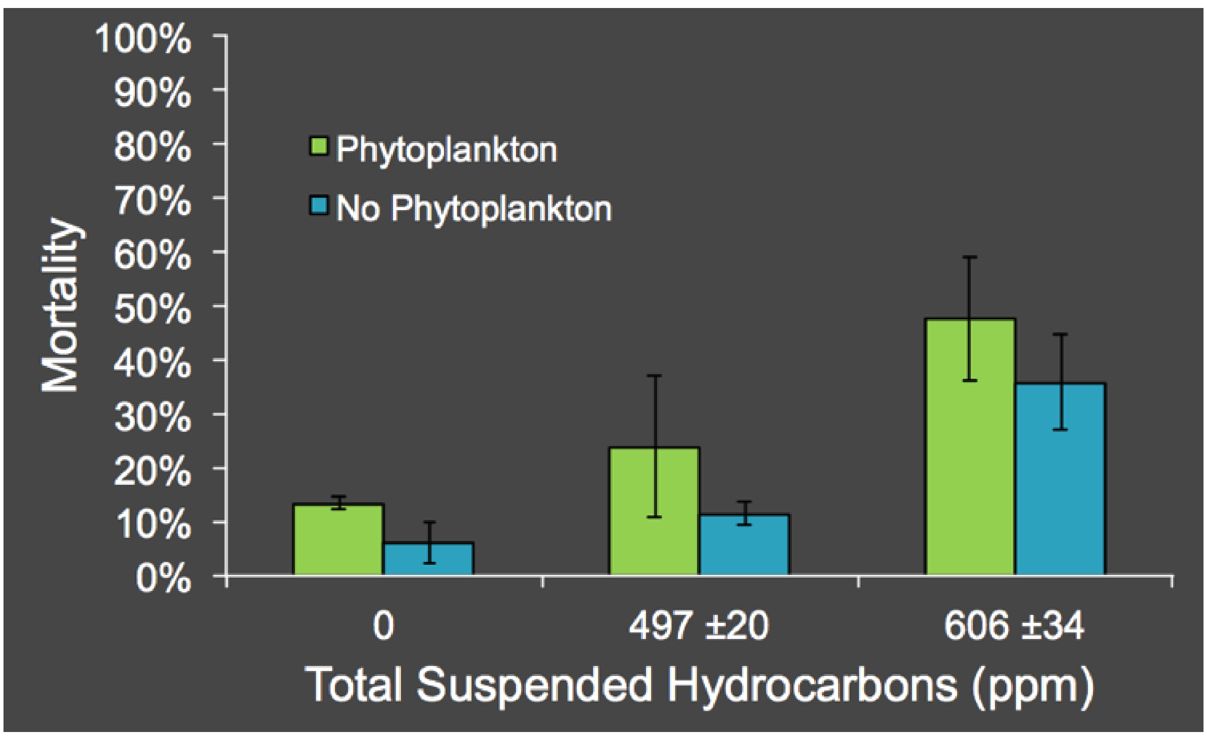


Figure 13. Mortality of Bay Anchovy in HEWAF Treatments containing only Phytoplankton [57]

In an experiment of identical HEWAF and phytoplankton concentrations to those analyzed above, Dr. Webb saw an increase in the mortality of Bay Anchovy with

increasing HEWAF concentration, as well as an elevated death rate in treatments containing phytoplankton (shown in Figure 13 above) [57]. These results do not correspond with the previous aromatic data showing higher levels of aromatics in samples without phytoplankton, which are most closely associated with oil toxicity. Because no trends could be detected from the alkane analysis, no connection could be made to Dr. Webb's results.

4.3 Trial 3: Evaluation of the Contribution of Rotifers to HEWAF Toxicity

Trial 3 was conducted to determine if the effects that plankton had on alkane and aromatic concentrations in trial 1 were due to the presence of zooplankton. Half of each beaker was filtered to remove the rotifer biomass, and the filtered and unfiltered waters were extracted to roughly estimate how much of the alkane and aromatic concentrations the rotifers might be sequestering. Trial 3 was not run in replicate, so no conclusions could be drawn from the data, which will be reported as trends. It used HEWAF concentrations of 0%, 7.5%, and 15%.

4.3.1 Alkane Concentration

There were minimal amounts of alkane found in the 0% HEWAF treatment. The 7.5% HEWAF samples had a higher level of alkanes in the samples without rotifers than with. 63.18 ppm (52%) of the alkanes were filtered out of the sample without rotifers, and 18.09 ppm (70%) of the sample with rotifers. The 15% HEWAF concentration group also had higher levels of alkanes in the sample without rotifers, than the sample with rotifers. In the sample without rotifers, the filter removed 40.61 ppm (61%) of the alkanes, and in the sample with rotifers, it removed 3.89 ppm (14%). There was no trend for the amount of alkanes in a sample versus the amount of HEWAF, or for the amount of

alkanes removed in samples with or without rotifers (Figure 14). There were more alkanes in the samples without rotifers.

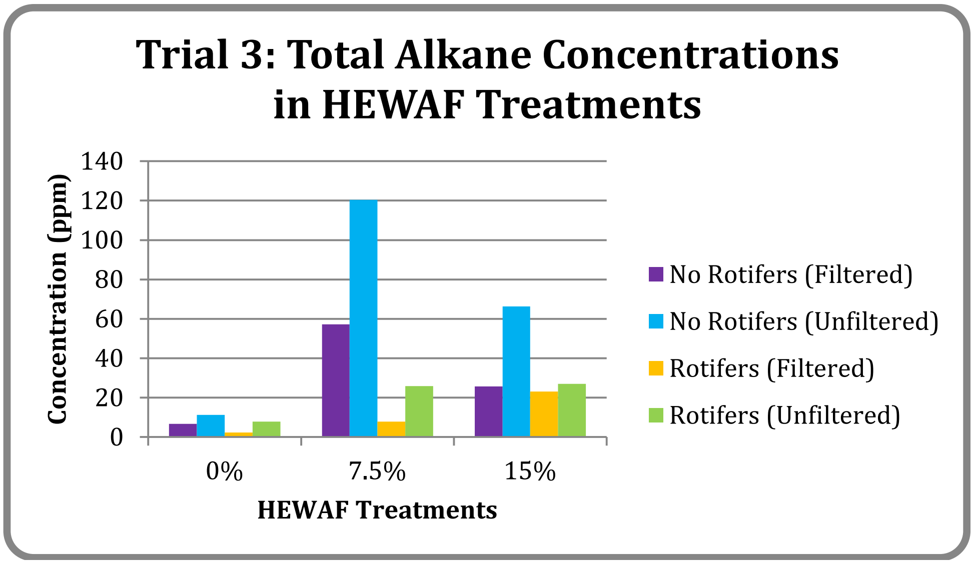


Figure 14. Trial 3: Alkane Concentration by HEWAF Treatment

4.3.2 Aromatic Concentrations

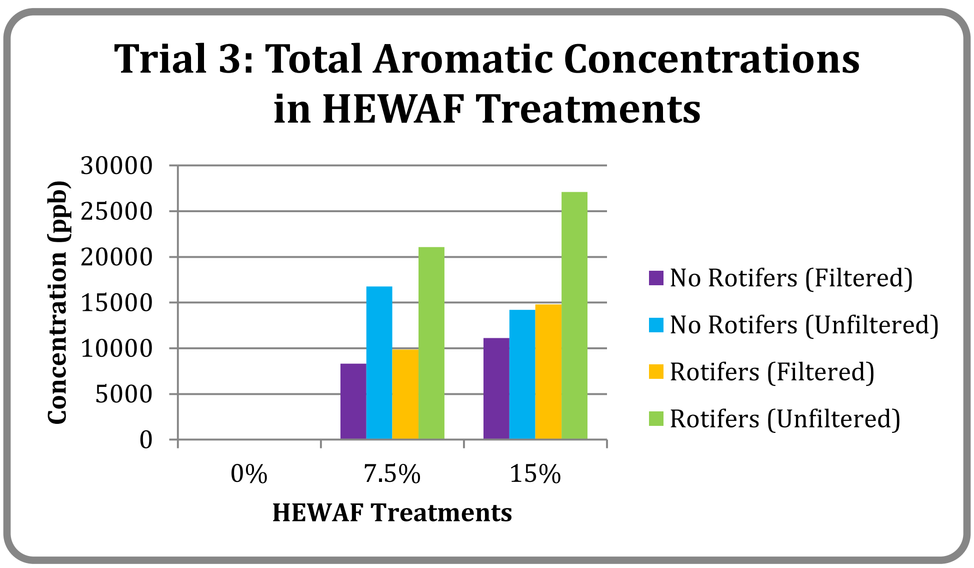


Figure 15. Trial 3: Aromatic Concentration by HEWAF Treatment

There were almost no aromatics found in the 0% HEWAF sample analysis. The 7.5% and 15% HEWAF samples with rotifers contained higher levels of aromatics than those without rotifers. Filtration removed 8430 ppb (50%) from the 7.5% HEWAF sample without rotifers, and 11200 ppb (53%) of the sample with rotifers. In the 15%

HEWAF samples, 3086 ppb (22%) of the aromatics were filtered out of the sample without rotifers, and 12298 ppb (45%) of the sample with rotifers. There was no trend relating HEWAF concentration to measured aromatic concentration; although there were more aromatics in samples with rotifers. Also, the filters removed similar percentages from the samples with and without rotifers. Figure 15 above depicts these findings.

4.3.3 Mortality

In a study conducted by Dr. Sarah Webb the presence of zooplankton had no statistically significant effect on the mortality rate of Bay Anchovy, this can be seen in figure 15 below [57]. Trial 3 showed that aromatics are higher in samples with rotifers, but alkanes are lower. Based on the aromatic results, we would expect a higher mortality rate, and the opposite based on alkanes.

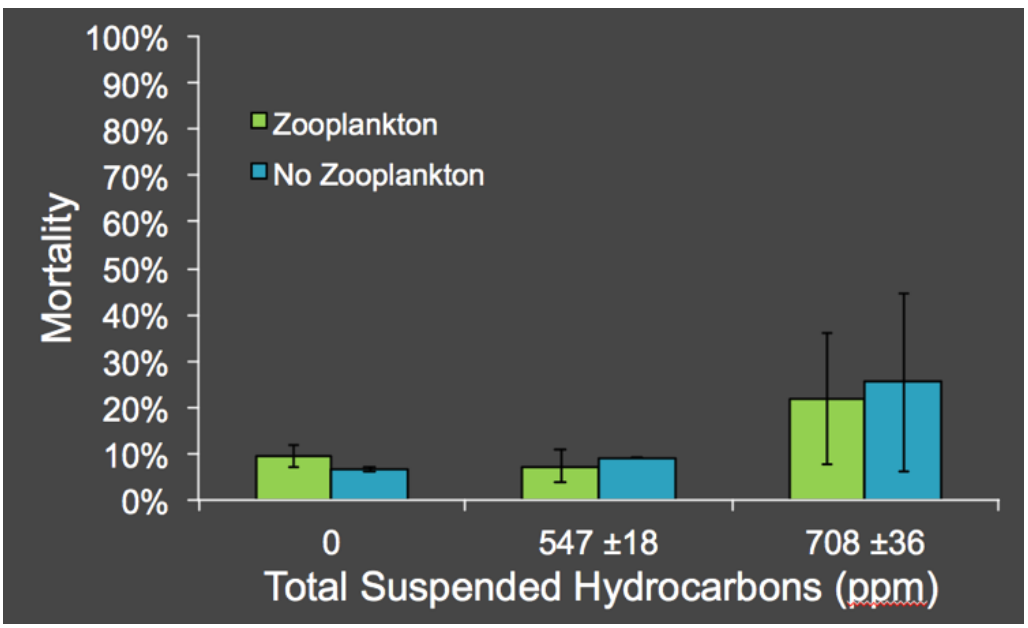


Figure 16. Mortality of Bay Anchovy in HEWAF Treatments Containing only Zooplankton [57]

4.4 Trial 4: Evaluation of the sequestration of hydrocarbons due to glass adherence

Trial 4 was conducted using HEWAF concentrations of 0%, 7.5%, and 15%. It included phytoplankton, rotifers, and Bay Anchovy. It was designed to determine if the increased level of petroleum hydrocarbons in the samples containing plankton (trial 1) was due to planktonic absorption of oil, and not due to oil constituents adhering to glassware in the absence of plankton. The samples were filtered to try and estimate planktonic absorption.

4.4.1 Alkane Concentration (Water)

In trial 4, the plankton effect was not significant (F-statistic of 0.16, p-value of 0.6970) for alkane concentrations. Treatments with and without plankton did not have statistically different alkane concentrations (both belonged to group A).

The HEWAF concentration effect was significant. It had an F-statistic of 28.09 and a p-value of <0.0001. Group A contained the 15% HEWAF and 7.5% HEWAF treatments, and Group B contained the 0% HEWAF treatments. The 7.5% HEWAF and 15% HEWAF treatment concentrations were not significantly different from each other, but their alkane concentrations were significantly higher than those in treatments with 0% HEWAF.

The HEWAF concentration/plankton interaction had a significant effect on alkane concentrations (F-statistic of 12.69, p-value of 0.0011). The treatments were divided into group A: 15% HEWAF with plankton; group AB: 7.5% HEWAF without plankton; group BC: 15% HEWAF without plankton; and group C: 7.5% HEWAF with plankton, 0% HEWAF with plankton, and 0% HEWAF without plankton.

There is no statistical connection between the concentration of alkane in the samples with plankton versus samples without plankton, and the plankton-HEWAF interaction grouping didn't reveal any trend in the data either. Figure 17 below summarizes the results from this analysis.

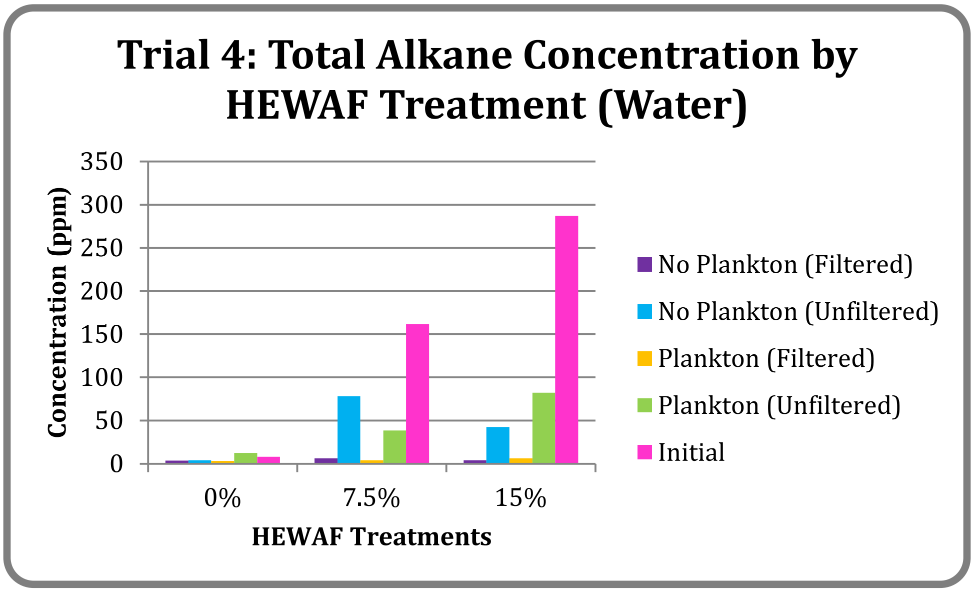


Figure 17. Trial 4: Alkane Concentration by HEWAF Treatment

4.4.2 Aromatic Concentration (Water)

Plankton did not have a significant effect on the aromatic concentrations in trial 4 water samples (F-statistic 0.11, p-value 0.7463).

The HEWAF concentrations had a statistically significant effect on aromatic concentrations (F-statistic 10.59, p-value 0.0022). Even though the 15% HEWAF treatment had a much higher average estimated aromatic concentration than the 7.5% HEWAF treatment, treatments containing 7.5% HEWAF and 15% HEWAF (group A) were not statistically different from each other; however, both had statistically higher aromatic concentrations than the 0% HEWAF treatments (group B).

The plankton/HEWAF concentration interaction was not significant. It had an F-statistic of 0.82 and a p-value of 0.4632. 15% HEWAF with and without plankton (group

A) and 7.5% HEWAF with and without plankton and 0% HEWAF with plankton treatments (group AB) were all statistically similar. The only significant difference was a higher alkane concentration in the 15% HEWAF treatments above the 0% HEWAF treatment without plankton. The log-normalized analysis agreed that plankton/HEWAF concentration interaction had no significant effect on aromatic concentration (F-statistic 8.46, p-value 0.0051), but shows a significant difference between group A (15% HEWAF with plankton, 15% HEWAF without plankton, and 7.5% HEWAF without plankton), group B (0% HEWAF with plankton), and group C (0% HEWAF without plankton). The aromatic concentration decreased from group A to group B. Also, 7.5 % HEWAF with plankton (group AB) had significantly higher alkane concentrations than 0% HEWAF without plankton. None of the samples containing HEWAF (7.5% and 15% HEWAF) were statistically different from each other. The results from this experiment can be seen in figure 18 below.

Because there was no planktonic effect in this trial, even though it was so pronounced in trial 1, it is possible that there is some threshold of oil (HEWAF) that must be reached before the plankton begins to have an effect on the alkane/aromatic concentrations. The partition coefficient may have favored that the aromatic and alkane hydrocarbons remain in water as opposed to entering planktonic tissue. It is also possible that less HEWAF equated less oil available for rotifer consumption.

The HEWAFs likely contained emulsions and non-homogenous oil concentrations in the stock. This would lead smaller HEWAF loadings to decrease the likelihood of having significant differences between alkane/aromatic concentrations in the treatments.

This could have played a part in the lack of difference between HEWAF treatment group concentrations in trial 3 and trial 4.

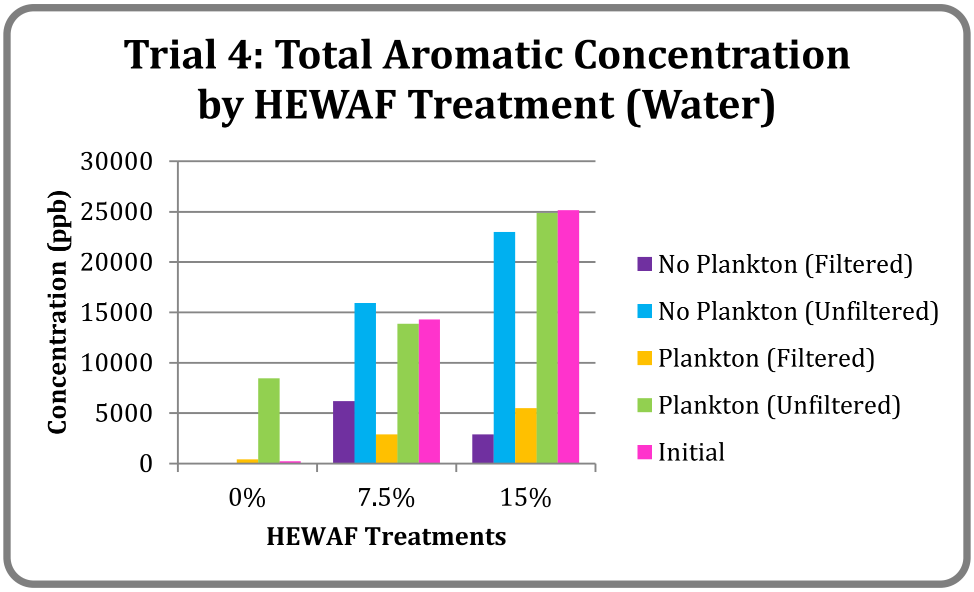


Figure 18. Trial 4: Aromatic Concentration by HEWAF Treatment (Water)

4.4.3 Mortality

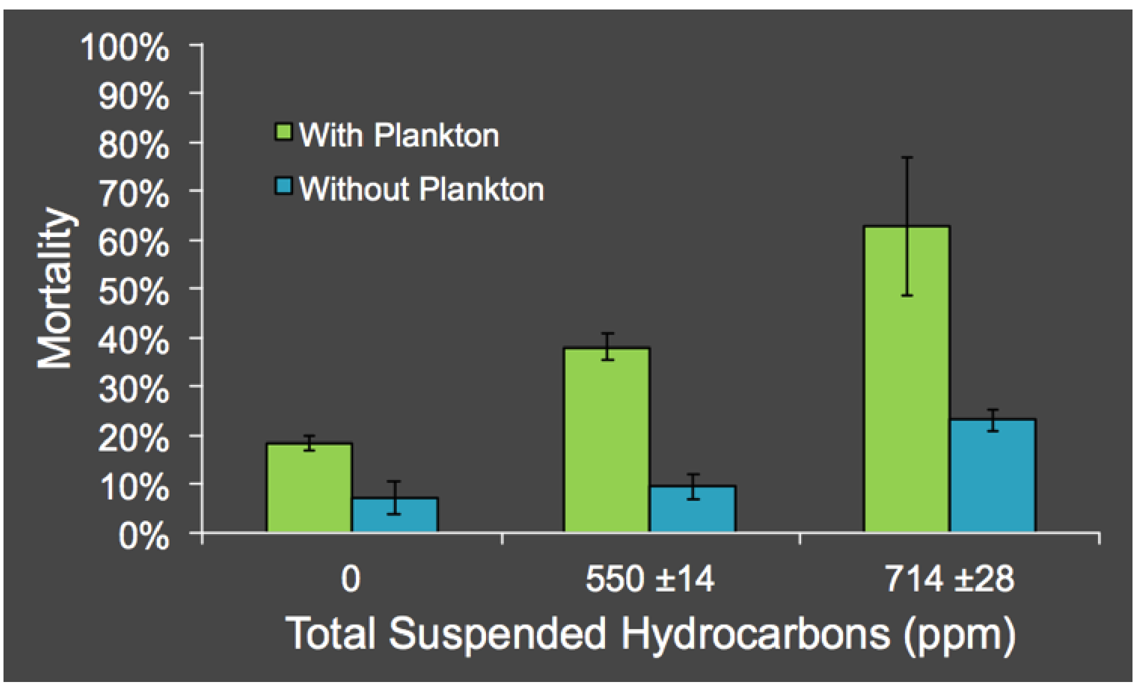


Figure 19. Mortality of Bay Anchovy in HEWAF treatments with Phytoplankton & Zooplankton [57]

Dr. Webb's studies showed an increase in mortality of Bay Anchovy in beakers containing plankton over those without plankton at the 0%, 7.5%, and 15% HEWAF concentrations. The trial 4 hydrocarbon water analysis did not imply that this would be a likely trend; however, there are thousands of other compounds present in crude oil that could be playing a role. The oil used in these exposures was not oiled prior to HEWAF preparation; so BTEX, the most toxic and volatile portion of oil, may have still been present and caused increased mortality. The plankton might have reduced the amount able to volatilize. Results from this study can be seen above in figure 19.

4.4.4 Alkane Concentration (Beaker)

Plankton did not have a significant effect on alkane concentrations extracted from the beakers used in the exposure trials (F-statistic 4.45, p-value 0.0793). This showed that the absence of plankton did not increase the amount of alkanes that adsorbed to the glassware.

The HEWAF concentration had a significant effect on alkane concentrations (F-statistic 41.02, p-value 0.0003). 15% HEWAF treatments had statistically higher alkane concentrations than 7.5% and 0% HEWAF treatments. 7.5% HEWAF treatments had higher alkane concentrations than 0% HEWAF treatments, but not significantly higher. When higher amounts of oil were present (15% HEWAF), higher amounts of alkanes adhered to the glass.

There was no significant plankton/HEWAF concentration interaction effect on alkane concentration (F-statistic 4.59, p-value 0.0620); although the log normalized data analysis disagreed (F-statistic 11.76, p-value 0.0084). It showed that beakers that contained 15% HEWAF with plankton had significantly higher alkane concentrations

than beakers that contained 7.5% HEWAF with and without plankton, as well as 0% HEWAF with and without plankton. The 15% HEWAF without plankton treatment was higher than the 0% HEWAF treatments with and without plankton, but not from both 7.5% HEWAF treatments. Treatments of 7.5% HEWAF with and without plankton were significantly higher than 0% HEWAF treatments with and without plankton. In summary, beakers that contained HEWAF concentrations above 0% retained greater levels of alkanes. Results from this experiment are graphed in figure 20 below.

