JUVENILE CRAWFISH (*PROCAMBARUS CLARKII*) LC_{50} MORTALITY FROM SOUTH LOUISIANA CRUDE, PEANUT AND MINERAL OIL

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Abstract

Nearly every continent has species of crawfish that inhabit wetlands or are near coastal areas where petroleum and other oils are produced, transported, and accidentally released. Most crawfish cannot tolerate polluted water. Thus, they could serve as biomonitors of fossil fuel, biofuel, and other oil releases and assess potential negative impacts such oils have on wetland and coastal ecosystems.

The objectives of this study were to estimate the acute toxicity of three oils to juvenile crawfish and determine if toxicity was due to poisoning or interference with oxygen transfer across the gills. Juvenile crawfish (*Procambarus clarkii*) were tested for acute toxicity (96-h) using South Louisiana crude, peanut and mineral oils. Pairs of randomized tanks, with ten crawfish per tank or twenty per treatment concentration, were used in each experiment. Temperature (22°C±1) and standard water quality parameters were maintained constant based on ASTM protocols. The acute toxicity of South Louisiana crude oil was compared with peanut oil, a representative of biofuels and similar biologically-based oils, and mineral oil, which has a higher molecular weight and represents the heavier components of crude oil and biofuels.

Two sets of experiments showed crude oil is acutely toxic to juvenile crawfish from 50 to 100 mg/L, with 84 mg/L being the LC_{50} (median lethal concentration at 96-h). Peanut oil acute toxicity, identified by another pair of experiments, was approximately one order-of-magnitude lower (LC_{50} = 600 mg/L) than crude oil. The heavier mineral oil produced another order-of-magnitude lower mortality, with the LC_{50} exceeding 10,000 mg/L. The gills of crawfish exposed to crude oil did not appear to be coated with hydrocarbons and the mortality observed is putatively due to poisoning, Peanut oil mortality appeared to be due to poisoning plus smothering, whereas mineral oil mortality appeared to be due primarily to smothering. This work suggests possible engineering techniques to reduce the toxic effects of oils and points to toxicity considerations of biofuels. Additional laboratory and field-based studies using juvenile crawfish are needed to provide understanding of the mechanisms of acute and chronic toxicity by hydrocarbon and biologically-based oils in sensitive wetland and coastal ecosystems.

Key words: crawfish, crude oil, biofuel, toxicity, ecology, engineering

Chapter 1

Introduction

Petroleum pollution specifically the toxicity to human and aquatic organisms is the most significant problems in areas of oil and gas extraction, shipping and processing. Petroleum refers to a group of naturally occurring hydrocarbons. Even a small petroleum spill into the ocean, atmosphere or terrestrial environment may be toxic and cause great material and ecological losses. The discharge of chemicals to different compartments of the ecosphere affects the aquatic environment (Hyland et al, 1999). Petroleum discharges from tankers and the resulting environmental impacts may exceed those from the extraction process and distribution via pipelines. For example the Exxon Valdez, which went aground in 1989, spilled nearly 11million gallons of North Slope crude oil into the waters of south central Alaska. The oil spread along the coastline and covered marine life and turned hundreds of miles into an area of ecological disaster (Williams and Iatropoulos, 1999). The federal government passed the Oil Pollution Act of 1990 which established lines of responsibility and a cleanup consortium for future oil spills (Davis and Guidry, 1996). The difficulty in determining petroleum pollution in an environmental sample is based on the petroleum's composition. This shows several sources of petroleum that can be used to determine the magnitude and amount of entry of substances into any particular sector of the environment. The most visible consequences of the numerous petroleum pollution incidents in Louisiana are the loss of both plants and animal.

Presently, petroleum pollution is described by location and time. This information provides an opportunity to bring together information, such as pollution volumes, petroleum properties, petroleum pollution process rates (e.g. evaporation, spreading, drifting), and environmental conditions to predict the overall location, properties, and impact of the pollution (Banks and Brown, 2002).