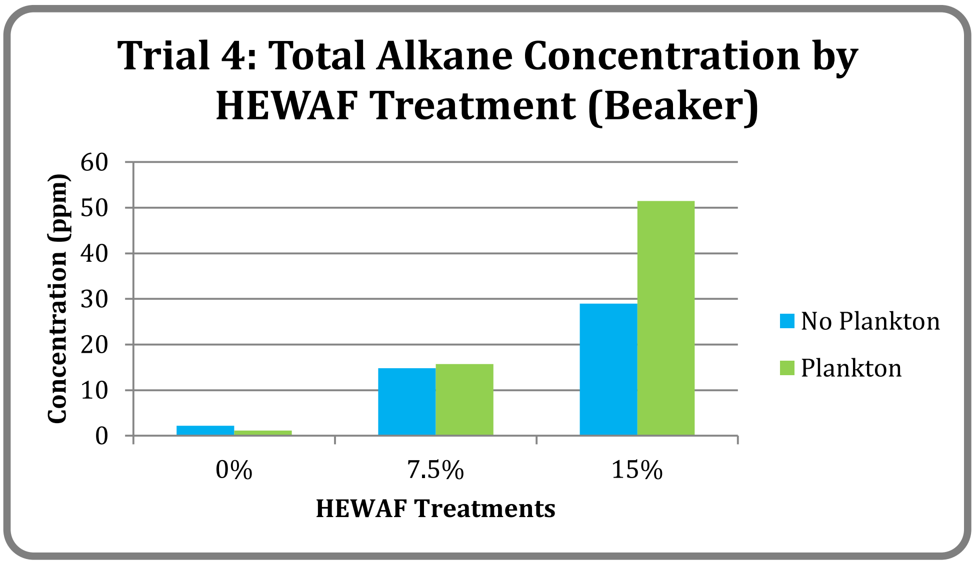


Figure 20. Trial 4: Alkane Concentration by HEWAF Treatment (Beaker)

4.4.5 Aromatic Concentration (Beaker)

The plankton effect on the aromatic concentrations in the exposure beakers was not significant. The F-statistic was 2.24 and the p-value was 0.2853. Whether a beaker did or did not contain plankton had no effect on the amount of aromatics that adsorbed to the glass.

The HEWAF concentration effect was significant (F-statistic 92.44, p-value <0.0001). The 15% HEWAF treatments had statistically higher aromatic concentrations than 7.5% HEWAF and 0% HEWAF treatments, and 7.5% HEWAF treatments had

higher aromatic concentrations than 0% HEWAF treatments; however, the difference was not significant.

The interaction between plankton and HEWAF concentration did not significantly affect aromatic concentrations in the beakers. The F-statistic was 3.37 and the p-value was 0.1402. Aromatic concentrations in treatments of 15% HEWAF with and without plankton were significantly higher than in treatments of 7.5% HEWAF with and without plankton, as well as 0% HEWAF with and without plankton, the lower HEWAF concentrations were not significantly different form each other. The results form this analysis are seen below in figure 21.

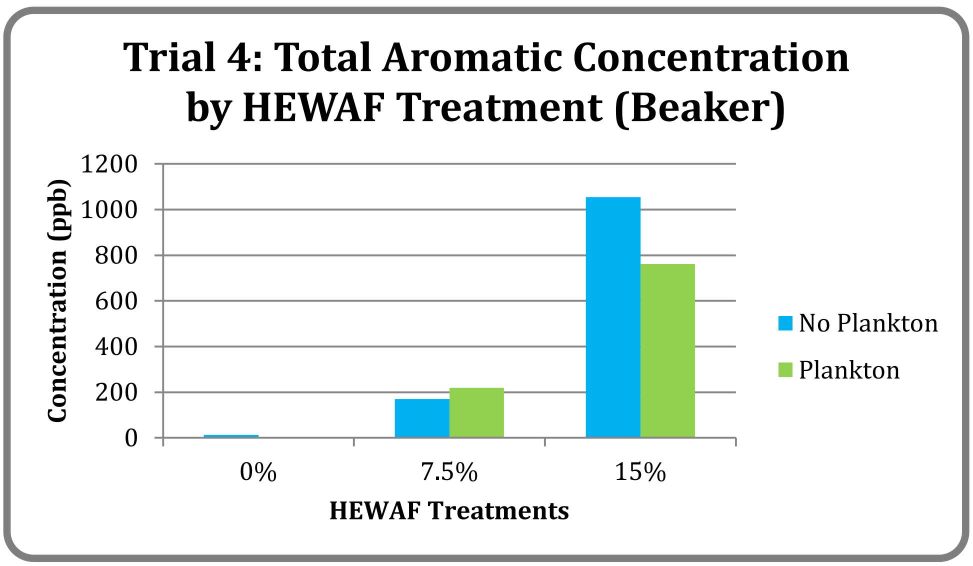


Figure 21. Trial 4: Aromatic Concentration by HEWAF Treatment (Beaker)

4.4.6 Alkane Concentration (Filter)

The plankton effect on alkane concentration in the filters used in experiment 4 was significant (F-statistic of 6.94, p-value of 0.0388). Treatments containing plankton had statistically higher alkane concentrations than treatments without. Because the filters removed the plankton from the exposure waters, the filter extractions most likely

contained a lot of biogenic alkanes which contributed to an elevated alkane level in the plankton versus no plankton filters.

HEWAF concentration had a significant effect on alkane concentrations as well. It had an F-statistic of 10.97 and a p-value of 0.0099. The 15% HEWAF treatments contained significantly higher alkane concentrations than treatments of 7.5% and 0% HEWAF. 7.5% HEWAF treatments had alkane concentrations that were elevated above the 0% HEWAF treatments; but, there wasn't a great enough difference for it to be statistically significant.

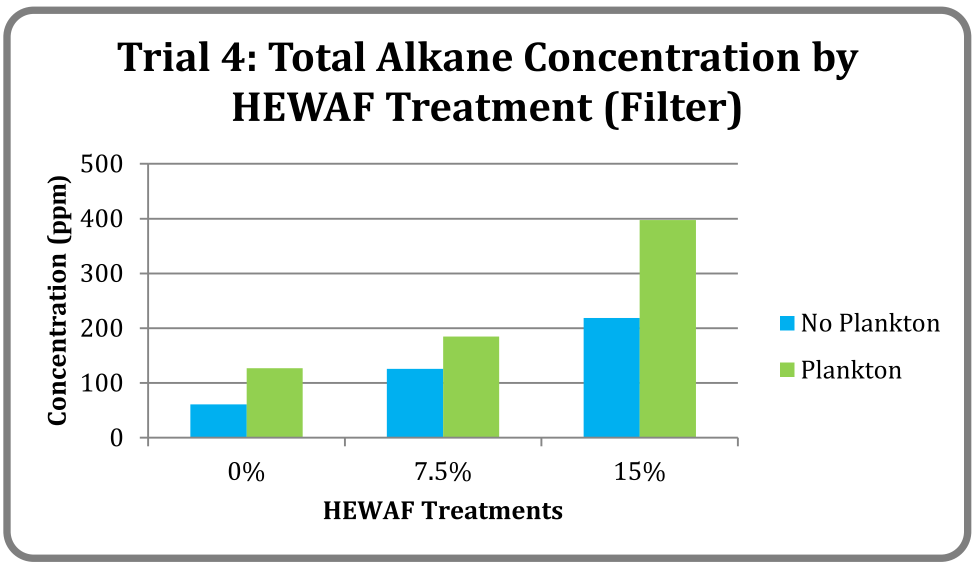


Figure 22. Trial 4: Alkane Concentration by HEWAF Treatment (Filter)

The interaction between plankton and HEWAF concentration did not significantly affect the alkane concentrations (F-statistic of 1.01, p-value of 0.4174). The 15% HEWAF with plankton treatment contained significantly higher levels of alkanes than treatments with 0% HEWAF with and without plankton, as well as 7.5% HEWAF without plankton. Group A contained 15% HEWAF with plankton; group AB: 15% HEWAF without plankton and 7.5% HEWAF with plankton; group B: 0% HEWAF with plankton, 7.5% HEWAF without plankton, and 0% HEWAF without plankton. All of the

treatments had similar alkane concentrations. Results from this analysis can be seen in figure 22 above.

4.4.7 Aromatic Concentration (Filter)

The plankton effect on the aromatic concentrations in the trial 4 filters was not significant (F-statistic 0.66, p-value 0.4474); however, analysis of the log-normalized data showed a significant difference (F-statistic of 39.47, p-value of 0.0008). According to log-normalized data, treatments containing plankton had statistically higher aromatic concentration than treatments without plankton.

HEWAF concentration had a significant effect on aromatic concentrations. It had an F-statistic of 86.80 and a p-value of <0.0001. HEWAF treatments of 15% had significantly higher aromatic concentrations than the two lower treatments, and 7.5% HEWAF had higher aromatic concentrations than 0% HEWAF treatments. Filters removed more aromatics from treatments containing 15% HEWAF than the 0% and 7.5% HEWAF treatments.

Plankton/HEWAF concentration interaction had no significant effect on aromatic concentrations (F-statistic of 0.03, p-value of 0.9663); however, analysis of the log normalized data showed significance (F-statistic of 26.48, p-value of 0.0011). 15% HEWAF with and without plankton (group A) had significantly higher aromatic concentrations than all the other treatments. Treatments of 7.5% HEWAF with and without plankton (group B) had significantly higher alkane concentrations than 0% HEWAF with plankton and without plankton. So, filters removed more aromatics from higher HEWAF loadings, but did not remove more or less from identical HEWAF loadings with plankton. Results from this analysis are below in figure 23.

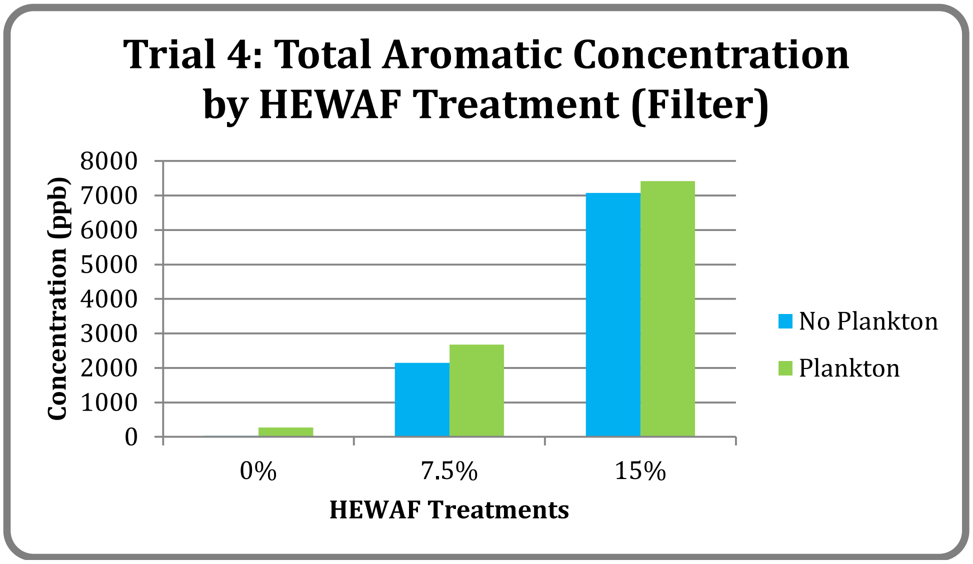


Figure 23. Trial 4: Aromatic Concentration by HEWAF Treatment (Filter)

4.4.8 Alkane Sequestration

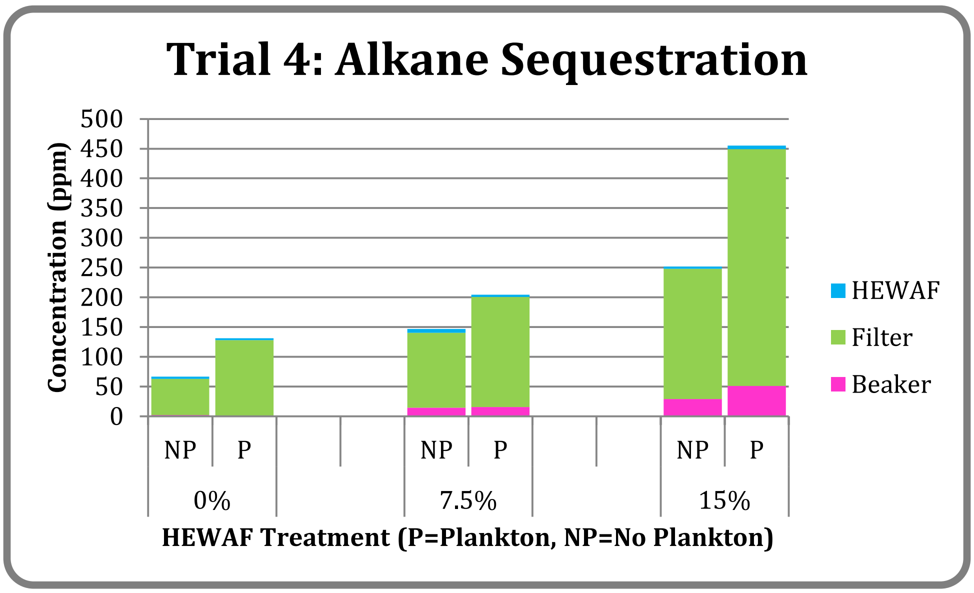


Figure 24. Trial 4: Alkane Sequestration

As shown in figure 24 above, the filters removed most of the alkanes from the exposure waters, regardless of if they contained plankton or not. This made filtering plankton an unreliable way of determining how much alkanes plankton biomass sequestered. The glassware retained very little of the alkanes, meaning it did not play a role in the planktonic effect visible in the first trial; although, this could be attributed to

the much lower HEWAF concentrations used in this trial. There was no connection between the amount of alkanes removed and the presence of plankton. It is also easy to see from this graph, that even though the plankton did not have a statistical effect on the alkane concentration, samples containing plankton had more alkanes present. This increase is most likely due to biogenic contribution by the lipid rich phytoplankton.

4.4.9 Aromatic Sequestration

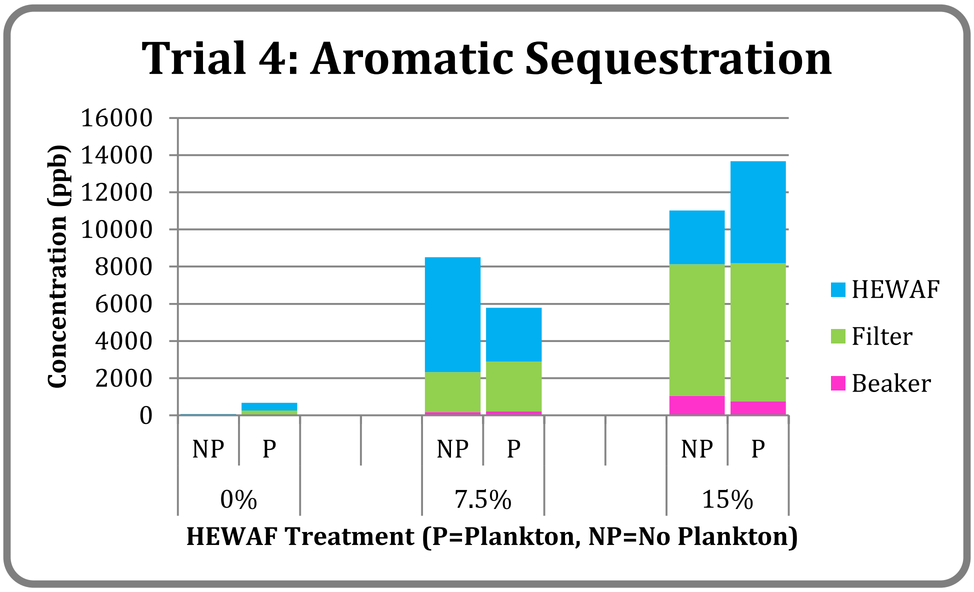


Figure 25. Trial 4: Aromatic Sequestration

As shown in figure 25 above, the filters removed most of the aromatics from the exposure waters, regardless of if they contained plankton or not. Increases in aromatic removal were due to HEWAF treatments; however, the log-normalized data showed a slight increase due to planktonic effect. Overall, filtering plankton was not a definitive way of determining how much aromatics were sequestered by plankton biomass. The glassware retained very little of the aromatics, meaning it did not play a role in the planktonic effect visible in the first trial. The only increase in glassware aromatics came with increasing HEWAF concentration, not from plankton. This could change with higher HEWAF loadings.

4.5 Trial 5: Re-evaluation of the plankton contribution to HEWAF Toxicity

Trial 5 was conducted to re-examine the effect of plankton on higher HEWAF concentrations.

4.5.1 Alkane Concentrations

The trial was not run in replicate, so no conclusions can be drawn from it; however, for alkanes it appears that the planktonic effect remained intact. There were more alkanes in samples with plankton than without plankton of the same HEWAF loading. Also, there is a trend for increasing alkanes with increasing HEWAF loading in samples with or without plankton. These results are evident in figure 26 below.

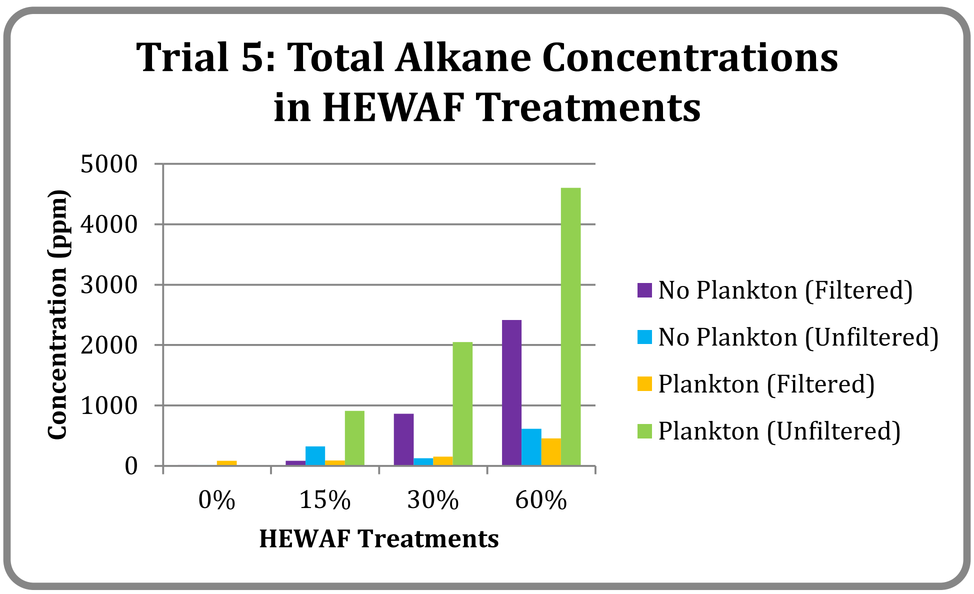


Figure 26. Trial 5: Alkane Concentration by HEWAF Treatment

4.5.2 Aromatic Concentrations

Aromatic concentrations seemed to increase in samples with increasing HEWAF loading. The samples with plankton contained more aromatics than the samples of the same HEWAF treatment without plankton. These results are shown in figure 27 below.

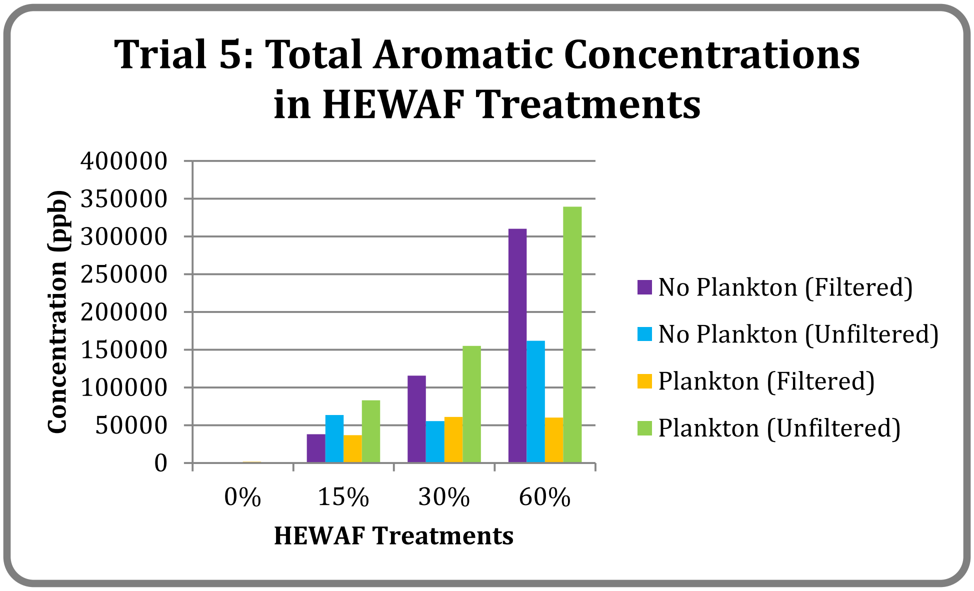


Figure 27. Trial 5: Aromatic Concentration by HEWAF Treatment

4.6 Initial Concentrations

Initial water samples were taken at various HEWAF concentrations (without plankton) to determine if HEWAF loading corresponded to changes in aromatic and alkane concentrations.

4.6.1 Alkane Initial Concentrations

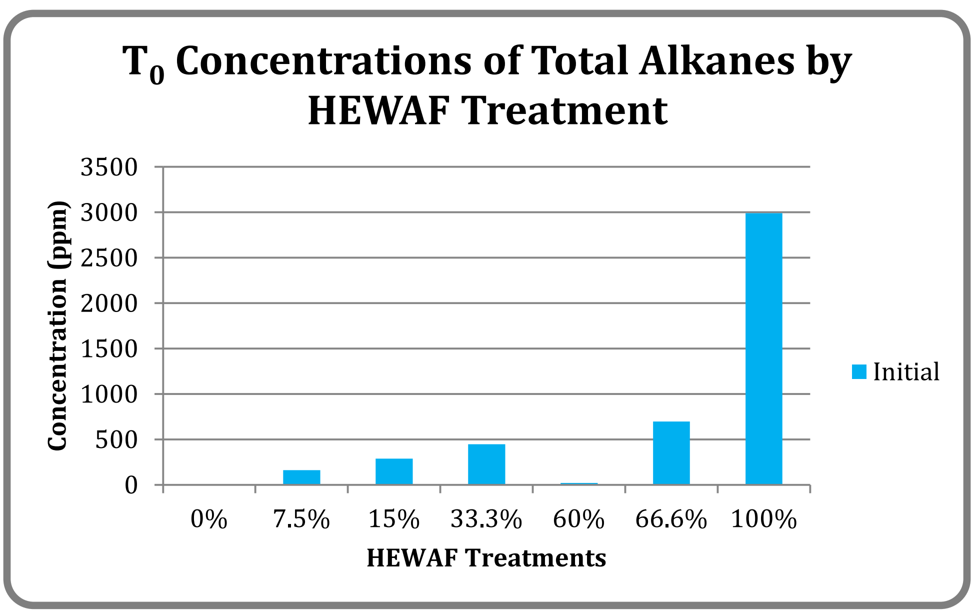


Figure 28. Initial Concentration of Alkanes by HEWAF Treatment

As seen in figure 28 above, with the exception of 60% HEWAF, alkane analysis of time zero water samples showed an increasing trend in alkane concentration with increasing HEWAF loading.

4.6.2 Aromatic Initial Concentrations

Figure 29 below shows that with the exception of 33.3%, there was an increasing trend in the amount of aromatics extracted from time zero water samples that corresponded to an increase in HEWAF concentration.

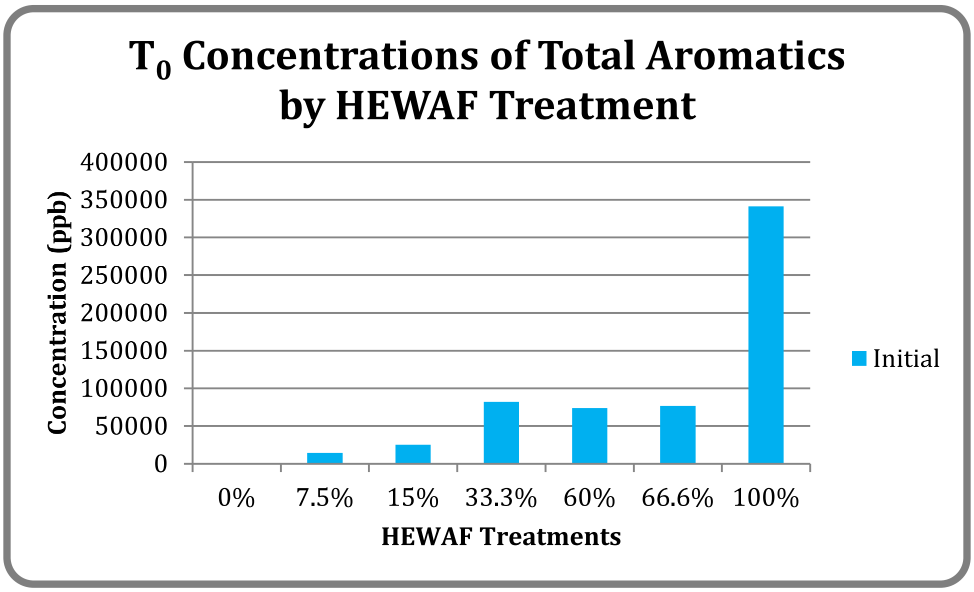


Figure 29. Initial Concentrations of Alkanes by HEWAF Treatment

CHAPTER 5. SUMMARY AND CONCLUSIONS

5.1 Summary

5.1.1 Trial 1: Evaluation of the Contributions of Plankton to HEWAF Toxicity

The presence of phytoplankton had a significant effect on both the alkane and aromatic concentrations in exposures conducted in trial 1; the concentrations were higher in water samples that contained plankton. It is likely that the high levels of alkanes in the samples containing plankton were due to the presence of phytoplankton, which is extremely lipid rich. Having a biogenic source of alkanes would also explain why increases in alkanes from the start to the end of the experiment occurred. The increased level of aromatics in samples with plankton was most likely due to the hydrophobic nature of PAHs, which imparts an affinity for particulate matter. The trial also implied that using loading volumes to achieve different HEWAF concentrations is not efficient if alkanes are being studied, but seemed to work well for aromatics. Aromatics are more soluble than alkanes, and were probably more homogenously dispersed throughout the HEWAF stock.

5.1.2 Trials 2 and 3: Contributions of Phytoplankton or Rotifers to HEWAF Toxicity

In trial 2, the study using 0%, 7.5%, and 15% HEWAF and containing phytoplankton without rotifers, there was no stepwise increase in alkanes with HEWAF concentration although there was for aromatics. The presence or absence of phytoplankton had no visible effect on the concentration of alkanes. In samples without phytoplankton there was an increased amount of aromatics. In a mortality study that used the same HEWAF and phytoplankton concentrations as this study, death increased with

increased loading of HEWAF, and also with the presence of phytoplankton [57]. These results contradict each other.

Trial 3 used the same HEWAF concentrations, but included rotifers without phytoplankton. There was no trend for an increase in aromatic or alkane concentrations with an increase in HEWAF loading. Higher levels of alkanes were found in samples of the same HEWAF concentrations containing rotifers than those without, but aromatic concentrations were higher in samples with rotifers. Dr. Webb conducted a mortality study under the same conditions and found that Bay Anchovy death rate was unaffected by rotifers.

The data from these trials was extremely inconsistent, and they would need to be repeated to determine the effect that each plankton species has on the concentration of alkanes and aromatics in the HEWAF exposures. The results indicated that filtering the water samples was not a good way to estimate the amount of oil that plankton sequestered, partially because oil compounds have a high affinity for organic matter (filters). The erratic results in alkane and aromatic concentrations were probably due to emulsions present in the HEWAFs; however, more trials would have to be conducted to see if this is really the case.

5.1.3 Trial 4: Evaluation of the sequestration of hydrocarbons due to glass adherence

Plankton did not have an effect on the alkane or aromatic hydrocarbon concentrations in the water samples from trial 4. This is probably due to the extremely low LD_{50}-based HEWAF loading. It is possible that the threshold at which plankton begins to sequester oil constituents had not been reached. Plankton had no effect on the amount of aromatics or alkanes sequestered by beakers, and only affected the amount of

alkanes removed by filtration. The increase in alkanes extracted from the filters in this experiment was probably due to biogenic donation. HEWAF concentration had a significant effect on the amount of alkanes and aromatics removed by filtration; it increased with increasing HEWAF loadings.

The plankton/HEWAF interaction was not significant for beakers or filter analysis of aromatics or alkanes, but was significant for the alkane analysis of water. There were statistically more alkanes in samples with higher HEWAF concentrations containing plankton. Again, this is most likely attributed to biogenic alkanes.

5.1.4 Trial 5: Re-evaluation of the plankton contribution to HEWAF Toxicity

Although trial 5 is not statistically relevant, this experiment's results agreed with trial 1. Water samples with plankton retained higher aromatic and alkane concentrations; and, hydrocarbon concentrations of both fractions increased with increased HEWAF loading.

5.1.5 Initial Concentrations

When the alkane and aromatic concentrations of water samples collected at the beginning of various trials were graphed, overall they seemed to increase with increasing HEWAF loading.

5.2 Conclusions

Plankton was at its maximum abundance in the Gulf of Mexico when the Deep Water Horizon oil spill occurred. For this reason, it was important to determine if plankton had an effect on the toxicity of the MC252 oil that was spilled. HEWAFs were chosen as the vehicle for the exposures because they most closely mimic how the spilled oil likely interacted with the GoM waters.

In HEWAF samples of high loadings (33%, 66.6%, 100% HEWAF), aromatic and alkane concentrations were elevated in samples that contained plankton, indicating that there was some binding mechanism taking place. This mechanism was probably driven by the attraction of lipophilic oil to lipid rich phytoplankton. This phenomenon prevented hydrocarbon constituents from leaving the exposure beakers by volatilization or other weathering processes. This trend does not hold true in trials that use low HEWAF loadings (7.5% and 15% HEWAF).

The attempt to determine which plankton species used in the exposures (rotifers or phytoplankton) was responsible for the "planktonic effect" in high loadings failed; however, it is strongly suspected that the effect can be attributed to phytoplankton. Regardless, this effect is an important finding because not only does their inclusion in oil exposures yield more environmentally relevant results, but it also shows that the consumption of plankton could concentrate these oil constituents in the higher trophic levels.

Although the post-exposure samples analyzed in all of the trials yielded aromatic and alkane concentrations that did not follow a consistent trend, pre-trial samples of each HEWAF concentration without plankton showed that aromatic and alkane concentrations steadily increased with increasing HEWAF loading. In addition, Dr. Webb's work with Bay Anchovy showed that mortality rates increased steadily with increasing HEWAF concentrations. The presence of plankton also increased the percentage of deaths over beakers without plankton of an identical HEWAF concentration (even in low concentrations). This shows that HEWAFs are a valuable tool for mortality studies, and plankton probably are playing some role in increasing HEWAF toxicity, but the GC/MS

analysis conducted in this study was not sufficient for determining what this role might be.

5.3 Future Research

All of the trials in this study excluded dispersants. Dispersants are key players in post oil spill dynamics since their use has become widespread. DWH cleanup included the use of dispersants on the sea floor, as well as the ocean surface, which makes them particularly relevant to the current study. The toxicities of dispersants varies significantly between different experiments and conditions, making trials similar to those analyzed in this thesis with the addition of dispersants necessary for understanding the true effect of DWH on the Gulf of Mexico. Because plankton appear to sequester crude oil compounds, especially the aromatic fraction, it is important to determine how dispersants affect the lipophilicity of these compounds for organic matter.

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APPENDIX

A. DATA

A.1 Trial 1

WAF	Sample	Alkanes (µg/L)	Aromatics (µg/L)
0% HEWAF	1A	15.43	171
0% HEWAF	1A (NR)	14.93	393
0% HEWAF	1B	29.98	870
0% HEWAF	1B (NR)	5.92	371
33.3% HEWAF	2A	2.91	4285
33.3% HEWAF	2A (NR)	379.99	29748
33.3% HEWAF	2B	5.18	7454
33.3% HEWAF	2B (NR)	801.09	52874
66.6% HEWAF	3A	25.11	20156
66.6% HEWAF	3A (NR)	1380.2	94320
66.6% HEWAF	3B	77.82	20213
66.6% HEWAF	3B (NR)	1414.79	94121
100% HEWAF	4A	130.57	33389
100% HEWAF	4A (NR)	247.69	45676
100% HEWAF	4B	1862.2	148293
100% HEWAF	4B (NR)	4105.04	255691
NR stands for with *Nannochloropsis* and Rotifers			
0% HEWAF	T_0 1A	5.96	100
0% HEWAF	T_0 1B	6.96	103
33.3% HEWAF	T_0 2A	368.75	71113
33.3% HEWAF	T_0 2B	522.54	93210
66.6% HEWAF	T_0 3A	1531.37	193580
66.6% HEWAF	T_0 3B	696.18	76722
100% HEWAF	T_0 4A	3577.73	347554
100% HEWAF	T_0 4B	2402.48	334573
All time zero contain no plankton			

A.2 Trial 2

WAF	Sample	Alkanes (µg/L)	Aromatics (µg/L)
15% HEWAF	with plankton/ filtered	19.95	4531
15% HEWAF	without plankton/ filtered	24.44	6140
15% HEWAF	without plankton/ unfiltered	66.59	33558
15% HEWAF	with plankton/ unfiltered	59	13208
7.5% HEWAF	without plankton/unfiltered	18.85	14182
7.5% HEWAF	without plankton/ filtered	1.89	4408
7.5% HEWAF	with plankton/ filtered	20.52	2525
7.5% HEWAF	with plankton/ unfiltered	27.63	6924
control	without plankton/ filtered	16.16	136
control	without plankton/ unifltered	19.07	61
control	with plankton/ unfiltered	5.29	659
control	with plankton/ filtered	37.45	122

A.3 Trial 3

WAF	Sample	Alkanes (µg/L)	Aromatics (µg/L)
15% HEWAF	without rotifers/ unfiltered	66.41	27094
15% HEWAF	without rotifers/filtered	25.8	14796
15% HEWAF	with rotifers/ unfiltered	23.14	14189
15% HEWAF	with rotifers/ filtered	27.03	11103
7.5% HEWAF	with rotifers/ unfiltered	25.95	21073
7.5% HEWAF	with rotifers/filtered	7.86	9873
7.5% HEWAF	without rotifers/ unfiltered	120.48	16750
7.5% HEWAF	without rotifers/ filtered	57.3	8320
control	with rotifers/ unfiltered	2.32	7
control	without rotifers/ unfiltered	6.66	10
control	without rotifers/ filtered	11.26	17
control	with rotifers/ filtered	7.93	40

A.4 Trial 4

A.4.1 Water

HEWAF Treatme	Samples	Medium	Alkanes (µg/L)	Aromatics (µg/L)
15% HEWAF	pre-trial	water	286.94	25150
7.5% HEWAF	pre-trial	water	161.48	14290
0% HEWAF	pre-trial	water	8.07	221

15% HEWAF	2 plankton/ unfiltered	water	75.63	15048
15% HEWAF	1 plankton/ unfiltered	water	88.54	34685
15% HEWAF	1 plankton/ filtered	water	4.81	5227
15% HEWAF	2 no planton/ unfiltered	water	43.77	19698
15% HEWAF	1 no plankton/ unfiltered	water	41.49	26293
15% HEWAF	1 no plankton/ filtered	water	3.99	6874
15% HEWAF	2 no plankton/ filtered	water	4.29	5685
15% HEWAF	2 plankton/ filtered	water	7.37	5753
7.5% HEWAF	2 no plankton/ unfiltered	water	76.83	16747
7.5% HEWAF	2 no plankton/ filtered	water	7.27	8667
7.5% HEWAF	2 plankton/ filtered	water	3.8	3259
7.5% HEWAF	1 plankton/ unfiltered	water	54.99	12214
7.5% HEWAF	2 plankton/ unfiltered	water	22.1	15535
7.5% HEWAF	1 no plankton/ unfiltered	water	79.21	15169
7.5% HEWAF	1 no plankton/ filtered	water	5.36	3714
7.5% HEWAF	1 plankton/ filtered	water	4.39	2521
control	1 no plankton/ filtered	water	3.74	32
control	1 no plankton/ unfiltered	water	4	29
control	2 plankton/ unfiltered	water	22.45	16625
control	2 plankton/ filtered	water	4.03	97
control	1 plankton/ filtered	water	2.39	702
control	2 no plankton/ filtered	water	3.6	33
control	2 no plankton/ unfiltered	water	3.61	37
control	1 plankton/ unfiltered	water	2.89	296

A.4.2 Beakers

15% HEWAF	2 plankton	glassware	41.26	905
15% HEWAF	1 plankton	glassware	61.72	619
15% HEWAF	2 no planton	glassware	27.41	1044
15% HEWAF	1 no plankton	glassware	30.56	1065
7.5% HEWAF	1 plankton	glassware	13.84	143
7.5% HEWAF	2 plankton	glassware	17.57	294
7.5% HEWAF	2 no plankton	glassware	13.65	226
7.5% HEWAF	1 no plankton	glassware	15.91	114
control	1 no plankton	glassware	2.58	12
control	2 plankton	glassware	1.17	0
control	2 no plankton	glassware	1.82	13
control	1 plankton	glassware	1.05	0

A.4.3 Filters

15% HEWAF	2 plankton	filter	398.81	7331
15% HEWAF	1 no plankton	filter	231.51	5861
15% HEWAF	2 no plankton	filter	206.27	8296
15% HEWAF	1 plankton	filter	396.29	7501
7.5% HEWAF	1 no plankton	filter	108.23	1712
7.5% HEWAF	2 no plankton	filter	143.06	2593
7.5% HEWAF	1 plankton	filter	230.19	3034
7.5% HEWAF	2 plankton	filter	139.7	2314
control	1 plankton	filter	230.52	210
control	2 no plankton	filter	51.54	31
control	1 no plankton	filter	70.66	34
control	2 plankton	filter	23.57	328

A.5 Trial 5

WAF	Sample	Alkanes (µg/L)	Aromatics (µg/L)
0%	no plankton/filtered	17.04	334
0%	no plankton/unfiltered	15.45	0
0%	plankton/filtered	84.2	1504
0%	plankton/unfiltered	9.07	304
15%	no plankton/filtered	81.34	38229
15%	no plankton/unfiltered	323.86	63441
15%	plankton/filtered	90.81	36703
15%	plankton/unfiltered	912.29	82786
30%	no plankton/filtered	860.02	115673
30%	no plankton/unfiltered	125.51	55279
30%	plankton/filtered	154.2	60964
30%	plankton/unfiltered	2052.24	155050
60%	no plankton/filtered	2414.87	310325
60%	no plankton/unfiltered	615.86	161824
60%	plankton/filtered	454.21	60178
60%	plankton/unfiltered	4602.24	339443
Time Zero			
60%	no plankton/unfiltered	21.56	73795
0%	no plankton/unfiltered	8.07	221

B. STATISTCAL ANALYSIS

B.1 Trial 1

plankton

Obs	Oil_con	plankton	Alk	aro
1	0	0	15.43	171
2	0	0	29.98	870
3	0	1	14.93	393
4	0	1	5.92	371
5	1	0	2.91	4285
6	1	0	5.18	7454
7	1	1	379.99	29748
8	1	1	801.09	52874
9	2	0	25.11	20156
10	2	0	77.82	20213
11	2	1	1380.20	94320
12	2	1	1414.79	94121
13	3	0	130.57	33389
14	3	0	247.69	45676
15	3	1	1862.20	148293
16	3	1	4105.04	255691

plankton

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	Alk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	4	0 1 2 3
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	15
Columns in Z	0
Subjects	1
Max Obs per Subject	16

Number of Observations	
Number of Observations Read	16
Number of Observations Used	16
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	326603

plankton

The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	129.8
AIC (Smaller is Better)	131.8
AICC (Smaller is Better)	132.5
BIC (Smaller is Better)	131.9

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	8	17.02	0.0033
Oil_con	3	8	5.75	0.0214
Oil_con*plankton	3	8	4.50	0.0395

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	66.8362	202.05	8	0.33	0.7493
plankton		1	1245.52	202.05	8	6.16	0.0003
Oil_con	0		16.5650	285.75	8	0.06	0.9552
Oil_con	1		297.29	285.75	8	1.04	0.3286
Oil_con	2		724.48	285.75	8	2.54	0.0350
Oil_con	3		1586.38	285.75	8	5.55	0.0005
Oil_con*plankton	0	0	22.7050	404.11	8	0.06	0.9566
Oil_con*plankton	0	1	10.4250	404.11	8	0.03	0.9801
Oil_con*plankton	1	0	4.0450	404.11	8	0.01	0.9923
Oil_con*plankton	1	1	590.54	404.11	8	1.46	0.1821
Oil_con*plankton	2	0	51.4650	404.11	8	0.13	0.9018
Oil_con*plankton	2	1	1397.50	404.11	8	3.46	0.0086
Oil_con*plankton	3	0	189.13	404.11	8	0.47	0.6523
Oil_con*plankton	3	1	2983.62	404.11	8	7.38	<.0001

plankton

The Mixed Procedure

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
plankton		0		1	-1178.68	285.75	8	-4.12	0.0033	Tukey
Oil_con	0		1		-280.73	404.11	8	-0.69	0.5069	Tukey
Oil_con	0		2		-707.91	404.11	8	-1.75	0.1179	Tukey
Oil_con	0		3		-1569.81	404.11	8	-3.88	0.0046	Tukey
Oil_con	1		2		-427.19	404.11	8	-1.06	0.3213	Tukey
Oil_con	1		3		-1289.08	404.11	8	-3.19	0.0128	Tukey
Oil_con	2		3		-861.89	404.11	8	-2.13	0.0655	Tukey
Oil_con*plankton	0	0	0	1	12.2800	571.49	8	0.02	0.9834	Tukey
Oil_con*plankton	0	0	1	0	18.6600	571.49	8	0.03	0.9748	Tukey
Oil_con*plankton	0	0	1	1	-567.84	571.49	8	-0.99	0.3495	Tukey
Oil_con*plankton	0	0	2	0	-28.7600	571.49	8	-0.05	0.9611	Tukey
Oil_con*plankton	0	0	2	1	-1374.79	571.49	8	-2.41	0.0428	Tukey
Oil_con*plankton	0	0	3	0	-166.43	571.49	8	-0.29	0.7783	Tukey
Oil_con*plankton	0	0	3	1	-2960.91	571.49	8	-5.18	0.0008	Tukey
Oil_con*plankton	0	1	1	0	6.3800	571.49	8	0.01	0.9914	Tukey
Oil_con*plankton	0	1	1	1	-580.12	571.49	8	-1.02	0.3398	Tukey
Oil_con*plankton	0	1	2	0	-41.0400	571.49	8	-0.07	0.9445	Tukey
Oil_con*plankton	0	1	2	1	-1387.07	571.49	8	-2.43	0.0414	Tukey
Oil_con*plankton	0	1	3	0	-178.70	571.49	8	-0.31	0.7625	Tukey
Oil_con*plankton	0	1	3	1	-2973.19	571.49	8	-5.20	0.0008	Tukey
Oil_con*plankton	1	0	1	1	-586.50	571.49	8	-1.03	0.3348	Tukey
Oil_con*plankton	1	0	2	0	-47.4200	571.49	8	-0.08	0.9359	Tukey
Oil_con*plankton	1	0	2	1	-1393.45	571.49	8	-2.44	0.0407	Tukey
Oil_con*plankton	1	0	3	0	-185.09	571.49	8	-0.32	0.7543	Tukey
Oil_con*plankton	1	0	3	1	-2979.57	571.49	8	-5.21	0.0008	Tukey
Oil_con*plankton	1	1	2	0	539.08	571.49	8	0.94	0.3731	Tukey
Oil_con*plankton	1	1	2	1	-806.95	571.49	8	-1.41	0.1956	Tukey
Oil_con*plankton	1	1	3	0	401.41	571.49	8	0.70	0.5024	Tukey
Oil_con*plankton	1	1	3	1	-2393.08	571.49	8	-4.19	0.0030	Tukey
Oil_con*plankton	2	0	2	1	-1346.03	571.49	8	-2.36	0.0463	Tukey
Oil_con*plankton	2	0	3	0	-137.67	571.49	8	-0.24	0.8157	Tukey
Oil_con*plankton	2	0	3	1	-2932.15	571.49	8	-5.13	0.0009	Tukey

plankton

The Mixed Procedure

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Oil_con*plankton	2	1	3	0	1208.37	571.49	8	2.11	0.0674	Tukey
Oil_con*plankton	2	1	3	1	-1586.12	571.49	8	-2.78	0.0241	Tukey
Oil_con*plankton	3	0	3	1	-2794.49	571.49	8	-4.89	0.0012	Tukey

Differences of Least Squares Means					
Effect	Oil_con	plankton	_Oil_con	_plankton	Adj P
plankton		0		1	0.0033
Oil_con	0		1		0.8963
Oil_con	0		2		0.3601
Oil_con	0		3		0.0195
Oil_con	1		2		0.7230
Oil_con	1		3		0.0509
Oil_con	2		3		0.2218
Oil_con*plankton	0	0	0	1	1.0000
Oil_con*plankton	0	0	1	0	1.0000
Oil_con*plankton	0	0	1	1	0.9629
Oil_con*plankton	0	0	2	0	1.0000
Oil_con*plankton	0	0	2	1	0.3474
Oil_con*plankton	0	0	3	0	1.0000
Oil_con*plankton	0	0	3	1	0.0113
Oil_con*plankton	0	1	1	0	1.0000
Oil_con*plankton	0	1	1	1	0.9588
Oil_con*plankton	0	1	2	0	1.0000
Oil_con*plankton	0	1	2	1	0.3390
Oil_con*plankton	0	1	3	0	1.0000
Oil_con*plankton	0	1	3	1	0.0110
Oil_con*plankton	1	0	1	1	0.9566
Oil_con*plankton	1	0	2	0	1.0000
Oil_con*plankton	1	0	2	1	0.3347
Oil_con*plankton	1	0	3	0	1.0000
Oil_con*plankton	1	0	3	1	0.0109
Oil_con*plankton	1	1	2	0	0.9714

plankton

The Mixed Procedure

Differences of Least Squares Means					
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Adj P**
Oil_con*plankton	1	1	2	1	0.8304
Oil_con*plankton	1	1	3	0	0.9943
Oil_con*plankton	1	1	3	1	0.0374
Oil_con*plankton	2	0	2	1	0.3676
Oil_con*plankton	2	0	3	0	1.0000
Oil_con*plankton	2	0	3	1	0.0119
Oil_con*plankton	2	1	3	0	0.4755
Oil_con*plankton	2	1	3	1	0.2241
Oil_con*plankton	3	0	3	1	0.0159

plankton

Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	1245.52	202.05	A
2		0	66.8362	202.05	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	3		1586.38	285.75	A
4	2		724.48	285.75	AB
5	1		297.29	285.75	AB
6	0		16.5650	285.75	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
7	3	1	2983.62	404.11	A
8	2	1	1397.50	404.11	AB
9	1	1	590.54	404.11	B
10	3	0	189.13	404.11	B
11	2	0	51.4650	404.11	B
12	0	0	22.7050	404.11	B
13	0	1	10.4250	404.11	B
14	1	0	4.0450	404.11	B

plankton

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	aro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	4	0 1 2 3
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	15
Columns in Z	0
Subjects	1
Max Obs per Subject	16

Number of Observations	
Number of Observations Read	16
Number of Observations Used	16
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	7.6442E8

plankton

The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	191.9
AIC (Smaller is Better)	193.9
AICC (Smaller is Better)	194.6
BIC (Smaller is Better)	194.0

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	8	24.16	0.0012
Oil_con	3	8	14.32	0.0014
Oil_con*plankton	3	8	6.39	0.0161