Petroleum activities have generated negative environmental impacts on aquatic species, and have caused public concern throughout the world. In the study of ecological effects of petroleum pollution, several factors need to be considered, such as the type and amount of petroleum, the frequency of exposure whether acute, chronic or sub chronic toxicity type of oil fraction (light or heavy), environmental conditions (e.g., water, temperature, dissolved oxygen, NH_3, salinity and pH), use of crude oil and their associated toxicity and the sensitivity of specific biological behavior to the toxic effects of hydrocarbons (Domagalski et al., 2004).

The toxicity of petroleum depends on its chemical composition. The most toxic component of petroleum is typically the aromatic fraction. Also toxicity occurs when petroleum has entered the environment and its chemical composition begins to change. The change depends on properties such as how easily they dissolved in water. The rate of change varies with environmental conditions (Chen et al., 2002).

When petroleum pollutes the environment, it affects aquatic species by altering essential elements of their habitat. For example, Petroleum spillage in water does not quickly dilute, but tends to remain in a concentrated mass on the surface, which is only slowly changed and degraded. Thus, its most pronounced effects on aquatic organisms are on those who make use of the water surface or inhabit the shorelines.

Petroleum pollution also causes high mortality in marsh grass, mangrove, and plant communities. Algae may die off or grow more abundantly in response to petroleum, depending on conditions and on the concentration of the petroleum (Albers, 1998). Petroleum may also contribute to deep sea pollution. Most petroleum pollution enters the deep sea from spills on land or rivers during transport of petroleum.

Petroleum also seeps into the deep sea naturally through cracks in the sea floor (Jessup et al., 2000).

Wetlands are one of the nature's richest habitats providing food, water, and cover for a diversity of aquatic species. When petroleum pollution reaches wetlands it causes extensive damage to aquatic species and vegetation. The impact of petroleum pollution on wetlands is compounded by toxicity and tainting effects resulting from the chemical composition of petroleum as well as diversity and variability of biological systems and their sensitivity to petroleum pollution (Bohannon, 2002).

Rand (1995) reported that to determine the relative toxicity of a chemical on aquatic species, an acute toxicity test using a lethal concentration of the compound or chemical is often administered for 48h or 96 hours to determine $LC_{50.}$ LC_{50} is the concentration estimated to produce mortality in 50% of a test population over a specified time period.

Objectives

The goals of this study were to evaluate the acute toxicity of South Louisiana crude oil pollution to indigenous aquatic populations focusing primarily on the pollution to juvenile crawfish (*Procambarus clarkii*) and to compare to peanut and mineral oil.

1. The toxicity and mortality (LC_{50}) of Louisiana crude oil to juvenile crawfish (*Procambarus clarkii*) and record the lethal effects of the petroleum;
2. Toxicity and mortality (LC_{50}) of juvenile crawfish of peanut and mineral oil;
3. Monitoring the levels of ammonia, pH, temperature, and dissolved oxygen in water.

All tests were carried out at the Department of Biological and Agricultural Engineering, Aquacultural Engineering Laboratory in the Louisiana State University AgCenter.

Chapter 2

Literature Review

Petroleum is a major contributor to our present standard of living. The activities for finding, transporting and processing petroleum, however, impact the environment. Thus petroleum pollution is recognized as a potential environmental contaminant around the world.

The general concern over the adverse effects of petroleum pollution stimulated the development of a variety of response techniques to contain or remove petroleum pollution before it could significantly harm the environment (Breuel, 1996). Petroleum inputs into the worldwide freshwater, marshes and wetland may come from four major sources: natural seeps, and releases that occur during extraction, transportation, and consumption of petroleum (Clark, 2000). The effects of petroleum in the environment are manifold, and generally only the acute effects are understood. This is because the effect of any petroleum contaminant depends on its toxicity and the quantity released. At higher concentrations can cause acute toxicity to aquatic organisms, whereas, at lower concentrations they may cause chronic effects.