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	16527	9775.08	8	1.69	0.1294
plankton		1	84476	9775.08	8	8.64	<.0001
Oil_con	0		451.25	13824	8	0.03	0.9748
Oil_con	1		23590	13824	8	1.71	0.1263
Oil_con	2		57203	13824	8	4.14	0.0033
Oil_con	3		120762	13824	8	8.74	<.0001
Oil_con*plankton	0	0	520.50	19550	8	0.03	0.9794
Oil_con*plankton	0	1	382.00	19550	8	0.02	0.9849
Oil_con*plankton	1	0	5869.50	19550	8	0.30	0.7717
Oil_con*plankton	1	1	41311	19550	8	2.11	0.0675
Oil_con*plankton	2	0	20185	19550	8	1.03	0.3321
Oil_con*plankton	2	1	94221	19550	8	4.82	0.0013
Oil_con*plankton	3	0	39533	19550	8	2.02	0.0778
Oil_con*plankton	3	1	201992	19550	8	10.33	<.0001

plankton

The Mixed Procedure

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
plankton		0		1	-67950	13824	8	-4.92	0.0012	Tukey
Oil_con	0		1		-23139	19550	8	-1.18	0.2706	Tukey
Oil_con	0		2		-56751	19550	8	-2.90	0.0198	Tukey
Oil_con	0		3		-120311	19550	8	-6.15	0.0003	Tukey
Oil_con	1		2		-33612	19550	8	-1.72	0.1239	Tukey
Oil_con	1		3		-97172	19550	8	-4.97	0.0011	Tukey
Oil_con	2		3		-63560	19550	8	-3.25	0.0117	Tukey
Oil_con*plankton	0	0	0	1	138.50	27648	8	0.01	0.9961	Tukey
Oil_con*plankton	0	0	1	0	-5349.00	27648	8	-0.19	0.8514	Tukey
Oil_con*plankton	0	0	1	1	-40791	27648	8	-1.48	0.1784	Tukey
Oil_con*plankton	0	0	2	0	-19664	27648	8	-0.71	0.4972	Tukey
Oil_con*plankton	0	0	2	1	-93700	27648	8	-3.39	0.0095	Tukey
Oil_con*plankton	0	0	3	0	-39012	27648	8	-1.41	0.1959	Tukey
Oil_con*plankton	0	0	3	1	-201472	27648	8	-7.29	<.0001	Tukey
Oil_con*plankton	0	1	1	0	-5487.50	27648	8	-0.20	0.8476	Tukey
Oil_con*plankton	0	1	1	1	-40929	27648	8	-1.48	0.1770	Tukey
Oil_con*plankton	0	1	2	0	-19802	27648	8	-0.72	0.4942	Tukey
Oil_con*plankton	0	1	2	1	-93838	27648	8	-3.39	0.0094	Tukey
Oil_con*plankton	0	1	3	0	-39150	27648	8	-1.42	0.1945	Tukey
Oil_con*plankton	0	1	3	1	-201610	27648	8	-7.29	<.0001	Tukey
Oil_con*plankton	1	0	1	1	-35442	27648	8	-1.28	0.2358	Tukey
Oil_con*plankton	1	0	2	0	-14315	27648	8	-0.52	0.6186	Tukey
Oil_con*plankton	1	0	2	1	-88351	27648	8	-3.20	0.0127	Tukey
Oil_con*plankton	1	0	3	0	-33663	27648	8	-1.22	0.2581	Tukey
Oil_con*plankton	1	0	3	1	-196123	27648	8	-7.09	0.0001	Tukey
Oil_con*plankton	1	1	2	0	21126	27648	8	0.76	0.4667	Tukey
Oil_con*plankton	1	1	2	1	-52910	27648	8	-1.91	0.0920	Tukey
Oil_con*plankton	1	1	3	0	1778.50	27648	8	0.06	0.9503	Tukey
Oil_con*plankton	1	1	3	1	-160681	27648	8	-5.81	0.0004	Tukey
Oil_con*plankton	2	0	2	1	-74036	27648	8	-2.68	0.0280	Tukey
Oil_con*plankton	2	0	3	0	-19348	27648	8	-0.70	0.5039	Tukey
Oil_con*plankton	2	0	3	1	-181808	27648	8	-6.58	0.0002	Tukey

plankton

The Mixed Procedure

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Oil_con*plankton	2	1	3	0	54688	27648	8	1.98	0.0833	Tukey
Oil_con*plankton	2	1	3	1	-107772	27648	8	-3.90	0.0046	Tukey
Oil_con*plankton	3	0	3	1	-162460	27648	8	-5.88	0.0004	Tukey

Differences of Least Squares Means					
Effect	Oil_con	plankton	_Oil_con	_plankton	Adj P
plankton		0		1	0.0012
Oil_con	0		1		0.6528
Oil_con	0		2		0.0763
Oil_con	0		3		0.0012
Oil_con	1		2		0.3744
Oil_con	1		3		0.0048
Oil_con	2		3		0.0467
Oil_con*plankton	0	0	0	1	1.0000
Oil_con*plankton	0	0	1	0	1.0000
Oil_con*plankton	0	0	1	1	0.8017
Oil_con*plankton	0	0	2	0	0.9939
Oil_con*plankton	0	0	2	1	0.1033
Oil_con*plankton	0	0	3	0	0.8308
Oil_con*plankton	0	0	3	1	0.0012
Oil_con*plankton	0	1	1	0	1.0000
Oil_con*plankton	0	1	1	1	0.7994
Oil_con*plankton	0	1	2	0	0.9937
Oil_con*plankton	0	1	2	1	0.1026
Oil_con*plankton	0	1	3	0	0.8286
Oil_con*plankton	0	1	3	1	0.0012
Oil_con*plankton	1	0	1	1	0.8831
Oil_con*plankton	1	0	2	0	0.9991
Oil_con*plankton	1	0	2	1	0.1323
Oil_con*plankton	1	0	3	0	0.9055
Oil_con*plankton	1	0	3	1	0.0015
Oil_con*plankton	1	1	2	0	0.9908

plankton

The Mixed Procedure

Differences of Least Squares Means					
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Adj P**
Oil_con*plankton	1	1	2	1	0.5768
Oil_con*plankton	1	1	3	0	1.0000
Oil_con*plankton	1	1	3	1	0.0055
Oil_con*plankton	2	0	2	1	0.2524
Oil_con*plankton	2	0	3	0	0.9945
Oil_con*plankton	2	0	3	1	0.0025
Oil_con*plankton	2	1	3	0	0.5435
Oil_con*plankton	2	1	3	1	0.0539
Oil_con*plankton	3	0	3	1	0.0052

plankton

Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	84476	9775.08	A
2		0	16527	9775.08	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	3		120762	13824	A
4	2		57203	13824	B
5	1		23590	13824	B
6	0		451.25	13824	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
7	3	1	201992	19550	A
8	2	1	94221	19550	AB
9	1	1	41311	19550	B
10	3	0	39533	19550	B
11	2	0	20185	19550	B
12	1	0	5869.50	19550	B
13	0	0	520.50	19550	B
14	0	1	382.00	19550	B

plankton

Obs	Oil_con	plankton	Alk	aro	logAlk	logaro
1	0	0	15.43	171	2.73631	5.1417
2	0	0	29.98	870	3.40053	6.7685
3	0	1	14.93	393	2.70337	5.9738
4	0	1	5.92	371	1.77834	5.9162
5	1	0	2.91	4285	1.06815	8.3629
6	1	0	5.18	7454	1.64481	8.9165
7	1	1	379.99	29748	5.94014	10.3005
8	1	1	801.09	52874	6.68597	10.8757
9	2	0	25.11	20156	3.22327	9.9113
10	2	0	77.82	20213	4.35440	9.9141
11	2	1	1380.20	94320	7.22998	11.4544
12	2	1	1414.79	94121	7.25474	11.4523
13	3	0	130.57	33389	4.87191	10.4160
14	3	0	247.69	45676	5.51218	10.7293
15	3	1	1862.20	148293	7.52951	11.9069
16	3	1	4105.04	255691	8.31997	12.4517

plankton

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logAlk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	4	0 1 2 3
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	15
Columns in Z	0
Subjects	1
Max Obs per Subject	16

Number of Observations	
Number of Observations Read	16
Number of Observations Used	16
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.2813

Fit Statistics	
-2 Res Log Likelihood	18.1
AIC (Smaller is Better)	20.1
AICC (Smaller is Better)	20.8
BIC (Smaller is Better)	20.2

Differences of Least Squares Means											
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
plankton		0		1	-2.5788	0.2652	8	-9.72	<.0001	Tukey	<.0001
Oil_con	0		1		-1.1801	0.3750	8	-3.15	0.0137	Tukey	0.0541
Oil_con	0		2		-2.8610	0.3750	8	-7.63	<.0001	Tukey	0.0003
Oil_con	0		3		-3.9038	0.3750	8	-10.41	<.0001	Tukey	<.0001
Oil_con	1		2		-1.6808	0.3750	8	-4.48	0.0021	Tukey	0.0088
Oil_con	1		3		-2.7236	0.3750	8	-7.26	<.0001	Tukey	0.0004
Oil_con	2		3		-1.0428	0.3750	8	-2.78	0.0239	Tukey	0.0907
Oil_con*plankton	0	0	0	1	0.8276	0.5304	8	1.56	0.1573	Tukey	0.7608
Oil_con*plankton	0	0	1	0	1.7119	0.5304	8	3.23	0.0121	Tukey	0.1269
Oil_con*plankton	0	0	1	1	-3.2446	0.5304	8	-6.12	0.0003	Tukey	0.0040
Oil_con*plankton	0	0	2	0	-0.7204	0.5304	8	-1.36	0.2114	Tukey	0.8532
Oil_con*plankton	0	0	2	1	-4.1739	0.5304	8	-7.87	<.0001	Tukey	0.0007
Oil_con*plankton	0	0	3	0	-2.1236	0.5304	8	-4.00	0.0039	Tukey	0.0471
Oil_con*plankton	0	0	3	1	-4.8563	0.5304	8	-9.16	<.0001	Tukey	0.0002
Oil_con*plankton	0	1	1	0	0.8844	0.5304	8	1.67	0.1340	Tukey	0.7063
Oil_con*plankton	0	1	1	1	-4.0722	0.5304	8	-7.68	<.0001	Tukey	0.0009
Oil_con*plankton	0	1	2	0	-1.5480	0.5304	8	-2.92	0.0193	Tukey	0.1876
Oil_con*plankton	0	1	2	1	-5.0015	0.5304	8	-9.43	<.0001	Tukey	0.0002
Oil_con*plankton	0	1	3	0	-2.9512	0.5304	8	-5.56	0.0005	Tukey	0.0073
Oil_con*plankton	0	1	3	1	-5.6839	0.5304	8	-10.72	<.0001	Tukey	<.0001
Oil_con*plankton	1	0	1	1	-4.9566	0.5304	8	-9.35	<.0001	Tukey	0.0002
Oil_con*plankton	1	0	2	0	-2.4324	0.5304	8	-4.59	0.0018	Tukey	0.0229
Oil_con*plankton	1	0	2	1	-5.8859	0.5304	8	-11.10	<.0001	Tukey	<.0001
Oil_con*plankton	1	0	3	0	-3.8356	0.5304	8	-7.23	<.0001	Tukey	0.0013
Oil_con*plankton	1	0	3	1	-6.5683	0.5304	8	-12.38	<.0001	Tukey	<.0001
Oil_con*plankton	1	1	2	0	2.5242	0.5304	8	4.76	0.0014	Tukey	0.0185
Oil_con*plankton	1	1	2	1	-0.9293	0.5304	8	-1.75	0.1178	Tukey	0.6619
Oil_con*plankton	1	1	3	0	1.1210	0.5304	8	2.11	0.0675	Tukey	0.4759
Oil_con*plankton	1	1	3	1	-1.6117	0.5304	8	-3.04	0.0161	Tukey	0.1614
Oil_con*plankton	2	0	2	1	-3.4535	0.5304	8	-6.51	0.0002	Tukey	0.0027
Oil_con*plankton	2	0	3	0	-1.4032	0.5304	8	-2.65	0.0294	Tukey	0.2623
Oil_con*plankton	2	0	3	1	-4.1359	0.5304	8	-7.80	<.0001	Tukey	0.0008
Oil_con*plankton	2	1	3	0	2.0503	0.5304	8	3.87	0.0048	Tukey	0.0561
Oil_con*plankton	2	1	3	1	-0.6824	0.5304	8	-1.29	0.2342	Tukey	0.8813
Oil_con*plankton	3	0	3	1	-2.7327	0.5304	8	-5.15	0.0009	Tukey	0.0116

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	8	94.57	<.0001
Oil_con	3	8	42.84	<.0001
Oil_con*plankton	3	8	21.39	0.0004

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	3.3514	0.1875	8	17.87	<.0001
plankton		1	5.9303	0.1875	8	31.63	<.0001
Oil_con	0		2.6546	0.2652	8	10.01	<.0001
Oil_con	1		3.8348	0.2652	8	14.46	<.0001
Oil_con	2		5.5156	0.2652	8	20.80	<.0001
Oil_con	3		6.5584	0.2652	8	24.73	<.0001
Oil_con*plankton	0	0	3.0684	0.3750	8	8.18	<.0001
Oil_con*plankton	0	1	2.2409	0.3750	8	5.98	0.0003
Oil_con*plankton	1	0	1.3565	0.3750	8	3.62	0.0068
Oil_con*plankton	1	1	6.3131	0.3750	8	16.83	<.0001
Oil_con*plankton	2	0	3.7888	0.3750	8	10.10	<.0001
Oil_con*plankton	2	1	7.2424	0.3750	8	19.31	<.0001
Oil_con*plankton	3	0	5.1920	0.3750	8	13.84	<.0001
Oil_con*plankton	3	1	7.9247	0.3750	8	21.13	<.0001

plankton

Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	5.9303	0.1875	A
2		0	3.3514	0.1875	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	3		6.5584	0.2652	A
4	2		5.5156	0.2652	A
5	1		3.8348	0.2652	B
6	0		2.6546	0.2652	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
7	3	1	7.9247	0.3750	A
8	2	1	7.2424	0.3750	AB
9	1	1	6.3131	0.3750	AB
10	3	0	5.1920	0.3750	BC
11	2	0	3.7888	0.3750	CD
12	0	0	3.0684	0.3750	DE
13	0	1	2.2409	0.3750	DE
14	1	0	1.3565	0.3750	E

plankton

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logaro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	4	0 1 2 3
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	15
Columns in Z	0
Subjects	1
Max Obs per Subject	16

Number of Observations	
Number of Observations Read	16
Number of Observations Used	16
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.2301

Fit Statistics	
-2 Res Log Likelihood	16.5
AIC (Smaller is Better)	18.5
AICC (Smaller is Better)	19.2
BIC (Smaller is Better)	18.6

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	8	28.10	0.0007
Oil_con	3	8	101.38	<.0001
Oil_con*plankton	3	8	3.31	0.0781

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	8.7700	0.1696	8	51.71	<.0001
plankton		1	10.0415	0.1696	8	59.20	<.0001
Oil_con	0		5.9500	0.2399	8	24.81	<.0001
Oil_con	1		9.6139	0.2399	8	40.08	<.0001
Oil_con	2		10.6830	0.2399	8	44.54	<.0001
Oil_con	3		11.3760	0.2399	8	47.43	<.0001
Oil_con*plankton	0	0	5.9551	0.3392	8	17.56	<.0001
Oil_con*plankton	0	1	5.9450	0.3392	8	17.53	<.0001
Oil_con*plankton	1	0	8.6397	0.3392	8	25.47	<.0001
Oil_con*plankton	1	1	10.5881	0.3392	8	31.21	<.0001
Oil_con*plankton	2	0	9.9127	0.3392	8	29.22	<.0001
Oil_con*plankton	2	1	11.4534	0.3392	8	33.76	<.0001
Oil_con*plankton	3	0	10.5727	0.3392	8	31.17	<.0001
Oil_con*plankton	3	1	12.1793	0.3392	8	35.90	<.0001

Differences of Least Squares Means											
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
plankton		0		1	-1.2714	0.2399	8	-5.30	0.0007	Tukey	0.0007
Oil_con	0		1		-3.6638	0.3392	8	-10.80	<.0001	Tukey	<.0001
Oil_con	0		2		-4.7330	0.3392	8	-13.95	<.0001	Tukey	<.0001
Oil_con	0		3		-5.4260	0.3392	8	-16.00	<.0001	Tukey	<.0001
Oil_con	1		2		-1.0691	0.3392	8	-3.15	0.0136	Tukey	0.0537
Oil_con	1		3		-1.7621	0.3392	8	-5.19	0.0008	Tukey	0.0037
Oil_con	2		3		-0.6930	0.3392	8	-2.04	0.0753	Tukey	0.2498
Oil_con*plankton	0	0	0	1	0.01007	0.4797	8	0.02	0.9838	Tukey	1.0000
Oil_con*plankton	0	0	1	0	-2.6846	0.4797	8	-5.60	0.0005	Tukey	0.0070
Oil_con*plankton	0	0	1	1	-4.6330	0.4797	8	-9.66	<.0001	Tukey	0.0002
Oil_con*plankton	0	0	2	0	-3.9576	0.4797	8	-8.25	<.0001	Tukey	0.0005
Oil_con*plankton	0	0	2	1	-5.4983	0.4797	8	-11.46	<.0001	Tukey	<.0001
Oil_con*plankton	0	0	3	0	-4.6176	0.4797	8	-9.63	<.0001	Tukey	0.0002
Oil_con*plankton	0	0	3	1	-6.2243	0.4797	8	-12.97	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	1	0	-2.6947	0.4797	8	-5.62	0.0005	Tukey	0.0069
Oil_con*plankton	0	1	1	1	-4.6431	0.4797	8	-9.68	<.0001	Tukey	0.0002
Oil_con*plankton	0	1	2	0	-3.9677	0.4797	8	-8.27	<.0001	Tukey	0.0005
Oil_con*plankton	0	1	2	1	-5.5084	0.4797	8	-11.48	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	3	0	-4.6276	0.4797	8	-9.65	<.0001	Tukey	0.0002
Oil_con*plankton	0	1	3	1	-6.2343	0.4797	8	-13.00	<.0001	Tukey	<.0001
Oil_con*plankton	1	0	1	1	-1.9484	0.4797	8	-4.06	0.0036	Tukey	0.0438
Oil_con*plankton	1	0	2	0	-1.2730	0.4797	8	-2.65	0.0291	Tukey	0.2598
Oil_con*plankton	1	0	2	1	-2.8137	0.4797	8	-5.87	0.0004	Tukey	0.0052
Oil_con*plankton	1	0	3	0	-1.9330	0.4797	8	-4.03	0.0038	Tukey	0.0456
Oil_con*plankton	1	0	3	1	-3.5396	0.4797	8	-7.38	<.0001	Tukey	0.0011
Oil_con*plankton	1	1	2	0	0.6754	0.4797	8	1.41	0.1968	Tukey	0.8322
Oil_con*plankton	1	1	2	1	-0.8653	0.4797	8	-1.80	0.1089	Tukey	0.6346
Oil_con*plankton	1	1	3	0	0.01544	0.4797	8	0.03	0.9751	Tukey	1.0000
Oil_con*plankton	1	1	3	1	-1.5912	0.4797	8	-3.32	0.0106	Tukey	0.1133
Oil_con*plankton	2	0	2	1	-1.5407	0.4797	8	-3.21	0.0124	Tukey	0.1296
Oil_con*plankton	2	0	3	0	-0.6600	0.4797	8	-1.38	0.2062	Tukey	0.8460
Oil_con*plankton	2	0	3	1	-2.2667	0.4797	8	-4.72	0.0015	Tukey	0.0193
Oil_con*plankton	2	1	3	0	0.8807	0.4797	8	1.84	0.1037	Tukey	0.6176
Oil_con*plankton	2	1	3	1	-0.7259	0.4797	8	-1.51	0.1687	Tukey	0.7838
Oil_con*plankton	3	0	3	1	-1.6067	0.4797	8	-3.35	0.0101	Tukey	0.1087

plankton

Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	10.0415	0.1696	A
2		0	8.7700	0.1696	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	3		11.3760	0.2399	A
4	2		10.6830	0.2399	AB
5	1		9.6139	0.2399	B
6	0		5.9500	0.2399	C

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
7	3	1	12.1793	0.3392	A
8	2	1	11.4534	0.3392	AB
9	1	1	10.5881	0.3392	AB
10	3	0	10.5727	0.3392	AB
11	2	0	9.9127	0.3392	BC
12	1	0	8.6397	0.3392	C
13	0	0	5.9551	0.3392	D
14	0	1	5.9450	0.3392	D

B.2 Trial 4

B.2.1 Water

water

Obs	Oil_con	plankton	filter	Alk	aro
1	0	0	0	4.00	29
2	0	0	0	3.61	37
3	0	0	1	3.74	32
4	0	0	1	3.60	33
5	0	1	0	2.89	296
6	0	1	0	22.45	16625
7	0	1	1	2.39	702
8	0	1	1	4.03	97
9	1	0	0	79.21	15169
10	1	0	0	76.83	16747
11	1	0	1	5.36	3714
12	1	0	1	7.27	8667
13	1	1	0	54.99	12214
14	1	1	0	22.10	15535
15	1	1	1	4.39	2521
16	1	1	1	3.80	3259
17	2	0	0	41.49	26293
18	2	0	0	43.77	19698
19	2	0	1	3.99	6874
20	2	0	1	4.29	5685
21	2	1	0	88.54	34685
22	2	1	0	75.63	15048
23	2	1	1	4.81	5227
24	2	1	1	7.37	5753

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The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	Alk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
Oil_con	3	0 1 2
plankton	2	0 1
filter	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	24

Number of Observations	
Number of Observations Read	24
Number of Observations Used	24
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	**Estimate**
Residual	68.9741

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The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	93.2
AIC (Smaller is Better)	95.2
AICC (Smaller is Better)	95.6
BIC (Smaller is Better)	95.7

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
plankton	1	12	0.16	0.6970
Oil_con	2	12	28.09	<.0001
Oil_con*plankton	2	12	12.69	0.0011
filter	1	12	128.09	<.0001
plankton*filter	1	12	0.22	0.6463
Oil_con*filter	2	12	24.64	<.0001
Oil_co*plankt*filter	2	12	10.33	0.0025

Least Squares Means								
Effect	**Oil_con**	**plankton**	**filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
plankton		0		23.0967	2.3975	12	9.63	<.0001
plankton		1		24.4492	2.3975	12	10.20	<.0001
Oil_con	0			5.8388	2.9363	12	1.99	0.0701
Oil_con	1			31.7438	2.9363	12	10.81	<.0001
Oil_con	2			33.7363	2.9363	12	11.49	<.0001
Oil_con*plankton	0	0		3.7375	4.1525	12	0.90	0.3858
Oil_con*plankton	0	1		7.9400	4.1525	12	1.91	0.0800
Oil_con*plankton	1	0		42.1675	4.1525	12	10.15	<.0001
Oil_con*plankton	1	1		21.3200	4.1525	12	5.13	0.0002
Oil_con*plankton	2	0		23.3850	4.1525	12	5.63	0.0001
Oil_con*plankton	2	1		44.0875	4.1525	12	10.62	<.0001
filter			0	42.9592	2.3975	12	17.92	<.0001
filter			1	4.5867	2.3975	12	1.91	0.0799
plankton*filter		0	0	41.4850	3.3905	12	12.24	<.0001

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The Mixed Procedure

Least Squares Means								
Effect	**Oil_con**	**plankton**	**filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
plankton*filter		0	1	4.7083	3.3905	12	1.39	0.1902
plankton*filter		1	0	44.4333	3.3905	12	13.11	<.0001
plankton*filter		1	1	4.4650	3.3905	12	1.32	0.2125
Oil_con*filter	0		0	8.2375	4.1525	12	1.98	0.0706
Oil_con*filter	0		1	3.4400	4.1525	12	0.83	0.4236
Oil_con*filter	1		0	58.2825	4.1525	12	14.04	<.0001
Oil_con*filter	1		1	5.2050	4.1525	12	1.25	0.2339
Oil_con*filter	2		0	62.3575	4.1525	12	15.02	<.0001
Oil_con*filter	2		1	5.1150	4.1525	12	1.23	0.2416
Oil_co*plankt*filter	0	0	0	3.8050	5.8726	12	0.65	0.5292
Oil_co*plankt*filter	0	0	1	3.6700	5.8726	12	0.62	0.5437
Oil_co*plankt*filter	0	1	0	12.6700	5.8726	12	2.16	0.0519
Oil_co*plankt*filter	0	1	1	3.2100	5.8726	12	0.55	0.5947
Oil_co*plankt*filter	1	0	0	78.0200	5.8726	12	13.29	<.0001
Oil_co*plankt*filter	1	0	1	6.3150	5.8726	12	1.08	0.3034
Oil_co*plankt*filter	1	1	0	38.5450	5.8726	12	6.56	<.0001
Oil_co*plankt*filter	1	1	1	4.0950	5.8726	12	0.70	0.4989
Oil_co*plankt*filter	2	0	0	42.6300	5.8726	12	7.26	<.0001
Oil_co*plankt*filter	2	0	1	4.1400	5.8726	12	0.70	0.4943
Oil_co*plankt*filter	2	1	0	82.0850	5.8726	12	13.98	<.0001
Oil_co*plankt*filter	2	1	1	6.0900	5.8726	12	1.04	0.3202

Differences of Least Squares Means											
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
plankton		0			1		-1.3525	3.3905	12	-0.40	0.6970
Oil_con	0			1			-25.9050	4.1525	12	-6.24	<.0001
Oil_con	0			2			-27.8975	4.1525	12	-6.72	<.0001
Oil_con	1			2			-1.9925	4.1525	12	-0.48	0.6400
Oil_con*plankton	0	0		0	1		-4.2025	5.8726	12	-0.72	0.4879
Oil_con*plankton	0	0		1	0		-38.4300	5.8726	12	-6.54	<.0001

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The Mixed Procedure

Differences of Least Squares Means											
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Oil_con*plankton	0	0		1	1		-17.5825	5.8726	12	-2.99	0.0112
Oil_con*plankton	0	0		2	0		-19.6475	5.8726	12	-3.35	0.0058
Oil_con*plankton	0	0		2	1		-40.3500	5.8726	12	-6.87	<.0001
Oil_con*plankton	0	1		1	0		-34.2275	5.8726	12	-5.83	<.0001
Oil_con*plankton	0	1		1	1		-13.3800	5.8726	12	-2.28	0.0418
Oil_con*plankton	0	1		2	0		-15.4450	5.8726	12	-2.63	0.0220
Oil_con*plankton	0	1		2	1		-36.1475	5.8726	12	-6.16	<.0001
Oil_con*plankton	1	0		1	1		20.8475	5.8726	12	3.55	0.0040
Oil_con*plankton	1	0		2	0		18.7825	5.8726	12	3.20	0.0077
Oil_con*plankton	1	0		2	1		-1.9200	5.8726	12	-0.33	0.7493
Oil_con*plankton	1	1		2	0		-2.0650	5.8726	12	-0.35	0.7312
Oil_con*plankton	1	1		2	1		-22.7675	5.8726	12	-3.88	0.0022
Oil_con*plankton	2	0		2	1		-20.7025	5.8726	12	-3.53	0.0042
filter			0			1	38.3725	3.3905	12	11.32	<.0001
plankton*filter		0	0		0	1	36.7767	4.7949	12	7.67	<.0001
plankton*filter		0	0		1	0	-2.9483	4.7949	12	-0.61	0.5501
plankton*filter		0	0		1	1	37.0200	4.7949	12	7.72	<.0001
plankton*filter		0	1		1	0	-39.7250	4.7949	12	-8.28	<.0001
plankton*filter		0	1		1	1	0.2433	4.7949	12	0.05	0.9604
plankton*filter		1	0		1	1	39.9683	4.7949	12	8.34	<.0001
Oil_con*filter	0		0	0		1	4.7975	5.8726	12	0.82	0.4299
Oil_con*filter	0		0	1		0	-50.0450	5.8726	12	-8.52	<.0001
Oil_con*filter	0		0	1		1	3.0325	5.8726	12	0.52	0.6150
Oil_con*filter	0		0	2		0	-54.1200	5.8726	12	-9.22	<.0001
Oil_con*filter	0		0	2		1	3.1225	5.8726	12	0.53	0.6046
Oil_con*filter	0		1	1		0	-54.8425	5.8726	12	-9.34	<.0001
Oil_con*filter	0		1	1		1	-1.7650	5.8726	12	-0.30	0.7689
Oil_con*filter	0		1	2		0	-58.9175	5.8726	12	-10.03	<.0001
Oil_con*filter	0		1	2		1	-1.6750	5.8726	12	-0.29	0.7803
Oil_con*filter	1		0	1		1	53.0775	5.8726	12	9.04	<.0001
Oil_con*filter	1		0	2		0	-4.0750	5.8726	12	-0.69	0.5010

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The Mixed Procedure

Differences of Least Squares Means											
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Oil_con*filter	1		0	2		1	53.1675	5.8726	12	9.05	<.0001
Oil_con*filter	1		1	2		0	-57.1525	5.8726	12	-9.73	<.0001
Oil_con*filter	1		1	2		1	0.09000	5.8726	12	0.02	0.9880
Oil_con*filter	2		0	2		1	57.2425	5.8726	12	9.75	<.0001
Oil_co*plankt*filter	0	0	0	0	0	1	0.1350	8.3051	12	0.02	0.9873
Oil_co*plankt*filter	0	0	0	0	1	0	-8.8650	8.3051	12	-1.07	0.3068
Oil_co*plankt*filter	0	0	0	0	1	1	0.5950	8.3051	12	0.07	0.9441
Oil_co*plankt*filter	0	0	0	1	0	0	-74.2150	8.3051	12	-8.94	<.0001
Oil_co*plankt*filter	0	0	0	1	0	1	-2.5100	8.3051	12	-0.30	0.7677
Oil_co*plankt*filter	0	0	0	1	1	0	-34.7400	8.3051	12	-4.18	0.0013
Oil_co*plankt*filter	0	0	0	1	1	1	-0.2900	8.3051	12	-0.03	0.9727
Oil_co*plankt*filter	0	0	0	2	0	0	-38.8250	8.3051	12	-4.67	0.0005
Oil_co*plankt*filter	0	0	0	2	0	1	-0.3350	8.3051	12	-0.04	0.9685
Oil_co*plankt*filter	0	0	0	2	1	0	-78.2800	8.3051	12	-9.43	<.0001
Oil_co*plankt*filter	0	0	0	2	1	1	-2.2850	8.3051	12	-0.28	0.7879
Oil_co*plankt*filter	0	0	1	0	1	0	-9.0000	8.3051	12	-1.08	0.2998
Oil_co*plankt*filter	0	0	1	0	1	1	0.4600	8.3051	12	0.06	0.9567
Oil_co*plankt*filter	0	0	1	1	0	0	-74.3500	8.3051	12	-8.95	<.0001
Oil_co*plankt*filter	0	0	1	1	0	1	-2.6450	8.3051	12	-0.32	0.7556
Oil_co*plankt*filter	0	0	1	1	1	0	-34.8750	8.3051	12	-4.20	0.0012
Oil_co*plankt*filter	0	0	1	1	1	1	-0.4250	8.3051	12	-0.05	0.9600
Oil_co*plankt*filter	0	0	1	2	0	0	-38.9600	8.3051	12	-4.69	0.0005
Oil_co*plankt*filter	0	0	1	2	0	1	-0.4700	8.3051	12	-0.06	0.9558
Oil_co*plankt*filter	0	0	1	2	1	0	-78.4150	8.3051	12	-9.44	<.0001
Oil_co*plankt*filter	0	0	1	2	1	1	-2.4200	8.3051	12	-0.29	0.7757
Oil_co*plankt*filter	0	1	0	0	1	1	9.4600	8.3051	12	1.14	0.2769
Oil_co*plankt*filter	0	1	0	1	0	0	-65.3500	8.3051	12	-7.87	<.0001
Oil_co*plankt*filter	0	1	0	1	0	1	6.3550	8.3051	12	0.77	0.4589
Oil_co*plankt*filter	0	1	0	1	1	0	-25.8750	8.3051	12	-3.12	0.0089
Oil_co*plankt*filter	0	1	0	1	1	1	8.5750	8.3051	12	1.03	0.3222
Oil_co*plankt*filter	0	1	0	2	0	0	-29.9600	8.3051	12	-3.61	0.0036

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The Mixed Procedure

Differences of Least Squares Means											
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Oil_co*plankt*filter	0	1	0	2	0	1	8.5300	8.3051	12	1.03	0.3246
Oil_co*plankt*filter	0	1	0	2	1	0	-69.4150	8.3051	12	-8.36	<.0001
Oil_co*plankt*filter	0	1	0	2	1	1	6.5800	8.3051	12	0.79	0.4436
Oil_co*plankt*filter	0	1	1	1	0	0	-74.8100	8.3051	12	-9.01	<.0001
Oil_co*plankt*filter	0	1	1	1	0	1	-3.1050	8.3051	12	-0.37	0.7150
Oil_co*plankt*filter	0	1	1	1	1	0	-35.3350	8.3051	12	-4.25	0.0011
Oil_co*plankt*filter	0	1	1	1	1	1	-0.8850	8.3051	12	-0.11	0.9169
Oil_co*plankt*filter	0	1	1	2	0	0	-39.4200	8.3051	12	-4.75	0.0005
Oil_co*plankt*filter	0	1	1	2	0	1	-0.9300	8.3051	12	-0.11	0.9127
Oil_co*plankt*filter	0	1	1	2	1	0	-78.8750	8.3051	12	-9.50	<.0001
Oil_co*plankt*filter	0	1	1	2	1	1	-2.8800	8.3051	12	-0.35	0.7348
Oil_co*plankt*filter	1	0	0	1	0	1	71.7050	8.3051	12	8.63	<.0001
Oil_co*plankt*filter	1	0	0	1	1	0	39.4750	8.3051	12	4.75	0.0005
Oil_co*plankt*filter	1	0	0	1	1	1	73.9250	8.3051	12	8.90	<.0001
Oil_co*plankt*filter	1	0	0	2	0	0	35.3900	8.3051	12	4.26	0.0011
Oil_co*plankt*filter	1	0	0	2	0	1	73.8800	8.3051	12	8.90	<.0001
Oil_co*plankt*filter	1	0	0	2	1	0	-4.0650	8.3051	12	-0.49	0.6333
Oil_co*plankt*filter	1	0	0	2	1	1	71.9300	8.3051	12	8.66	<.0001
Oil_co*plankt*filter	1	0	1	1	1	0	-32.2300	8.3051	12	-3.88	0.0022
Oil_co*plankt*filter	1	0	1	1	1	1	2.2200	8.3051	12	0.27	0.7938
Oil_co*plankt*filter	1	0	1	2	0	0	-36.3150	8.3051	12	-4.37	0.0009
Oil_co*plankt*filter	1	0	1	2	0	1	2.1750	8.3051	12	0.26	0.7978
Oil_co*plankt*filter	1	0	1	2	1	0	-75.7700	8.3051	12	-9.12	<.0001
Oil_co*plankt*filter	1	0	1	2	1	1	0.2250	8.3051	12	0.03	0.9788
Oil_co*plankt*filter	1	1	0	1	1	1	34.4500	8.3051	12	4.15	0.0014
Oil_co*plankt*filter	1	1	0	2	0	0	-4.0850	8.3051	12	-0.49	0.6317
Oil_co*plankt*filter	1	1	0	2	0	1	34.4050	8.3051	12	4.14	0.0014
Oil_co*plankt*filter	1	1	0	2	1	0	-43.5400	8.3051	12	-5.24	0.0002
Oil_co*plankt*filter	1	1	0	2	1	1	32.4550	8.3051	12	3.91	0.0021
Oil_co*plankt*filter	1	1	1	2	0	0	-38.5350	8.3051	12	-4.64	0.0006
Oil_co*plankt*filter	1	1	1	2	0	1	-0.04500	8.3051	12	-0.01	0.9958

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The Mixed Procedure

Differences of Least Squares Means											
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Estimate	Standard Error	DF	t Value	Pr > |t|
Oil_co*plankt*filter	1	1	1	2	1	0	-77.9900	8.3051	12	-9.39	<.0001
Oil_co*plankt*filter	1	1	1	2	1	1	-1.9950	8.3051	12	-0.24	0.8142
Oil_co*plankt*filter	2	0	0	2	0	1	38.4900	8.3051	12	4.63	0.0006
Oil_co*plankt*filter	2	0	0	2	1	0	-39.4550	8.3051	12	-4.75	0.0005
Oil_co*plankt*filter	2	0	0	2	1	1	36.5400	8.3051	12	4.40	0.0009
Oil_co*plankt*filter	2	0	1	2	1	0	-77.9450	8.3051	12	-9.39	<.0001
Oil_co*plankt*filter	2	0	1	2	1	1	-1.9500	8.3051	12	-0.23	0.8183
Oil_co*plankt*filter	2	1	0	2	1	1	75.9950	8.3051	12	9.15	<.0001

Differences of Least Squares Means								
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Adjustment	Adj P
plankton		0			1		Tukey	0.6970
Oil_con	0			1			Tukey	0.0001
Oil_con	0			2			Tukey	<.0001
Oil_con	1			2			Tukey	0.8820
Oil_con*plankton	0	0		0	1		Tukey	0.9762
Oil_con*plankton	0	0		1	0		Tukey	0.0003
Oil_con*plankton	0	0		1	1		Tukey	0.0914
Oil_con*plankton	0	0		2	0		Tukey	0.0511
Oil_con*plankton	0	0		2	1		Tukey	0.0002
Oil_con*plankton	0	1		1	0		Tukey	0.0009
Oil_con*plankton	0	1		1	1		Tukey	0.2736
Oil_con*plankton	0	1		2	0		Tukey	0.1630
Oil_con*plankton	0	1		2	1		Tukey	0.0005
Oil_con*plankton	1	0		1	1		Tukey	0.0363
Oil_con*plankton	1	0		2	0		Tukey	0.0653
Oil_con*plankton	1	0		2	1		Tukey	0.9994
Oil_con*plankton	1	1		2	0		Tukey	0.9991
Oil_con*plankton	1	1		2	1		Tukey	0.0209
Oil_con*plankton	2	0		2	1		Tukey	0.0378
filter			0			1	Tukey	<.0001

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The Mixed Procedure

Differences of Least Squares Means								
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Adjustment	Adj P
plankton*filter		0	0		0	1	Tukey	<.0001
plankton*filter		0	0		1	0	Tukey	0.9254
plankton*filter		0	0		1	1	Tukey	<.0001
plankton*filter		0	1		1	0	Tukey	<.0001
plankton*filter		0	1		1	1	Tukey	1.0000
plankton*filter		1	0		1	1	Tukey	<.0001
Oil_con*filter	0		0	0		1	Tukey	0.9587
Oil_con*filter	0		0	1		0	Tukey	<.0001
Oil_con*filter	0		0	1		1	Tukey	0.9944
Oil_con*filter	0		0	2		0	Tukey	<.0001
Oil_con*filter	0		0	2		1	Tukey	0.9936
Oil_con*filter	0		1	1		0	Tukey	<.0001
Oil_con*filter	0		1	1		1	Tukey	0.9996
Oil_con*filter	0		1	2		0	Tukey	<.0001
Oil_con*filter	0		1	2		1	Tukey	0.9997
Oil_con*filter	1		0	1		1	Tukey	<.0001
Oil_con*filter	1		0	2		0	Tukey	0.9792
Oil_con*filter	1		0	2		1	Tukey	<.0001
Oil_con*filter	1		1	2		0	Tukey	<.0001
Oil_con*filter	1		1	2		1	Tukey	1.0000
Oil_con*filter	2		0	2		1	Tukey	<.0001
Oil_co*plankt*filter	0	0	0	0	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	0	1	0	Tukey	0.9908
Oil_co*plankt*filter	0	0	0	0	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	1	0	0	Tukey	<.0001
Oil_co*plankt*filter	0	0	0	1	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	1	1	0	Tukey	0.0357
Oil_co*plankt*filter	0	0	0	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	2	0	0	Tukey	0.0164
Oil_co*plankt*filter	0	0	0	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	0	0	0	2	1	1	Tukey	1.0000

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The Mixed Procedure

Differences of Least Squares Means								
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Adjustment**	**Adj P**
Oil_co*plankt*filter	0	0	1	0	1	0	Tukey	0.9897
Oil_co*plankt*filter	0	0	1	0	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	1	1	0	0	Tukey	<.0001
Oil_co*plankt*filter	0	0	1	1	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	1	1	1	0	Tukey	0.0348
Oil_co*plankt*filter	0	0	1	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	1	2	0	0	Tukey	0.0160
Oil_co*plankt*filter	0	0	1	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	1	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	0	0	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	0	0	1	1	Tukey	0.9852
Oil_co*plankt*filter	0	1	0	1	0	0	Tukey	0.0002
Oil_co*plankt*filter	0	1	0	1	0	1	Tukey	0.9994
Oil_co*plankt*filter	0	1	0	1	1	0	Tukey	0.1845
Oil_co*plankt*filter	0	1	0	1	1	1	Tukey	0.9929
Oil_co*plankt*filter	0	1	0	2	0	0	Tukey	0.0881
Oil_co*plankt*filter	0	1	0	2	0	1	Tukey	0.9932
Oil_co*plankt*filter	0	1	0	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	0	1	0	2	1	1	Tukey	0.9992
Oil_co*plankt*filter	0	1	1	1	0	0	Tukey	<.0001
Oil_co*plankt*filter	0	1	1	1	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	1	1	1	0	Tukey	0.0319
Oil_co*plankt*filter	0	1	1	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	1	2	0	0	Tukey	0.0147
Oil_co*plankt*filter	0	1	1	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	1	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	0	1	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	1	0	0	1	0	1	Tukey	<.0001
Oil_co*plankt*filter	1	0	0	1	1	0	Tukey	0.0145
Oil_co*plankt*filter	1	0	0	1	1	1	Tukey	<.0001
Oil_co*plankt*filter	1	0	0	2	0	0	Tukey	0.0316
Oil_co*plankt*filter	1	0	0	2	0	1	Tukey	<.0001

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The Mixed Procedure

Differences of Least Squares Means								
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Adjustment**	**Adj P**
Oil_co*plankt*filter	1	0	0	2	1	0	Tukey	1.0000
Oil_co*plankt*filter	1	0	0	2	1	1	Tukey	<.0001
Oil_co*plankt*filter	1	0	1	1	1	0	Tukey	0.0576
Oil_co*plankt*filter	1	0	1	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	1	0	1	2	0	0	Tukey	0.0265
Oil_co*plankt*filter	1	0	1	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	1	0	1	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	1	0	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	1	1	0	1	1	1	Tukey	0.0377
Oil_co*plankt*filter	1	1	0	2	0	0	Tukey	1.0000
Oil_co*plankt*filter	1	1	0	2	0	1	Tukey	0.0381
Oil_co*plankt*filter	1	1	0	2	1	0	Tukey	0.0068
Oil_co*plankt*filter	1	1	0	2	1	1	Tukey	0.0552
Oil_co*plankt*filter	1	1	1	2	0	0	Tukey	0.0173
Oil_co*plankt*filter	1	1	1	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	1	1	1	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	1	1	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	2	0	0	2	0	1	Tukey	0.0175
Oil_co*plankt*filter	2	0	0	2	1	0	Tukey	0.0146
Oil_co*plankt*filter	2	0	0	2	1	1	Tukey	0.0253
Oil_co*plankt*filter	2	0	1	2	1	0	Tukey	<.0001
Oil_co*plankt*filter	2	0	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	2	1	0	2	1	1	Tukey	<.0001

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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
1		1		24.4492	2.3975	A
2		0		23.0967	2.3975	A

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
3	2			33.7363	2.9363	A
4	1			31.7438	2.9363	A
5	0			5.8388	2.9363	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
6	2	1		44.0875	4.1525	A
7	1	0		42.1675	4.1525	AB
8	2	0		23.3850	4.1525	BC
9	1	1		21.3200	4.1525	C
10	0	1		7.9400	4.1525	C
11	0	0		3.7375	4.1525	C

Effect=filter Method=Tukey(P<.05) Set=4

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
12			0	42.9592	2.3975	A
13			1	4.5867	2.3975	B

Effect=plankton*filter Method=Tukey(P<.05) Set=5

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
14		1	0	44.4333	3.3905	A
15		0	0	41.4850	3.3905	A

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Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
16		0	1	4.7083	3.3905	B
17		1	1	4.4650	3.3905	B

Effect=Oil_con*filter Method=Tukey(P<.05) Set=6

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
18	2		0	62.3575	4.1525	A
19	1		0	58.2825	4.1525	A
20	0		0	8.2375	4.1525	B
21	1		1	5.2050	4.1525	B
22	2		1	5.1150	4.1525	B
23	0		1	3.4400	4.1525	B

Effect=Oil_co*plankt*filter Method=Tukey(P<.05) Set=7

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
24	2	1	0	82.0850	5.8726	A
25	1	0	0	78.0200	5.8726	A
26	2	0	0	42.6300	5.8726	B
27	1	1	0	38.5450	5.8726	BC
28	0	1	0	12.6700	5.8726	BCD
29	1	0	1	6.3150	5.8726	CD
30	2	1	1	6.0900	5.8726	CD
31	2	0	1	4.1400	5.8726	D
32	1	1	1	4.0950	5.8726	D
33	0	0	0	3.8050	5.8726	D
34	0	0	1	3.6700	5.8726	D
35	0	1	1	3.2100	5.8726	D

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The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	aro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1
filter	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	24

Number of Observations	
Number of Observations Read	24
Number of Observations Used	24
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	30683104

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Fit Statistics	
-2 Res Log Likelihood	249.2
AIC (Smaller is Better)	251.2
AICC (Smaller is Better)	251.6
BIC (Smaller is Better)	251.7

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	12	0.11	0.7463
Oil_con	2	12	10.59	0.0022
Oil_con*plankton	2	12	0.82	0.4632
filter	1	12	22.88	0.0004
plankton*filter	1	12	0.77	0.3962
Oil_con*filter	2	12	3.21	0.0764
Oil_co*plankt*filter	2	12	0.21	0.8119

Least Squares Means								
Effect	Oil_con	plankton	filter	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0		8581.50	1599.04	12	5.37	0.0002
plankton		1		9330.17	1599.04	12	5.83	<.0001
Oil_con	0			2231.38	1958.41	12	1.14	0.2768
Oil_con	1			9728.25	1958.41	12	4.97	0.0003
Oil_con	2			14908	1958.41	12	7.61	<.0001
Oil_con*plankton	0	0		32.7500	2769.62	12	0.01	0.9908
Oil_con*plankton	0	1		4430.00	2769.62	12	1.60	0.1357
Oil_con*plankton	1	0		11074	2769.62	12	4.00	0.0018
Oil_con*plankton	1	1		8382.25	2769.62	12	3.03	0.0105
Oil_con*plankton	2	0		14638	2769.62	12	5.29	0.0002
Oil_con*plankton	2	1		15178	2769.62	12	5.48	0.0001
filter			0	14365	1599.04	12	8.98	<.0001
filter			1	3547.00	1599.04	12	2.22	0.0466
plankton*filter		0	0	12996	2261.38	12	5.75	<.0001