Petroleum Pollution

The effects of petroleum spills in the ocean environment depend upon several factors, such as the amount of petroleum spilled, its initial physical and chemical characteristics, the prevailing weather and sea conditions, and whether the petroleum remains at sea or comes ashore National Academies, 2002). Spilled petroleum is a complex mixture of hydrocarbons that can impact all aquatic and plant species in the affected area. Petroleum contamination often leads to public disquiet and interferes with recreational activities such as bathing and boating. Industries that rely on a clean supply of water for their normal operations are adversely affected by petroleum spills. The threat posed to aquatic species by the persistent residues of spilled petroleum and water in petroleum emulsions includes physical (Brassard, 1996). Animals may be exposed to petroleum's compounds by inhalation, direct contact with the skin, or ingestion. During the earliest stages of an oil spill, evaporation results in high concentrations of volatile compounds in the atmosphere. In addition to outright toxicity, the threat posed to aquatic species by the persistent residues of spilled petroleum and water in petroleum emulsions is one of physical smothering (Brassard, 1996).

Water Quality Issues

Water quality should be related to the anticipated beneficial use of the water, such as for aquatic species protection, drinking water, or agriculture. Also, water should be managed so that its use at one location will not be detrimental to its use at another location. Any addition to water that changes its natural state, so that the downstream user does not obtain the natural water, may be considered pollution. Analysts determine water quality by testing for specific chemicals or pollutants. The type of water determines the types of parameters being tested (Hurst, 1997). For example, EPA (2002) stated that the most important water quality variables are dissolved oxygen and temperature. Water temperature has a major influence on oxygen levels, and low dissolved oxygen causes serious problems in aquatic species. Water quality is one of the more important factors to consider. It is closely linked to water use and economic development. Water is used in industry for production and when contaminated, it becomes toxic (Ball, 2000). The complexity of water quality is reflected in biological, chemical and physical parameters

Pollution

Pollution is the release of chemical, physical, biological or radioactive contaminants to the environment. Hickey et al (2003) stated that pollution is" the contamination by man, of any natural resource but it can also be broken into different types, such as air pollution, land and water pollution''. The size and type of the source may be responsible for causing multiple forms of pollution. The interaction of pollutants can also yield complex results (Clean Ocean Foundation, 2001). Pollution can also be the consequence of a natural disaster. For example, hurricanes often cause water contamination from sewage and petrochemical spills. Larger scale environmental damage is not uncommon when coastal oil rigs or refineries are involved. Some sources of pollution, such as nuclear power plants or oil tankers, can produce widespread and potentially hazardous releases when accidents occur (Sinkule, 1995).

Accidental Petroleum Spills

Petroleum spills occur from discharges of oil from shipping, offshore extraction of petroleum and transport of petroleum in pipelines. These spills are usually a result of either accidents or normal deliberate operational discharges (Banerjee, 2003). Banerjee further reported that accidental petroleum spills are less frequent than other kinds of petroleum spills, but typically involve larger volumes of spilled petroleum compared to other kinds of petroleum spills. On the other hand, there are more oil slicks from illegal discharges of petroleum than accidental spills (Board, 1996).

Tankers

Very large petroleum spills from tankers are the most obvious and dramatic cause of acute petroleum pollution of the marine environment. Apobka (2000) reported that the largest petroleum spill was caused by Exxon Valdez, which exposed the Alaskan North Slope to gallons (tons, barrels) of crude oil. The Exxon Valdez oil spill had an acute and immediate effect on marine life and terrestrial communities. According to some estimates, accidents involving oil tankers account for about 10 percent of the annual total amount of petroleum entering worldwide ecosystems (Alongi, 2004).

Pipelines

Pipeline spills can occur due to material defects, pipe corrosion from ground erosion, tectonic movement, and damage due to ship anchors and bottom trawls.

The extent of the damage caused by pipeline spills depends on the nature of the damage, such as the size of cracks or ruptures (The Oil Daily, 2000). A pipeline can be either a source of small and long-term leakage, or from the pipeline rupturing near the bottom.

Drilling

Drilling mud pits are probably the most significant source of petroleum contamination during the drilling process. Farajat (2002) reported that if an influx of pressurized petroleum does occur during drilling, well control is maintained through the rig's blowout prevention system, which consists of a set of hydraulically operated valves and other closure devices that seal off the well and route the well bore fluids to specialized pressure controlling equipment. The main hazard of drilling spill is connected with the spills and blowout of petroleum, gas, and numerous other chemical substances and compounds entering the marine environment.