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Least Squares Means								
Effect	Oil_con	plankton	filter	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton*filter		0	1	4167.50	2261.38	12	1.84	0.0902
plankton*filter		1	0	15734	2261.38	12	6.96	<.0001
plankton*filter		1	1	2926.50	2261.38	12	1.29	0.2200
Oil_con*filter	0		0	4246.75	2769.62	12	1.53	0.1511
Oil_con*filter	0		1	216.00	2769.62	12	0.08	0.9391
Oil_con*filter	1		0	14916	2769.62	12	5.39	0.0002
Oil_con*filter	1		1	4540.25	2769.62	12	1.64	0.1271
Oil_con*filter	2		0	23931	2769.62	12	8.64	<.0001
Oil_con*filter	2		1	5884.75	2769.62	12	2.12	0.0551
Oil_co*plankt*filter	0	0	0	33.0000	3916.83	12	0.01	0.9934
Oil_co*plankt*filter	0	0	1	32.5000	3916.83	12	0.01	0.9935
Oil_co*plankt*filter	0	1	0	8460.50	3916.83	12	2.16	0.0517
Oil_co*plankt*filter	0	1	1	399.50	3916.83	12	0.10	0.9204
Oil_co*plankt*filter	1	0	0	15958	3916.83	12	4.07	0.0015
Oil_co*plankt*filter	1	0	1	6190.50	3916.83	12	1.58	0.1400
Oil_co*plankt*filter	1	1	0	13875	3916.83	12	3.54	0.0041
Oil_co*plankt*filter	1	1	1	2890.00	3916.83	12	0.74	0.4748
Oil_co*plankt*filter	2	0	0	22996	3916.83	12	5.87	<.0001
Oil_co*plankt*filter	2	0	1	6279.50	3916.83	12	1.60	0.1349
Oil_co*plankt*filter	2	1	0	24867	3916.83	12	6.35	<.0001
Oil_co*plankt*filter	2	1	1	5490.00	3916.83	12	1.40	0.1864

Differences of Least Squares Means												
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0			1		-748.67	2261.38	12	-0.33	0.7463
Oil_con	0			1			-7496.88	2769.62	12	-2.71	0.0191
Oil_con	0			2			-12676	2769.62	12	-4.58	0.0006
Oil_con	1			2			-5179.62	2769.62	12	-1.87	0.0860
Oil_con*plankton	0	0		0	1		-4397.25	3916.83	12	-1.12	0.2835
Oil_con*plankton	0	0		1	0		-11041	3916.83	12	-2.82	0.0155

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Differences of Least Squares Means											
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Estimate	Standard Error	DF	t Value	Pr > |t|
Oil_con*plankton	0	0		1	1		-8349.50	3916.83	12	-2.13	0.0544
Oil_con*plankton	0	0		2	0		-14605	3916.83	12	-3.73	0.0029
Oil_con*plankton	0	0		2	1		-15145	3916.83	12	-3.87	0.0022
Oil_con*plankton	0	1		1	0		-6644.25	3916.83	12	-1.70	0.1156
Oil_con*plankton	0	1		1	1		-3952.25	3916.83	12	-1.01	0.3329
Oil_con*plankton	0	1		2	0		-10207	3916.83	12	-2.61	0.0230
Oil_con*plankton	0	1		2	1		-10748	3916.83	12	-2.74	0.0178
Oil_con*plankton	1	0		1	1		2692.00	3916.83	12	0.69	0.5050
Oil_con*plankton	1	0		2	0		-3563.25	3916.83	12	-0.91	0.3809
Oil_con*plankton	1	0		2	1		-4104.00	3916.83	12	-1.05	0.3154
Oil_con*plankton	1	1		2	0		-6255.25	3916.83	12	-1.60	0.1362
Oil_con*plankton	1	1		2	1		-6796.00	3916.83	12	-1.74	0.1083
Oil_con*plankton	2	0		2	1		-540.75	3916.83	12	-0.14	0.8925
filter			0			1	10818	2261.38	12	4.78	0.0004
plankton*filter		0	0		0	1	8828.00	3198.08	12	2.76	0.0173
plankton*filter		0	0		1	0	-2738.33	3198.08	12	-0.86	0.4086
plankton*filter		0	0		1	1	10069	3198.08	12	3.15	0.0084
plankton*filter		0	1		1	0	-11566	3198.08	12	-3.62	0.0035
plankton*filter		0	1		1	1	1241.00	3198.08	12	0.39	0.7048
plankton*filter		1	0		1	1	12807	3198.08	12	4.00	0.0017
Oil_con*filter	0		0	0		1	4030.75	3916.83	12	1.03	0.3237
Oil_con*filter	0		0	1		0	-10669	3916.83	12	-2.72	0.0185
Oil_con*filter	0		0	1		1	-293.50	3916.83	12	-0.07	0.9415
Oil_con*filter	0		0	2		0	-19684	3916.83	12	-5.03	0.0003
Oil_con*filter	0		0	2		1	-1638.00	3916.83	12	-0.42	0.6832
Oil_con*filter	0		1	1		0	-14700	3916.83	12	-3.75	0.0028
Oil_con*filter	0		1	1		1	-4324.25	3916.83	12	-1.10	0.2912
Oil_con*filter	0		1	2		0	-23715	3916.83	12	-6.05	<.0001
Oil_con*filter	0		1	2		1	-5668.75	3916.83	12	-1.45	0.1734
Oil_con*filter	1		0	1		1	10376	3916.83	12	2.65	0.0212
Oil_con*filter	1		0	2		0	-9014.75	3916.83	12	-2.30	0.0401

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The Mixed Procedure

Differences of Least Squares Means											
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Oil_con*filter	1		0	2		1	9031.50	3916.83	12	2.31	0.0398
Oil_con*filter	1		1	2		0	-19391	3916.83	12	-4.95	0.0003
Oil_con*filter	1		1	2		1	-1344.50	3916.83	12	-0.34	0.7373
Oil_con*filter	2		0	2		1	18046	3916.83	12	4.61	0.0006
Oil_co*plankt*filter	0	0	0	0	0	1	0.5000	5539.23	12	0.00	0.9999
Oil_co*plankt*filter	0	0	0	0	1	0	-8427.50	5539.23	12	-1.52	0.1541
Oil_co*plankt*filter	0	0	0	0	1	1	-366.50	5539.23	12	-0.07	0.9483
Oil_co*plankt*filter	0	0	0	1	0	0	-15925	5539.23	12	-2.87	0.0140
Oil_co*plankt*filter	0	0	0	1	0	1	-6157.50	5539.23	12	-1.11	0.2881
Oil_co*plankt*filter	0	0	0	1	1	0	-13842	5539.23	12	-2.50	0.0280
Oil_co*plankt*filter	0	0	0	1	1	1	-2857.00	5539.23	12	-0.52	0.6154
Oil_co*plankt*filter	0	0	0	2	0	0	-22963	5539.23	12	-4.15	0.0014
Oil_co*plankt*filter	0	0	0	2	0	1	-6246.50	5539.23	12	-1.13	0.2815
Oil_co*plankt*filter	0	0	0	2	1	0	-24834	5539.23	12	-4.48	0.0007
Oil_co*plankt*filter	0	0	0	2	1	1	-5457.00	5539.23	12	-0.99	0.3440
Oil_co*plankt*filter	0	0	1	0	1	0	-8428.00	5539.23	12	-1.52	0.1540
Oil_co*plankt*filter	0	0	1	0	1	1	-367.00	5539.23	12	-0.07	0.9483
Oil_co*plankt*filter	0	0	1	1	0	0	-15926	5539.23	12	-2.88	0.0140
Oil_co*plankt*filter	0	0	1	1	0	1	-6158.00	5539.23	12	-1.11	0.2880
Oil_co*plankt*filter	0	0	1	1	1	0	-13842	5539.23	12	-2.50	0.0280
Oil_co*plankt*filter	0	0	1	1	1	1	-2857.50	5539.23	12	-0.52	0.6153
Oil_co*plankt*filter	0	0	1	2	0	0	-22963	5539.23	12	-4.15	0.0014
Oil_co*plankt*filter	0	0	1	2	0	1	-6247.00	5539.23	12	-1.13	0.2815
Oil_co*plankt*filter	0	0	1	2	1	0	-24834	5539.23	12	-4.48	0.0007
Oil_co*plankt*filter	0	0	1	2	1	1	-5457.50	5539.23	12	-0.99	0.3440
Oil_co*plankt*filter	0	1	0	0	1	1	8061.00	5539.23	12	1.46	0.1713
Oil_co*plankt*filter	0	1	0	1	0	0	-7497.50	5539.23	12	-1.35	0.2008
Oil_co*plankt*filter	0	1	0	1	0	1	2270.00	5539.23	12	0.41	0.6892
Oil_co*plankt*filter	0	1	0	1	1	0	-5414.00	5539.23	12	-0.98	0.3477
Oil_co*plankt*filter	0	1	0	1	1	1	5570.50	5539.23	12	1.01	0.3344
Oil_co*plankt*filter	0	1	0	2	0	0	-14535	5539.23	12	-2.62	0.0222

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Differences of Least Squares Means											
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Estimate	Standard Error	DF	t Value	Pr > |t|
Oil_co*plankt*filter	0	1	0	2	0	1	2181.00	5539.23	12	0.39	0.7007
Oil_co*plankt*filter	0	1	0	2	1	0	-16406	5539.23	12	-2.96	0.0119
Oil_co*plankt*filter	0	1	0	2	1	1	2970.50	5539.23	12	0.54	0.6016
Oil_co*plankt*filter	0	1	1	1	0	0	-15559	5539.23	12	-2.81	0.0158
Oil_co*plankt*filter	0	1	1	1	0	1	-5791.00	5539.23	12	-1.05	0.3164
Oil_co*plankt*filter	0	1	1	1	1	0	-13475	5539.23	12	-2.43	0.0316
Oil_co*plankt*filter	0	1	1	1	1	1	-2490.50	5539.23	12	-0.45	0.6610
Oil_co*plankt*filter	0	1	1	2	0	0	-22596	5539.23	12	-4.08	0.0015
Oil_co*plankt*filter	0	1	1	2	0	1	-5880.00	5539.23	12	-1.06	0.3094
Oil_co*plankt*filter	0	1	1	2	1	0	-24467	5539.23	12	-4.42	0.0008
Oil_co*plankt*filter	0	1	1	2	1	1	-5090.50	5539.23	12	-0.92	0.3762
Oil_co*plankt*filter	1	0	0	1	0	1	9767.50	5539.23	12	1.76	0.1033
Oil_co*plankt*filter	1	0	0	1	1	0	2083.50	5539.23	12	0.38	0.7134
Oil_co*plankt*filter	1	0	0	1	1	1	13068	5539.23	12	2.36	0.0361
Oil_co*plankt*filter	1	0	0	2	0	0	-7037.50	5539.23	12	-1.27	0.2280
Oil_co*plankt*filter	1	0	0	2	0	1	9678.50	5539.23	12	1.75	0.1061
Oil_co*plankt*filter	1	0	0	2	1	0	-8908.50	5539.23	12	-1.61	0.1338
Oil_co*plankt*filter	1	0	0	2	1	1	10468	5539.23	12	1.89	0.0832
Oil_co*plankt*filter	1	0	1	1	1	0	-7684.00	5539.23	12	-1.39	0.1906
Oil_co*plankt*filter	1	0	1	1	1	1	3300.50	5539.23	12	0.60	0.5624
Oil_co*plankt*filter	1	0	1	2	0	0	-16805	5539.23	12	-3.03	0.0104
Oil_co*plankt*filter	1	0	1	2	0	1	-89.0000	5539.23	12	-0.02	0.9874
Oil_co*plankt*filter	1	0	1	2	1	0	-18676	5539.23	12	-3.37	0.0056
Oil_co*plankt*filter	1	0	1	2	1	1	700.50	5539.23	12	0.13	0.9015
Oil_co*plankt*filter	1	1	0	1	1	1	10985	5539.23	12	1.98	0.0707
Oil_co*plankt*filter	1	1	0	2	0	0	-9121.00	5539.23	12	-1.65	0.1256
Oil_co*plankt*filter	1	1	0	2	0	1	7595.00	5539.23	12	1.37	0.1954
Oil_co*plankt*filter	1	1	0	2	1	0	-10992	5539.23	12	-1.98	0.0706
Oil_co*plankt*filter	1	1	0	2	1	1	8384.50	5539.23	12	1.51	0.1560
Oil_co*plankt*filter	1	1	1	2	0	0	-20106	5539.23	12	-3.63	0.0035
Oil_co*plankt*filter	1	1	1	2	0	1	-3389.50	5539.23	12	-0.61	0.5520

Differences of Least Squares Means											
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Estimate	Standard Error	DF	t Value	Pr > |t|
Oil_co*plankt*filter	1	1	1	2	1	0	-21977	5539.23	12	-3.97	0.0019
Oil_co*plankt*filter	1	1	1	2	1	1	-2600.00	5539.23	12	-0.47	0.6472
Oil_co*plankt*filter	2	0	0	2	0	1	16716	5539.23	12	3.02	0.0107
Oil_co*plankt*filter	2	0	0	2	1	0	-1871.00	5539.23	12	-0.34	0.7414
Oil_co*plankt*filter	2	0	0	2	1	1	17506	5539.23	12	3.16	0.0082
Oil_co*plankt*filter	2	0	1	2	1	0	-18587	5539.23	12	-3.36	0.0057
Oil_co*plankt*filter	2	0	1	2	1	1	789.50	5539.23	12	0.14	0.8890
Oil_co*plankt*filter	2	1	0	2	1	1	19377	5539.23	12	3.50	0.0044

Differences of Least Squares Means								
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Adjustment	Adj P
plankton		0			1		Tukey	0.7463
Oil_con	0			1			Tukey	0.0467
Oil_con	0			2			Tukey	0.0017
Oil_con	1			2			Tukey	0.1896
Oil_con*plankton	0	0		0	1		Tukey	0.8630
Oil_con*plankton	0	0		1	0		Tukey	0.1212
Oil_con*plankton	0	0		1	1		Tukey	0.3340
Oil_con*plankton	0	0		2	0		Tukey	0.0268
Oil_con*plankton	0	0		2	1		Tukey	0.0212
Oil_con*plankton	0	1		1	0		Tukey	0.5582
Oil_con*plankton	0	1		1	1		Tukey	0.9062
Oil_con*plankton	0	1		2	0		Tukey	0.1691
Oil_con*plankton	0	1		2	1		Tukey	0.1364
Oil_con*plankton	1	0		1	1		Tukey	0.9800
Oil_con*plankton	1	0		2	0		Tukey	0.9366
Oil_con*plankton	1	0		2	1		Tukey	0.8925
Oil_con*plankton	1	1		2	0		Tukey	0.6152
Oil_con*plankton	1	1		2	1		Tukey	0.5363
Oil_con*plankton	2	0		2	1		Tukey	1.0000
filter			0			1	Tukey	0.0004

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Differences of Least Squares Means								
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Adjustment**	**Adj P**
plankton*filter		0	0		0	1	Tukey	0.0715
plankton*filter		0	0		1	0	Tukey	0.8268
plankton*filter		0	0		1	1	Tukey	0.0366
plankton*filter		0	1		1	0	Tukey	0.0161
plankton*filter		0	1		1	1	Tukey	0.9792
plankton*filter		1	0		1	1	Tukey	0.0082
Oil_con*filter	0		0	0		1	Tukey	0.8992
Oil_con*filter	0		0	1		0	Tukey	0.1408
Oil_con*filter	0		0	1		1	Tukey	1.0000
Oil_con*filter	0		0	2		0	Tukey	0.0031
Oil_con*filter	0		0	2		1	Tukey	0.9979
Oil_con*filter	0		1	1		0	Tukey	0.0257
Oil_con*filter	0		1	1		1	Tukey	0.8707
Oil_con*filter	0		1	2		0	Tukey	0.0006
Oil_con*filter	0		1	2		1	Tukey	0.7005
Oil_con*filter	1		0	1		1	Tukey	0.1582
Oil_con*filter	1		0	2		0	Tukey	0.2648
Oil_con*filter	1		0	2		1	Tukey	0.2632
Oil_con*filter	1		1	2		0	Tukey	0.0035
Oil_con*filter	1		1	2		1	Tukey	0.9992
Oil_con*filter	2		0	2		1	Tukey	0.0061
Oil_co*plankt*filter	0	0	0	0	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	0	1	0	Tukey	0.9078
Oil_co*plankt*filter	0	0	0	0	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	1	0	0	Tukey	0.2587
Oil_co*plankt*filter	0	0	0	1	0	1	Tukey	0.9876
Oil_co*plankt*filter	0	0	0	1	1	0	Tukey	0.4178
Oil_co*plankt*filter	0	0	0	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	0	2	0	0	Tukey	0.0379
Oil_co*plankt*filter	0	0	0	2	0	1	Tukey	0.9862
Oil_co*plankt*filter	0	0	0	2	1	0	Tukey	0.0222
Oil_co*plankt*filter	0	0	0	2	1	1	Tukey	0.9951

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Differences of Least Squares Means								
Effect	**Oil_con**	**plankton**	**filter**	**_Oil_con**	**_plankton**	**_filter**	**Adjustment**	**Adj P**
Oil_co*plankt*filter	0	0	1	0	1	0	Tukey	0.9078
Oil_co*plankt*filter	0	0	1	0	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	1	1	0	0	Tukey	0.2587
Oil_co*plankt*filter	0	0	1	1	0	1	Tukey	0.9876
Oil_co*plankt*filter	0	0	1	1	1	0	Tukey	0.4177
Oil_co*plankt*filter	0	0	1	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	0	1	2	0	0	Tukey	0.0379
Oil_co*plankt*filter	0	0	1	2	0	1	Tukey	0.9862
Oil_co*plankt*filter	0	0	1	2	1	0	Tukey	0.0222
Oil_co*plankt*filter	0	0	1	2	1	1	Tukey	0.9951
Oil_co*plankt*filter	0	1	0	0	1	1	Tukey	0.9280
Oil_co*plankt*filter	0	1	0	1	0	0	Tukey	0.9531
Oil_co*plankt*filter	0	1	0	1	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	0	1	1	0	Tukey	0.9954
Oil_co*plankt*filter	0	1	0	1	1	1	Tukey	0.9942
Oil_co*plankt*filter	0	1	0	2	0	0	Tukey	0.3590
Oil_co*plankt*filter	0	1	0	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	0	2	1	0	Tukey	0.2295
Oil_co*plankt*filter	0	1	0	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	1	1	0	0	Tukey	0.2828
Oil_co*plankt*filter	0	1	1	1	0	1	Tukey	0.9922
Oil_co*plankt*filter	0	1	1	1	1	0	Tukey	0.4509
Oil_co*plankt*filter	0	1	1	1	1	1	Tukey	1.0000
Oil_co*plankt*filter	0	1	1	2	0	0	Tukey	0.0421
Oil_co*plankt*filter	0	1	1	2	0	1	Tukey	0.9912
Oil_co*plankt*filter	0	1	1	2	1	0	Tukey	0.0247
Oil_co*plankt*filter	0	1	1	2	1	1	Tukey	0.9972
Oil_co*plankt*filter	1	0	0	1	0	1	Tukey	0.8102
Oil_co*plankt*filter	1	0	0	1	1	0	Tukey	1.0000
Oil_co*plankt*filter	1	0	0	1	1	1	Tukey	0.4891
Oil_co*plankt*filter	1	0	0	2	0	0	Tukey	0.9686
Oil_co*plankt*filter	1	0	0	2	0	1	Tukey	0.8177

water
aromatics

The Mixed Procedure

Differences of Least Squares Means								
Effect	Oil_con	plankton	filter	_Oil_con	_plankton	_filter	Adjustment	Adj P
Oil_co*plankt*filter	1	0	0	2	1	0	Tukey	0.8769
Oil_co*plankt*filter	1	0	0	2	1	1	Tukey	0.7469
Oil_co*plankt*filter	1	0	1	1	1	0	Tukey	0.9455
Oil_co*plankt*filter	1	0	1	1	1	1	Tukey	0.9999
Oil_co*plankt*filter	1	0	1	2	0	0	Tukey	0.2074
Oil_co*plankt*filter	1	0	1	2	0	1	Tukey	1.0000
Oil_co*plankt*filter	1	0	1	2	1	0	Tukey	0.1264
Oil_co*plankt*filter	1	0	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	1	1	0	1	1	1	Tukey	0.6969
Oil_co*plankt*filter	1	1	0	2	0	0	Tukey	0.8617
Oil_co*plankt*filter	1	1	0	2	0	1	Tukey	0.9492
Oil_co*plankt*filter	1	1	0	2	1	0	Tukey	0.6962
Oil_co*plankt*filter	1	1	0	2	1	1	Tukey	0.9103
Oil_co*plankt*filter	1	1	1	2	0	0	Tukey	0.0852
Oil_co*plankt*filter	1	1	1	2	0	1	Tukey	0.9999
Oil_co*plankt*filter	1	1	1	2	1	0	Tukey	0.0502
Oil_co*plankt*filter	1	1	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	2	0	0	2	0	1	Tukey	0.2122
Oil_co*plankt*filter	2	0	0	2	1	0	Tukey	1.0000
Oil_co*plankt*filter	2	0	0	2	1	1	Tukey	0.1729
Oil_co*plankt*filter	2	0	1	2	1	0	Tukey	0.1295
Oil_co*plankt*filter	2	0	1	2	1	1	Tukey	1.0000
Oil_co*plankt*filter	2	1	0	2	1	1	Tukey	0.1043

water
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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
1		1		9330.17	1599.04	A
2		0		8581.50	1599.04	A

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
3	2			14908	1958.41	A
4	1			9728.25	1958.41	A
5	0			2231.38	1958.41	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
6	2	1		15178	2769.62	A
7	2	0		14638	2769.62	A
8	1	0		11074	2769.62	AB
9	1	1		8382.25	2769.62	AB
10	0	1		4430.00	2769.62	AB
11	0	0		32.7500	2769.62	B

Effect=filter Method=Tukey(P<.05) Set=4

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
12			0	14365	1599.04	A
13			1	3547.00	1599.04	B

Effect=plankton*filter Method=Tukey(P<.05) Set=5

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
14		1	0	15734	2261.38	A
15		0	0	12996	2261.38	AB

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Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
16		0	1	4167.50	2261.38	BC
17		1	1	2926.50	2261.38	C

Effect=Oil_con*filter   Method=Tukey(P<.05)   Set=6

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
18	2		0	23931	2769.62	A
19	1		0	14916	2769.62	AB
20	2		1	5884.75	2769.62	BC
21	1		1	4540.25	2769.62	BC
22	0		0	4246.75	2769.62	BC
23	0		1	216.00	2769.62	C

Effect=Oil_co*plankt*filter   Method=Tukey(P<.05)   Set=7

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
24	2	1	0	24867	3916.83	A
25	2	0	0	22996	3916.83	A
26	1	0	0	15958	3916.83	AB
27	1	1	0	13875	3916.83	AB
28	0	1	0	8460.50	3916.83	AB
29	2	0	1	6279.50	3916.83	AB
30	1	0	1	6190.50	3916.83	AB
31	2	1	1	5490.00	3916.83	AB
32	1	1	1	2890.00	3916.83	AB
33	0	1	1	399.50	3916.83	B
34	0	0	0	33.0000	3916.83	B
35	0	0	1	32.5000	3916.83	B

water

Obs	Oil_con	plankton	filter	Alk	aro	logAlk	logaro
1	0	0	0	4.00	29	1.38629	3.3673
2	0	0	0	3.61	37	1.28371	3.6109
3	0	0	1	3.74	32	1.31909	3.4657
4	0	0	1	3.60	33	1.28093	3.4965
5	0	1	0	2.89	296	1.06126	5.6904
6	0	1	0	22.45	16625	3.11129	9.7187
7	0	1	1	2.39	702	0.87129	6.5539
8	0	1	1	4.03	97	1.39377	4.5747
9	1	0	0	79.21	15169	4.37210	9.6270
10	1	0	0	76.83	16747	4.34160	9.7260
11	1	0	1	5.36	3714	1.67896	8.2199
12	1	0	1	7.27	8667	1.98376	9.0673
13	1	1	0	54.99	12214	4.00715	9.4103
14	1	1	0	22.10	15535	3.09558	9.6509
15	1	1	1	4.39	2521	1.47933	7.8324
16	1	1	1	3.80	3259	1.33500	8.0892
17	2	0	0	41.49	26293	3.72545	10.1771
18	2	0	0	43.77	19698	3.77895	9.8883
19	2	0	1	3.99	6874	1.38379	8.8355
20	2	0	1	4.29	5685	1.45629	8.6456
21	2	1	0	88.54	34685	4.48345	10.4541
22	2	1	0	75.63	15048	4.32585	9.6190
23	2	1	1	4.81	5227	1.57070	8.5616
24	2	1	1	7.37	5753	1.99742	8.6575

water
alkanes

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logAlk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1
filter	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	24

Number of Observations	
Number of Observations Read	24
Number of Observations Used	24
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.2353

Fit Statistics	
-2 Res Log Likelihood	25.0
AIC (Smaller is Better)	27.0
AICC (Smaller is Better)	27.4
BIC (Smaller is Better)	27.5

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	12	0.10	0.7605
Oil_con	2	12	20.68	0.0001
Oil_con*plankton	2	12	3.02	0.0868
filter	1	12	79.74	<.0001
plankton*filter	1	12	0.48	0.5003
Oil_con*filter	2	12	10.39	0.0024
Oil_co*plankt*filter	2	12	0.90	0.4331

Least Squares Means								
Effect	Oil_con	plankton	filter	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0		2.3326	0.1400	12	16.66	<.0001
plankton		1		2.3943	0.1400	12	17.10	<.0001
Oil_con	0			1.4635	0.1715	12	8.53	<.0001
Oil_con	1			2.7867	0.1715	12	16.25	<.0001
Oil_con	2			2.8402	0.1715	12	16.56	<.0001
Oil_con*plankton	0	0		1.3175	0.2426	12	5.43	0.0002
Oil_con*plankton	0	1		1.6094	0.2426	12	6.64	<.0001
Oil_con*plankton	1	0		3.0941	0.2426	12	12.76	<.0001
Oil_con*plankton	1	1		2.4793	0.2426	12	10.22	<.0001
Oil_con*plankton	2	0		2.5861	0.2426	12	10.66	<.0001
Oil_con*plankton	2	1		3.0944	0.2426	12	12.76	<.0001
filter			0	3.2477	0.1400	12	23.19	<.0001
filter			1	1.4792	0.1400	12	10.56	<.0001
plankton*filter		0	0	3.1480	0.1981	12	15.90	<.0001
plankton*filter		0	1	1.5171	0.1981	12	7.66	<.0001
plankton*filter		1	0	3.3474	0.1981	12	16.90	<.0001
plankton*filter		1	1	1.4413	0.1981	12	7.28	<.0001
Oil_con*filter	0		0	1.7106	0.2426	12	7.05	<.0001
Oil_con*filter	0		1	1.2163	0.2426	12	5.01	0.0003
Oil_con*filter	1		0	3.9541	0.2426	12	16.30	<.0001
Oil_con*filter	1		1	1.6193	0.2426	12	6.68	<.0001
Oil_con*filter	2		0	4.0784	0.2426	12	16.81	<.0001
Oil_con*filter	2		1	1.6020	0.2426	12	6.60	<.0001
Oil_co*plankt*filter	0	0	0	1.3350	0.3430	12	3.89	0.0021
Oil_co*plankt*filter	0	0	1	1.3000	0.3430	12	3.79	0.0026
Oil_co*plankt*filter	0	1	0	2.0863	0.3430	12	6.08	<.0001
Oil_co*plankt*filter	0	1	1	1.1325	0.3430	12	3.30	0.0063
Oil_co*plankt*filter	1	0	0	4.3568	0.3430	12	12.70	<.0001
Oil_co*plankt*filter	1	0	1	1.8314	0.3430	12	5.34	0.0002
Oil_co*plankt*filter	1	1	0	3.5514	0.3430	12	10.35	<.0001
Oil_co*plankt*filter	1	1	1	1.4072	0.3430	12	4.10	0.0015
Oil_co*plankt*filter	2	0	0	3.7522	0.3430	12	10.94	<.0001
Oil_co*plankt*filter	2	0	1	1.4200	0.3430	12	4.14	0.0014
Oil_co*plankt*filter	2	1	0	4.4047	0.3430	12	12.84	<.0001
Oil_co*plankt*filter	2	1	1	1.7841	0.3430	12	5.20	0.0002

Effect=Oil_con*filter Method=Tukey(P<.05) Set=6

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
18	2		0	4.0784	0.2426	A
19	1		0	3.9541	0.2426	A
20	0		0	1.7106	0.2426	B
21	1		1	1.6193	0.2426	B
22	2		1	1.6020	0.2426	B
23	0		1	1.2163	0.2426	B

Effect=Oil_co*plankt*filter Method=Tukey(P<.05) Set=7

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
24	2	1	0	4.4047	0.3430	A
25	1	0	0	4.3568	0.3430	A
26	2	0	0	3.7522	0.3430	AB
27	1	1	0	3.5514	0.3430	ABC
28	0	1	0	2.0863	0.3430	BCD
29	1	0	1	1.8314	0.3430	BCD
30	2	1	1	1.7841	0.3430	CD
31	2	0	1	1.4200	0.3430	D
32	1	1	1	1.4072	0.3430	D
33	0	0	0	1.3350	0.3430	D
34	0	0	1	1.3000	0.3430	D
35	0	1	1	1.1325	0.3430	D

water aromatics

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logaro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1
filter	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	24

Number of Observations	
Number of Observations Read	24
Number of Observations Used	24
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.9118

Fit Statistics	
-2 Res Log Likelihood	41.3
AIC (Smaller is Better)	43.3
AICC (Smaller is Better)	43.7
BIC (Smaller is Better)	43.7

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	12	5.22	0.0414
Oil_con	2	12	49.38	<.0001
Oil_con*plankton	2	12	8.46	0.0051
filter	1	12	10.20	0.0077
plankton*filter	1	12	1.44	0.2537
Oil_con*filter	2	12	0.05	0.9515
Oil_co*plankt*filter	2	12	0.61	0.5584

water
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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
1		1		2.3943	0.1400	A
2		0		2.3326	0.1400	A

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
3	2			2.8402	0.1715	A
4	1			2.7867	0.1715	A
5	0			1.4635	0.1715	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
6	2	1		3.0944	0.2426	A
7	1	0		3.0941	0.2426	A
8	2	0		2.5861	0.2426	AB
9	1	1		2.4793	0.2426	AB
10	0	1		1.6094	0.2426	BC
11	0	0		1.3175	0.2426	C

Effect=filter Method=Tukey(P<.05) Set=4

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
12			0	3.2477	0.1400	A
13			1	1.4792	0.1400	B

Effect=plankton*filter Method=Tukey(P<.05) Set=5

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
14		1	0	3.3474	0.1981	A
15		0	0	3.1480	0.1981	A
16		0	1	1.5171	0.1981	B
17		1	1	1.4413	0.1981	B

water
aromatics

Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
1		1		8.2344	0.2756	A
2		0		7.3439	0.2756	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
3	2			9.3548	0.3376	A
4	1			8.9529	0.3376	A
5	0			5.0598	0.3376	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
6	2	0		9.3866	0.4774	A
7	2	1		9.3230	0.4774	A
8	1	0		9.1600	0.4774	A
9	1	1		8.7457	0.4774	AB
10	0	1		6.6344	0.4774	B
11	0	0		3.4851	0.4774	C

Effect=filter Method=Tukey(P<.05) Set=4

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
12			0	8.4117	0.2756	A
13			1	7.1666	0.2756	B

Effect=plankton*filter Method=Tukey(P<.05) Set=5

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
14		1	0	9.0905	0.3898	A
15		0	0	7.7328	0.3898	AB
16		1	1	7.3782	0.3898	B
17		0	1	6.9551	0.3898	B

Effect=Oil_con*filter Method=Tukey(P<.05) Set=6

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
18	2		0	10.0346	0.4774	A
19	1		0	9.6035	0.4774	A
20	2		1	8.6750	0.4774	A
21	1		1	8.3022	0.4774	A
22	0		0	5.5968	0.4774	B
23	0		1	4.5227	0.4774	B

Effect=Oil_co*plankt*filter Method=Tukey(P<.05) Set=7

Obs	Oil_con	plankton	filter	Estimate	Standard Error	Letter Group
24	2	1	0	10.0365	0.6752	A
25	2	0	0	10.0327	0.6752	A
26	1	0	0	9.6765	0.6752	A
27	1	1	0	9.5306	0.6752	A
28	2	0	1	8.7405	0.6752	AB
29	1	0	1	8.6436	0.6752	AB
30	2	1	1	8.6095	0.6752	AB
31	1	1	1	7.9608	0.6752	AB
32	0	1	0	7.7045	0.6752	AB
33	0	1	1	5.5643	0.6752	BC
34	0	0	0	3.4891	0.6752	C
35	0	0	1	3.4811	0.6752	C

B.2.2 Beakers

beaker

Obs	Oil_con	plankton	Alk	aro
1	0	0	2.58	12
2	0	0	1.82	13
3	0	1	1.05	0
4	0	1	1.17	0
5	1	0	15.91	114
6	1	0	13.65	226
7	1	1	13.84	143
8	1	1	17.57	294
9	2	0	30.56	1065
10	2	0	27.41	1044
11	2	1	61.72	619
12	2	1	41.26	905

beaker
Alkanes

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	Alk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	37.3456

beaker
Alkanes

The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	42.9
AIC (Smaller is Better)	44.9
AICC (Smaller is Better)	45.9
BIC (Smaller is Better)	44.7

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	4.45	0.0793
Oil_con	2	6	41.02	0.0003
Oil_con*plankton	2	6	4.58	0.0620

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	15.3217	2.4948	6	6.14	0.0009
plankton		1	22.7683	2.4948	6	9.13	<.0001
Oil_con	0		1.6550	3.0556	6	0.54	0.6076
Oil_con	1		15.2425	3.0556	6	4.99	0.0025
Oil_con	2		40.2375	3.0556	6	13.17	<.0001
Oil_con*plankton	0	0	2.2000	4.3212	6	0.51	0.6288
Oil_con*plankton	0	1	1.1100	4.3212	6	0.26	0.8059
Oil_con*plankton	1	0	14.7800	4.3212	6	3.42	0.0141
Oil_con*plankton	1	1	15.7050	4.3212	6	3.63	0.0109
Oil_con*plankton	2	0	28.9850	4.3212	6	6.71	0.0005
Oil_con*plankton	2	1	51.4900	4.3212	6	11.92	<.0001

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
plankton		0		1	-7.4467	3.5282	6	-2.11	0.0793	Tukey
Oil_con	0		1		-13.5875	4.3212	6	-3.14	0.0200	Tukey
Oil_con	0		2		-38.5825	4.3212	6	-8.93	0.0001	Tukey

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The Mixed Procedure

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Oil_con	1		2		-24.9950	4.3212	6	-5.78	0.0012	Tukey
Oil_con*plankton	0	0	0	1	1.0900	6.1111	6	0.18	0.8643	Tukey
Oil_con*plankton	0	0	1	0	-12.5800	6.1111	6	-2.06	0.0852	Tukey
Oil_con*plankton	0	0	1	1	-13.5050	6.1111	6	-2.21	0.0692	Tukey
Oil_con*plankton	0	0	2	0	-26.7850	6.1111	6	-4.38	0.0047	Tukey
Oil_con*plankton	0	0	2	1	-49.2900	6.1111	6	-8.07	0.0002	Tukey
Oil_con*plankton	0	1	1	0	-13.6700	6.1111	6	-2.24	0.0666	Tukey
Oil_con*plankton	0	1	1	1	-14.5950	6.1111	6	-2.39	0.0542	Tukey
Oil_con*plankton	0	1	2	0	-27.8750	6.1111	6	-4.56	0.0038	Tukey
Oil_con*plankton	0	1	2	1	-50.3800	6.1111	6	-8.24	0.0002	Tukey
Oil_con*plankton	1	0	1	1	-0.9250	6.1111	6	-0.15	0.8846	Tukey
Oil_con*plankton	1	0	2	0	-14.2050	6.1111	6	-2.32	0.0591	Tukey
Oil_con*plankton	1	0	2	1	-36.7100	6.1111	6	-6.01	0.0010	Tukey
Oil_con*plankton	1	1	2	0	-13.2800	6.1111	6	-2.17	0.0728	Tukey
Oil_con*plankton	1	1	2	1	-35.7850	6.1111	6	-5.86	0.0011	Tukey
Oil_con*plankton	2	0	2	1	-22.5050	6.1111	6	-3.68	0.0103	Tukey

Differences of Least Squares Means					
Effect	Oil_con	plankton	_Oil_con	_plankton	Adj P
plankton		0		1	0.0793
Oil_con	0		1		0.0455
Oil_con	0		2		0.0003
Oil_con	1		2		0.0028
Oil_con*plankton	0	0	0	1	1.0000
Oil_con*plankton	0	0	1	0	0.4095
Oil_con*plankton	0	0	1	1	0.3502
Oil_con*plankton	0	0	2	0	0.0329
Oil_con*plankton	0	0	2	1	0.0015
Oil_con*plankton	0	1	1	0	0.3403
Oil_con*plankton	0	1	1	1	0.2893
Oil_con*plankton	0	1	2	0	0.0275

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The Mixed Procedure

Differences of Least Squares Means					
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Adj P**
Oil_con*plankton	0	1	2	1	0.0014
Oil_con*plankton	1	0	1	1	1.0000
Oil_con*plankton	1	0	2	0	0.3100
Oil_con*plankton	1	0	2	1	0.0073
Oil_con*plankton	1	1	2	0	0.3640
Oil_con*plankton	1	1	2	1	0.0083
Oil_con*plankton	2	0	2	1	0.0688

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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	22.7683	2.4948	A
2		0	15.3217	2.4948	A

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		40.2375	3.0556	A
4	1		15.2425	3.0556	B
5	0		1.6550	3.0556	C

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	1	51.4900	4.3212	A
7	2	0	28.9850	4.3212	AB
8	1	1	15.7050	4.3212	BC
9	1	0	14.7800	4.3212	BC
10	0	0	2.2000	4.3212	C
11	0	1	1.1100	4.3212	C

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The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	aro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	9798.58

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The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	76.3
AIC (Smaller is Better)	78.3
AICC (Smaller is Better)	79.3
BIC (Smaller is Better)	78.1

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	2.24	0.1853
Oil_con	2	6	92.44	<.0001
Oil_con*plankton	2	6	3.37	0.1042

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	412.33	40.4116	6	10.20	<.0001
plankton		1	326.83	40.4116	6	8.09	0.0002
Oil_con	0		6.2500	49.4939	6	0.13	0.9036
Oil_con	1		194.25	49.4939	6	3.92	0.0078
Oil_con	2		908.25	49.4939	6	18.35	<.0001
Oil_con*plankton	0	0	12.5000	69.9949	6	0.18	0.8641
Oil_con*plankton	0	1	0	69.9949	6	0.00	1.0000
Oil_con*plankton	1	0	170.00	69.9949	6	2.43	0.0512
Oil_con*plankton	1	1	218.50	69.9949	6	3.12	0.0205
Oil_con*plankton	2	0	1054.50	69.9949	6	15.07	<.0001
Oil_con*plankton	2	1	762.00	69.9949	6	10.89	<.0001

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
plankton		0		1	85.5000	57.1506	6	1.50	0.1853	Tukey
Oil_con	0		1		-188.00	69.9949	6	-2.69	0.0362	Tukey
Oil_con	0		2		-902.00	69.9949	6	-12.89	<.0001	Tukey

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The Mixed Procedure

Differences of Least Squares Means										
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**
Oil_con	1		2		-714.00	69.9949	6	-10.20	<.0001	Tukey
Oil_con*plankton	0	0	0	1	12.5000	98.9878	6	0.13	0.9036	Tukey
Oil_con*plankton	0	0	1	0	-157.50	98.9878	6	-1.59	0.1627	Tukey
Oil_con*plankton	0	0	1	1	-206.00	98.9878	6	-2.08	0.0826	Tukey
Oil_con*plankton	0	0	2	0	-1042.00	98.9878	6	-10.53	<.0001	Tukey
Oil_con*plankton	0	0	2	1	-749.50	98.9878	6	-7.57	0.0003	Tukey
Oil_con*plankton	0	1	1	0	-170.00	98.9878	6	-1.72	0.1367	Tukey
Oil_con*plankton	0	1	1	1	-218.50	98.9878	6	-2.21	0.0694	Tukey
Oil_con*plankton	0	1	2	0	-1054.50	98.9878	6	-10.65	<.0001	Tukey
Oil_con*plankton	0	1	2	1	-762.00	98.9878	6	-7.70	0.0003	Tukey
Oil_con*plankton	1	0	1	1	-48.5000	98.9878	6	-0.49	0.6416	Tukey
Oil_con*plankton	1	0	2	0	-884.50	98.9878	6	-8.94	0.0001	Tukey
Oil_con*plankton	1	0	2	1	-592.00	98.9878	6	-5.98	0.0010	Tukey
Oil_con*plankton	1	1	2	0	-836.00	98.9878	6	-8.45	0.0002	Tukey
Oil_con*plankton	1	1	2	1	-543.50	98.9878	6	-5.49	0.0015	Tukey
Oil_con*plankton	2	0	2	1	292.50	98.9878	6	2.95	0.0255	Tukey

Differences of Least Squares Means					
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Adj P**
plankton		0		1	0.1853
Oil_con	0		1		0.0806
Oil_con	0		2		<.0001
Oil_con	1		2		0.0001
Oil_con*plankton	0	0	0	1	1.0000
Oil_con*plankton	0	0	1	0	0.6306
Oil_con*plankton	0	0	1	1	0.4002
Oil_con*plankton	0	0	2	0	0.0003
Oil_con*plankton	0	0	2	1	0.0022
Oil_con*plankton	0	1	1	0	0.5667
Oil_con*plankton	0	1	1	1	0.3511
Oil_con*plankton	0	1	2	0	0.0003

Differences of Least Squares Means					
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Adj P**
Oil_con*plankton	0	1	2	1	0.0020
Oil_con*plankton	1	0	1	1	0.9948
Oil_con*plankton	1	0	2	0	0.0009
Oil_con*plankton	1	0	2	1	0.0074
Oil_con*plankton	1	1	2	0	0.0012
Oil_con*plankton	1	1	2	1	0.0114
Oil_con*plankton	2	0	2	1	0.1542

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Effect=plankton   Method=Tukey(P<.05)   Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		0	412.33	40.4116	A
2		1	326.83	40.4116	A

Effect=Oil_con   Method=Tukey(P<.05)   Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		908.25	49.4939	A
4	1		194.25	49.4939	B
5	0		6.2500	49.4939	B

Effect=Oil_con*plankton   Method=Tukey(P<.05)   Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	0	1054.50	69.9949	A
7	2	1	762.00	69.9949	A
8	1	1	218.50	69.9949	B
9	1	0	170.00	69.9949	B
10	0	0	12.5000	69.9949	B
11	0	1	0	69.9949	B

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Obs	Oil_con	plankton	Alk	aro	logAlk	logaro
1	0	0	2.58	12	0.94779	2.48491
2	0	0	1.82	13	0.59884	2.56495
3	0	1	1.05	0	0.04879	.
4	0	1	1.17	0	0.15700	.
5	1	0	15.91	114	2.76695	4.73620
6	1	0	13.65	226	2.61374	5.42053
7	1	1	13.84	143	2.62756	4.96284
8	1	1	17.57	294	2.86619	5.68358
9	2	0	30.56	1065	3.41969	6.97073
10	2	0	27.41	1044	3.31091	6.95081
11	2	1	61.72	619	4.12261	6.42811
12	2	1	41.26	905	3.71989	6.80793

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The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logAlk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.03233

Fit Statistics	
-2 Res Log Likelihood	0.6
AIC (Smaller is Better)	2.6
AICC (Smaller is Better)	3.6
BIC (Smaller is Better)	2.4

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	0.03	0.8586
Oil_con	2	6	336.76	<.0001
Oil_con*plankton	2	6	11.76	0.0084

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	2.2763	0.07340	6	31.01	<.0001
plankton		1	2.2570	0.07340	6	30.75	<.0001
Oil_con	0		0.4381	0.08990	6	4.87	0.0028
Oil_con	1		2.7186	0.08990	6	30.24	<.0001
Oil_con	2		3.6433	0.08990	6	40.53	<.0001
Oil_con*plankton	0	0	0.7733	0.1271	6	6.08	0.0009
Oil_con*plankton	0	1	0.1029	0.1271	6	0.81	0.4492
Oil_con*plankton	1	0	2.6903	0.1271	6	21.16	<.0001
Oil_con*plankton	1	1	2.7469	0.1271	6	21.61	<.0001
Oil_con*plankton	2	0	3.3653	0.1271	6	26.47	<.0001
Oil_con*plankton	2	1	3.9213	0.1271	6	30.84	<.0001

Differences of Least Squares Means											
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
plankton		0		1	0.01931	0.1038	6	0.19	0.8586	Tukey	0.8586
Oil_con	0		1		-2.2805	0.1271	6	-17.94	<.0001	Tukey	<.0001
Oil_con	0		2		-3.2052	0.1271	6	-25.21	<.0001	Tukey	<.0001
Oil_con	1		2		-0.9247	0.1271	6	-7.27	0.0003	Tukey	0.0008
Oil_con*plankton	0	0	0	1	0.6704	0.1798	6	3.73	0.0098	Tukey	0.0654
Oil_con*plankton	0	0	1	0	-1.9170	0.1798	6	-10.66	<.0001	Tukey	0.0003
Oil_con*plankton	0	0	1	1	-1.9736	0.1798	6	-10.98	<.0001	Tukey	0.0003
Oil_con*plankton	0	0	2	0	-2.5920	0.1798	6	-14.42	<.0001	Tukey	<.0001
Oil_con*plankton	0	0	2	1	-3.1479	0.1798	6	-17.51	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	1	0	-2.5874	0.1798	6	-14.39	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	1	1	-2.6440	0.1798	6	-14.71	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	2	0	-3.2624	0.1798	6	-18.15	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	2	1	-3.8184	0.1798	6	-21.24	<.0001	Tukey	<.0001
Oil_con*plankton	1	0	1	1	-0.05653	0.1798	6	-0.31	0.7638	Tukey	0.9993
Oil_con*plankton	1	0	2	0	-0.6750	0.1798	6	-3.75	0.0095	Tukey	0.0637
Oil_con*plankton	1	0	2	1	-1.2309	0.1798	6	-6.85	0.0005	Tukey	0.0037
Oil_con*plankton	1	1	2	0	-0.6184	0.1798	6	-3.44	0.0138	Tukey	0.0897
Oil_con*plankton	1	1	2	1	-1.1744	0.1798	6	-6.53	0.0006	Tukey	0.0047
Oil_con*plankton	2	0	2	1	-0.5560	0.1798	6	-3.09	0.0213	Tukey	0.1322

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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		0	2.2763	0.07340	A
2		1	2.2570	0.07340	A

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		3.6433	0.08990	A
4	1		2.7186	0.08990	B
5	0		0.4381	0.08990	C

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	1	3.9213	0.1271	A
7	2	0	3.3653	0.1271	AB
8	1	1	2.7469	0.1271	B
9	1	0	2.6903	0.1271	B
10	0	0	0.7733	0.1271	C
11	0	1	0.1029	0.1271	C

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The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logaro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	11
Columns in Z	0
Subjects	1
Max Obs per Subject	10

Number of Observations	
Number of Observations Read	12
Number of Observations Used	10
Number of Observations Not Used	2

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.1139

Fit Statistics	
-2 Res Log Likelihood	6.8
AIC (Smaller is Better)	8.8
AICC (Smaller is Better)	10.1
BIC (Smaller is Better)	8.4

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	5	0.04	0.8455
Oil_con	2	5	93.65	0.0001
Oil_con*plankton	1	5	1.52	0.2730

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	4.8547	0.1378	5	35.24	<.0001
plankton		1	Non-est	.	.	.	.
Oil_con	0		Non-est	.	.	.	.
Oil_con	1		5.2008	0.1687	5	30.82	<.0001
Oil_con	2		6.7894	0.1687	5	40.24	<.0001
Oil_con*plankton	0	0	2.5249	0.2386	5	10.58	0.0001
Oil_con*plankton	1	0	5.0784	0.2386	5	21.28	<.0001
Oil_con*plankton	1	1	5.3232	0.2386	5	22.31	<.0001
Oil_con*plankton	2	0	6.9608	0.2386	5	29.17	<.0001
Oil_con*plankton	2	1	6.6180	0.2386	5	27.73	<.0001

Differences of Least Squares Means											
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
plankton		0		1	Non-est	.	.	.	.	Tukey-Kramer	.
Oil_con	0		1		Non-est	.	.	.	.	Tukey-Kramer	.
Oil_con	0		2		Non-est	.	.	.	.	Tukey-Kramer	.
Oil_con	1		2		-1.5886	0.2386	5	-6.66	0.0012	Tukey-Kramer	0.0012
Oil_con*plankton	0	0	1	0	-2.5534	0.3375	5	-7.57	0.0006	Tukey	0.0034
Oil_con*plankton	0	0	1	1	-2.7983	0.3375	5	-8.29	0.0004	Tukey	0.0022
Oil_con*plankton	0	0	2	0	-4.4358	0.3375	5	-13.14	<.0001	Tukey	0.0003
Oil_con*plankton	0	0	2	1	-4.0931	0.3375	5	-12.13	<.0001	Tukey	0.0004
Oil_con*plankton	1	0	1	1	-0.2448	0.3375	5	-0.73	0.5006	Tukey	0.9414
Oil_con*plankton	1	0	2	0	-1.8824	0.3375	5	-5.58	0.0026	Tukey	0.0133
Oil_con*plankton	1	0	2	1	-1.5397	0.3375	5	-4.56	0.0060	Tukey	0.0304
Oil_con*plankton	1	1	2	0	-1.6376	0.3375	5	-4.85	0.0047	Tukey	0.0237
Oil_con*plankton	1	1	2	1	-1.2948	0.3375	5	-3.84	0.0122	Tukey	0.0590
Oil_con*plankton	2	0	2	1	0.3428	0.3375	5	1.02	0.3564	Tukey	0.8391

beaker
Aromatics

Effect=plankton Method=LSD(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		0	4.8547	0.1378	A
2		1	.	.	

Effect=Oil_con Method=LSD(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		6.7894	0.1687	A
4	1		5.2008	0.1687	B
5	0		.	.	

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	0	6.9608	0.2386	A
7	2	1	6.6180	0.2386	AB
8	1	1	5.3232	0.2386	BC
9	1	0	5.0784	0.2386	C
10	0	0	2.5249	0.2386	D

B.2.3 Filters

filter

Obs	Oil_con	plankton	Alk	aro
1	0	0	70.66	34
2	0	0	51.54	31
3	0	1	230.52	210
4	0	1	23.57	328
5	1	0	108.23	1712
6	1	0	143.06	2593
7	1	1	230.19	3034
8	1	1	139.70	2314
9	2	0	231.51	5861
10	2	0	206.27	8296
11	2	1	396.29	7501
12	2	1	398.81	7331

filter
alkanes

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	Alk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	4436.57

filter
alkanes

The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	71.6
AIC (Smaller is Better)	73.6
AICC (Smaller is Better)	74.6
BIC (Smaller is Better)	73.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	6.94	0.0388
Oil_con	2	6	10.97	0.0099
Oil_con*plankton	2	6	1.01	0.4174

Least Squares Means

Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	135.21	27.1924	6	4.97	0.0025
plankton		1	236.51	27.1924	6	8.70	0.0001
Oil_con	0		94.0725	33.3038	6	2.82	0.0302
Oil_con	1		155.30	33.3038	6	4.66	0.0035
Oil_con	2		308.22	33.3038	6	9.25	<.0001
Oil_con*plankton	0	0	61.1000	47.0987	6	1.30	0.2422
Oil_con*plankton	0	1	127.05	47.0987	6	2.70	0.0357
Oil_con*plankton	1	0	125.65	47.0987	6	2.67	0.0371
Oil_con*plankton	1	1	184.95	47.0987	6	3.93	0.0077
Oil_con*plankton	2	0	218.89	47.0987	6	4.65	0.0035
Oil_con*plankton	2	1	397.55	47.0987	6	8.44	0.0002

Differences of Least Squares Means

Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
plankton		0		1	-101.30	38.4559	6	-2.63	0.0388	Tukey
Oil_con	0		1		-61.2225	47.0987	6	-1.30	0.2413	Tukey
Oil_con	0		2		-214.15	47.0987	6	-4.55	0.0039	Tukey

filter
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The Mixed Procedure

Differences of Least Squares Means

Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Oil_con	1		2		-152.92	47.0987	6	-3.25	0.0175	Tukey
Oil_con*plankton	0	0	0	1	-65.9450	66.6076	6	-0.99	0.3604	Tukey
Oil_con*plankton	0	0	1	0	-64.5450	66.6076	6	-0.97	0.3700	Tukey
Oil_con*plankton	0	0	1	1	-123.85	66.6076	6	-1.86	0.1123	Tukey
Oil_con*plankton	0	0	2	0	-157.79	66.6076	6	-2.37	0.0556	Tukey
Oil_con*plankton	0	0	2	1	-336.45	66.6076	6	-5.05	0.0023	Tukey
Oil_con*plankton	0	1	1	0	1.4000	66.6076	6	0.02	0.9839	Tukey
Oil_con*plankton	0	1	1	1	-57.9000	66.6076	6	-0.87	0.4181	Tukey
Oil_con*plankton	0	1	2	0	-91.8450	66.6076	6	-1.38	0.2171	Tukey
Oil_con*plankton	0	1	2	1	-270.50	66.6076	6	-4.06	0.0066	Tukey
Oil_con*plankton	1	0	1	1	-59.3000	66.6076	6	-0.89	0.4076	Tukey
Oil_con*plankton	1	0	2	0	-93.2450	66.6076	6	-1.40	0.2111	Tukey
Oil_con*plankton	1	0	2	1	-271.90	66.6076	6	-4.08	0.0065	Tukey
Oil_con*plankton	1	1	2	0	-33.9450	66.6076	6	-0.51	0.6285	Tukey
Oil_con*plankton	1	1	2	1	-212.60	66.6076	6	-3.19	0.0188	Tukey
Oil_con*plankton	2	0	2	1	-178.66	66.6076	6	-2.68	0.0364	Tukey

Differences of Least Squares Means

Effect	Oil_con	plankton	_Oil_con	_plankton	Adj P
plankton		0		1	0.0388
Oil_con	0		1		0.4454
Oil_con	0		2		0.0093
Oil_con	1		2		0.0402
Oil_con*plankton	0	0	0	1	0.9057
Oil_con*plankton	0	0	1	0	0.9126
Oil_con*plankton	0	0	1	1	0.4978
Oil_con*plankton	0	0	2	0	0.2955
Oil_con*plankton	0	0	2	1	0.0171
Oil_con*plankton	0	1	1	0	1.0000
Oil_con*plankton	0	1	1	1	0.9413
Oil_con*plankton	0	1	2	0	0.7386

filter
alkanes

The Mixed Procedure

Differences of Least Squares Means					
Effect	**Oil_con**	**plankton**	**_Oil_con**	**_plankton**	**Adj P**
Oil_con*plankton	0	1	2	1	0.0459
Oil_con*plankton	1	0	1	1	0.9358
Oil_con*plankton	1	0	2	0	0.7281
Oil_con*plankton	1	0	2	1	0.0449
Oil_con*plankton	1	1	2	0	0.9937
Oil_con*plankton	1	1	2	1	0.1182
Oil_con*plankton	2	0	2	1	0.2092

filter
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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	236.51	27.1924	A
2		0	135.21	27.1924	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		308.22	33.3038	A
4	1		155.30	33.3038	B
5	0		94.0725	33.3038	B

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	1	397.55	47.0987	A
7	2	0	218.89	47.0987	AB
8	1	1	184.95	47.0987	AB
9	0	1	127.05	47.0987	B
10	1	0	125.65	47.0987	B
11	0	0	61.1000	47.0987	B

filter
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The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	aro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	605552

filter
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The Mixed Procedure

Fit Statistics	
-2 Res Log Likelihood	101.1
AIC (Smaller is Better)	103.1
AICC (Smaller is Better)	104.1
BIC (Smaller is Better)	102.9

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	0.66	0.4474
Oil_con	2	6	86.80	<.0001
Oil_con*plankton	2	6	0.03	0.9663

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	3087.83	317.69	6	9.72	<.0001
plankton		1	3453.00	317.69	6	10.87	<.0001
Oil_con	0		150.75	389.09	6	0.39	0.7118
Oil_con	1		2413.25	389.09	6	6.20	0.0008
Oil_con	2		7247.25	389.09	6	18.63	<.0001
Oil_con*plankton	0	0	32.5000	550.25	6	0.06	0.9548
Oil_con*plankton	0	1	269.00	550.25	6	0.49	0.6423
Oil_con*plankton	1	0	2152.50	550.25	6	3.91	0.0079
Oil_con*plankton	1	1	2674.00	550.25	6	4.86	0.0028
Oil_con*plankton	2	0	7078.50	550.25	6	12.86	<.0001
Oil_con*plankton	2	1	7416.00	550.25	6	13.48	<.0001

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
plankton		0		1	-365.17	449.28	6	-0.81	0.4474	Tukey
Oil_con	0		1		-2262.50	550.25	6	-4.11	0.0063	Tukey
Oil_con	0		2		-7096.50	550.25	6	-12.90	<.0001	Tukey

filter
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The Mixed Procedure

Differences of Least Squares Means										
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Oil_con	1		2		-4834.00	550.25	6	-8.79	0.0001	Tukey
Oil_con*plankton	0	0	0	1	-236.50	778.17	6	-0.30	0.7715	Tukey
Oil_con*plankton	0	0	1	0	-2120.00	778.17	6	-2.72	0.0344	Tukey
Oil_con*plankton	0	0	1	1	-2641.50	778.17	6	-3.39	0.0146	Tukey
Oil_con*plankton	0	0	2	0	-7046.00	778.17	6	-9.05	0.0001	Tukey
Oil_con*plankton	0	0	2	1	-7383.50	778.17	6	-9.49	<.0001	Tukey
Oil_con*plankton	0	1	1	0	-1883.50	778.17	6	-2.42	0.0518	Tukey
Oil_con*plankton	0	1	1	1	-2405.00	778.17	6	-3.09	0.0214	Tukey
Oil_con*plankton	0	1	2	0	-6809.50	778.17	6	-8.75	0.0001	Tukey
Oil_con*plankton	0	1	2	1	-7147.00	778.17	6	-9.18	<.0001	Tukey
Oil_con*plankton	1	0	1	1	-521.50	778.17	6	-0.67	0.5277	Tukey
Oil_con*plankton	1	0	2	0	-4926.00	778.17	6	-6.33	0.0007	Tukey
Oil_con*plankton	1	0	2	1	-5263.50	778.17	6	-6.76	0.0005	Tukey
Oil_con*plankton	1	1	2	0	-4404.50	778.17	6	-5.66	0.0013	Tukey
Oil_con*plankton	1	1	2	1	-4742.00	778.17	6	-6.09	0.0009	Tukey
Oil_con*plankton	2	0	2	1	-337.50	778.17	6	-0.43	0.6797	Tukey

Differences of Least Squares Means					
Effect	Oil_con	plankton	_Oil_con	_plankton	Adj P
plankton		0		1	0.4474
Oil_con	0		1		0.0148
Oil_con	0		2		<.0001
Oil_con	1		2		0.0003
Oil_con*plankton	0	0	0	1	0.9994
Oil_con*plankton	0	0	1	0	0.1996
Oil_con*plankton	0	0	1	1	0.0943
Oil_con*plankton	0	0	2	0	0.0008
Oil_con*plankton	0	0	2	1	0.0006
Oil_con*plankton	0	1	1	0	0.2794
Oil_con*plankton	0	1	1	1	0.1324
Oil_con*plankton	0	1	2	0	0.0010

filter
aromatics

The Mixed Procedure

Differences of Least Squares Means					
Effect	Oil_con	plankton	_Oil_con	_plankton	Adj P
Oil_con*plankton	0	1	2	1	0.0007
Oil_con*plankton	1	0	1	1	0.9792
Oil_con*plankton	1	0	2	0	0.0056
Oil_con*plankton	1	0	2	1	0.0039
Oil_con*plankton	1	1	2	0	0.0098
Oil_con*plankton	1	1	2	1	0.0068
Oil_con*plankton	2	0	2	1	0.9970

filter
aromatics

Effect=plankton   Method=Tukey(P<.05)   Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	3453.00	317.69	A
2		0	3087.83	317.69	A

Effect=Oil_con   Method=Tukey(P<.05)   Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		7247.25	389.09	A
4	1		2413.25	389.09	B
5	0		150.75	389.09	C

Effect=Oil_con*plankton   Method=Tukey(P<.05)   Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	1	7416.00	550.25	A
7	2	0	7078.50	550.25	A
8	1	1	2674.00	550.25	B
9	1	0	2152.50	550.25	B
10	0	1	269.00	550.25	B
11	0	0	32.5000	550.25	B

filter

Obs	Oil_con	plankton	Alk	aro	logAlk	logaro
1	0	0	70.66	34	4.25788	3.52636
2	0	0	51.54	31	3.94236	3.43399
3	0	1	230.52	210	5.44034	5.34711
4	0	1	23.57	328	3.15997	5.79301
5	1	0	108.23	1712	4.68426	7.44542
6	1	0	143.06	2593	4.96326	7.86057
7	1	1	230.19	3034	5.43891	8.01764
8	1	1	139.70	2314	4.93950	7.74673
9	2	0	231.51	5861	5.44462	8.67608
10	2	0	206.27	8296	5.32919	9.02353
11	2	1	396.29	7501	5.98215	8.92279
12	2	1	398.81	7331	5.98849	8.89987

filter
alkanes

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logAlk
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.4700

Fit Statistics	
-2 Res Log Likelihood	16.7
AIC (Smaller is Better)	18.7
AICC (Smaller is Better)	19.7
BIC (Smaller is Better)	18.4

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	0.96	0.3649
Oil_con	2	6	4.71	0.0589
Oil_con*plankton	2	6	0.09	0.9194

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	4.7703	0.2799	6	17.04	<.0001
plankton		1	5.1582	0.2799	6	18.43	<.0001
Oil_con	0		4.2001	0.3428	6	12.25	<.0001
Oil_con	1		5.0065	0.3428	6	14.61	<.0001
Oil_con	2		5.6861	0.3428	6	16.59	<.0001
Oil_con*plankton	0	0	4.1001	0.4848	6	8.46	0.0001
Oil_con*plankton	0	1	4.3002	0.4848	6	8.87	0.0001
Oil_con*plankton	1	0	4.8238	0.4848	6	9.95	<.0001
Oil_con*plankton	1	1	5.1892	0.4848	6	10.70	<.0001
Oil_con*plankton	2	0	5.3869	0.4848	6	11.11	<.0001
Oil_con*plankton	2	1	5.9853	0.4848	6	12.35	<.0001

Differences of Least Squares Means											
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
plankton		0		1	-0.3880	0.3958	6	-0.98	0.3649	Tukey	0.3649
Oil_con	0		1		-0.8063	0.4848	6	-1.66	0.1473	Tukey	0.2928
Oil_con	0		2		-1.4860	0.4848	6	-3.07	0.0221	Tukey	0.0502
Oil_con	1		2		-0.6796	0.4848	6	-1.40	0.2105	Tukey	0.3977
Oil_con*plankton	0	0	0	1	-0.2000	0.6856	6	-0.29	0.7803	Tukey	0.9995
Oil_con*plankton	0	0	1	0	-0.7236	0.6856	6	-1.06	0.3318	Tukey	0.8827
Oil_con*plankton	0	0	1	1	-1.0891	0.6856	6	-1.59	0.1633	Tukey	0.6319
Oil_con*plankton	0	0	2	0	-1.2868	0.6856	6	-1.88	0.1096	Tukey	0.4896
Oil_con*plankton	0	0	2	1	-1.8852	0.6856	6	-2.75	0.0333	Tukey	0.1940
Oil_con*plankton	0	1	1	0	-0.5236	0.6856	6	-0.76	0.4740	Tukey	0.9645
Oil_con*plankton	0	1	1	1	-0.8890	0.6856	6	-1.30	0.2423	Tukey	0.7788
Oil_con*plankton	0	1	2	0	-1.0867	0.6856	6	-1.59	0.1640	Tukey	0.6336
Oil_con*plankton	0	1	2	1	-1.6852	0.6856	6	-2.46	0.0493	Tukey	0.2682
Oil_con*plankton	1	0	1	1	-0.3654	0.6856	6	-0.53	0.6132	Tukey	0.9923
Oil_con*plankton	1	0	2	0	-0.5631	0.6856	6	-0.82	0.4428	Tukey	0.9527
Oil_con*plankton	1	0	2	1	-1.1616	0.6856	6	-1.69	0.1412	Tukey	0.5782
Oil_con*plankton	1	1	2	0	-0.1977	0.6856	6	-0.29	0.7828	Tukey	0.9996
Oil_con*plankton	1	1	2	1	-0.7961	0.6856	6	-1.16	0.2897	Tukey	0.8404
Oil_con*plankton	2	0	2	1	-0.5984	0.6856	6	-0.87	0.4163	Tukey	0.9403

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Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	5.1582	0.2799	A
2		0	4.7703	0.2799	A

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		5.6861	0.3428	A
4	1		5.0065	0.3428	A
5	0		4.2001	0.3428	A

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	1	5.9853	0.4848	A
7	2	0	5.3869	0.4848	A
8	1	1	5.1892	0.4848	A
9	1	0	4.8238	0.4848	A
10	0	1	4.3002	0.4848	A
11	0	0	4.1001	0.4848	A

filter
aromatics

The Mixed Procedure

Model Information	
Data Set	WORK.PLANK
Dependent Variable	logaro
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
Oil_con	3	0 1 2
plankton	2	0 1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	12

Number of Observations	
Number of Observations Read	12
Number of Observations Used	12
Number of Observations Not Used	0

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.04786

Fit Statistics	
-2 Res Log Likelihood	2.9
AIC (Smaller is Better)	4.9
AICC (Smaller is Better)	5.9
BIC (Smaller is Better)	4.7

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
plankton	1	6	39.47	0.0008
Oil_con	2	6	427.92	<.0001
Oil_con*plankton	2	6	26.48	0.0011

Least Squares Means							
Effect	Oil_con	plankton	Estimate	Standard Error	DF	t Value	Pr > |t|
plankton		0	6.6610	0.08931	6	74.58	<.0001
plankton		1	7.4545	0.08931	6	83.46	<.0001
Oil_con	0		4.5251	0.1094	6	41.37	<.0001
Oil_con	1		7.7676	0.1094	6	71.01	<.0001
Oil_con	2		8.8806	0.1094	6	81.18	<.0001
Oil_con*plankton	0	0	3.4802	0.1547	6	22.50	<.0001
Oil_con*plankton	0	1	5.5701	0.1547	6	36.01	<.0001
Oil_con*plankton	1	0	7.6530	0.1547	6	49.47	<.0001
Oil_con*plankton	1	1	7.8822	0.1547	6	50.95	<.0001
Oil_con*plankton	2	0	8.8498	0.1547	6	57.21	<.0001
Oil_con*plankton	2	1	8.9113	0.1547	6	57.60	<.0001

Differences of Least Squares Means											
Effect	Oil_con	plankton	_Oil_con	_plankton	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
plankton		0		1	-0.7935	0.1263	6	-6.28	0.0008	Tukey	0.0008
Oil_con	0		1		-3.2425	0.1547	6	-20.96	<.0001	Tukey	<.0001
Oil_con	0		2		-4.3554	0.1547	6	-28.15	<.0001	Tukey	<.0001
Oil_con	1		2		-1.1130	0.1547	6	-7.19	0.0004	Tukey	0.0009
Oil_con*plankton	0	0	0	1	-2.0899	0.2188	6	-9.55	<.0001	Tukey	0.0006
Oil_con*plankton	0	0	1	0	-4.1728	0.2188	6	-19.07	<.0001	Tukey	<.0001
Oil_con*plankton	0	0	1	1	-4.4020	0.2188	6	-20.12	<.0001	Tukey	<.0001
Oil_con*plankton	0	0	2	0	-5.3696	0.2188	6	-24.54	<.0001	Tukey	<.0001
Oil_con*plankton	0	0	2	1	-5.4312	0.2188	6	-24.83	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	1	0	-2.0829	0.2188	6	-9.52	<.0001	Tukey	0.0006
Oil_con*plankton	0	1	1	1	-2.3121	0.2188	6	-10.57	<.0001	Tukey	0.0003
Oil_con*plankton	0	1	2	0	-3.2797	0.2188	6	-14.99	<.0001	Tukey	<.0001
Oil_con*plankton	0	1	2	1	-3.3413	0.2188	6	-15.27	<.0001	Tukey	<.0001
Oil_con*plankton	1	0	1	1	-0.2292	0.2188	6	-1.05	0.3352	Tukey	0.8856
Oil_con*plankton	1	0	2	0	-1.1968	0.2188	6	-5.47	0.0016	Tukey	0.0116
Oil_con*plankton	1	0	2	1	-1.2583	0.2188	6	-5.75	0.0012	Tukey	0.0090
Oil_con*plankton	1	1	2	0	-0.9676	0.2188	6	-4.42	0.0045	Tukey	0.0316
Oil_con*plankton	1	1	2	1	-1.0291	0.2188	6	-4.70	0.0033	Tukey	0.0239
Oil_con*plankton	2	0	2	1	-0.06153	0.2188	6	-0.28	0.7880	Tukey	0.9996

filter aromatics

Effect=plankton Method=Tukey(P<.05) Set=1

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
1		1	7.4545	0.08931	A
2		0	6.6610	0.08931	B

Effect=Oil_con Method=Tukey(P<.05) Set=2

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
3	2		8.8806	0.1094	A
4	1		7.7676	0.1094	B
5	0		4.5251	0.1094	C

Effect=Oil_con*plankton Method=Tukey(P<.05) Set=3

Obs	Oil_con	plankton	Estimate	Standard Error	Letter Group
6	2	1	8.9113	0.1547	A
7	2	0	8.8498	0.1547	A
8	1	1	7.8822	0.1547	B
9	1	0	7.6530	0.1547	B
10	0	1	5.5701	0.1547	C
11	0	0	3.4802	0.1547	D

C. COMPOUNDS ANALYZED

Naphthalene-d8 IS #1
nC-10 Decane
nC-11 Undecane
nC-12 Dodecane
nC-13 Tridecane
nC-14 Tetradecane
Naphthalene
C1-Naphthalenes
C2-Naphthalenes
C3-Naphthalenes
C4-Naphthalenes
Acenaphthene-d10 IS #2
nC-15 Pentadecane
nC-16 Hexadecane
nC-17 Heptadecane
Pristane
nC-18 Octadecane
Phytane
nC-19 Nonadecane
nC-20 Eicosane
nC-21 Heneicosane
nC-22 Docosane
nC-23 Tricosane
nC-24 Tetracosane
Fluorene
C1-Fluorenes
C2-Fluorenes
C3- Fluorenes
Dibenzothiophene
C1-Dibenzothiophenes
C2-Dibenzothiophenes
C3- Dibenzothiophenes
Phenanthrene
C1-Phenanthrenes
C2-Phenanthrenes
C3-Phenanthrenes
C4-Phenanthrenes
Anthracene
Phenanthrene-d10 SS #1
5-alpha Androstane SS #2
Chrysene-d12 IS #3

nC-25 Pentacosane
nC-26 Hexacosane
nC-27 Heptacosane
nC-28 Octacosane
nC-29 Nonacosane
Fluoranthene
Pyrene
C1- Pyrenes
C2- Pyrenes
C3- Pyrenes
C4- Pyrenes
Naphthobenzothiophene
C-1 Naphthobenzothiophenes
C-2 Naphthobenzothiophenes
C-3 Naphthobenzothiophenes
Benzo (a) Anthracene
Chrysene
C1- Chrysenes
C2- Chrysenes
C3- Chrysenes
C4- Chrysenes
Perylene-d12 IS #4
nC-30 Triacontane
nC-31 Hentriacontane
nC-32 Dotriacontane
nC-33 Tritriacontane
nC-34 Tetratriacontane
nC-35 Pentatriacontane
Benzo (b) Fluoranthene
Benzo (k) Fluoranthene
Benzo (e) Pyrene
Benzo (a) Pyrene
Perylene
Indeno (1,2,3 - cd) Pyrene
Dibenzo (a,h) anthracene
Benzo (g,h,i) perylene

VITA

Erin Elizabeth Saal is a native of Gueydan, Louisiana. She received her B.S. degree at Louisiana State University in Biological Sciences in 2010. Upon graduation, she continued to work in the cell culture lab of TransGenRx where she performed clone selections and gained experience with automated cell culture systems. Thereafter, she entered graduate school in the Department of Environmental Sciences at Louisiana State University. She will receive her M.S. in December 2015.