Effects of Petroleum Pollution on Aquatic Species

Petroleum spills often occur in water and aquatic species are most often affected by the petroleum contamination. Petroleum pollution in the environment can affect organisms by direct physical coating, altering essential elements of the habitat, and by the direct toxic effects of chemicals in the petroleum (National Geographic, 2000). Petroleum is an unusual pollutant because when it is spilled into water it does not quickly become diluted but remains in a concentrated mass on the surface, which only slowly changes and degrades (Peterson and Lubchenco, 2000). The effects of petroleum in water are manifold, however only the acute effects are understood and the long-term chronic effects are only slightly comprehended, if at all (Mitchell, 1999). The greatest impact arises from petroleum pollution at concentrations that are not naturally found.

Use of Crawfish (Juvenile) for Biomonitoring

Crawfish production is a part of Louisiana culture. The production of crawfish in Louisiana has increased 90% in USA and consumes 70% (McClain et al, 1998). The demand for quantity seafood, crawfish sales has increased both nationally and internationally.

The production of crawfish has attained economic importance as a commercial food product for human consumption, fish bait and laboratory organisms for biological studies. Essential metals are always found in high concentrations independently of their quantities in the environment because of the ability of crawfish to manipulate their levels for their own metabolic profit (Allinson et al, 2000). Anderson et al (1997) stated that crawfish are used as a biomonitoring of petroleum pollution because their dose and time- dependent accumulation are reflective of the levels of non-essential metals present in contaminated wetland.

Juvenile crawfish grow rapidly and reach harvestable size in three to four months, if their growing conditions are favorable (Banks and Brown, 2002). Petroleum toxicity studies have focused on adult stages of test species. Early stages of development in test species are more sensitive than adult's stages but there is exception. For example, post larval white shrimp *Litopenaeus setiferus* and brown shrimp *Penaeus aztecus* apparently are more tolerant than juveniles on exposure to fuel oil (Gerhardt and Schunidt, 2002). Crawfish species used in bio monitoring of several studies indicated that it can integrate pollutants into their body load over time (Ciarelli et al., 1997).

Madigosky et al (2003) stated that the crawfish was recorded as a fairly common species for bio monitoring studies, measurement of a DNA adducts, and providing more suitable biological environmental exposure than measurement of the parent compounds. Aquatic species and water samples were used for pollution monitoring purposes (Telisman, 1995). It is limited for the relevance of sediment measurements because its contaminants bound to the substrate by adsorption which may not be immediately uptake by aquatic species.

Welle and Kleinjans (1998) reported that concentrations on the sediment may reflect changes in the sediments rather than variations in the pollution levels of the sediments. Therefore, aquatic species should be used to monitor environmental contaminations since they will reflect the contamination of a particular location; they are exposed during their entire lifetime and are capable of accumulating these toxic substances in their tissue fractions.

Crawfish are grown throughout Louisiana with production concentrated in the south-central portion of the state. Improvements in production systems over the years through experience and application of research findings have improved yields, and today Louisiana is the top crawfish producing state in the nation (Louisiana State University AgCenter, 2005). Gerhardt (2000) reported that *Procambarus clarkii* produces more consistent yields and mate in open water in all months but mating starts in May/June. The female *Procambarus clarkii* crawfish, usually stores the spermatophore in a seminal receptacle for 2 to 8 months until spawning, while the males occupy a burrow with the family (Kirkpatrick, 2001).

Juvenile crawfish of *Procambarus clarkii* grow rapidly and obtain harvestable size in 2 to 4 months. This species hatches in late fall or mid-winter, which requires 4 to 5 months to attain harvestable size. Egg development within the female crawfish takes place over a period of 2 to 5 months depending on the temperature. Crawfish have a natural life span of no more than 2 or 3 years (McClain et al., 2001).

Toxicity of Hydrocarbon to Aquatic Organisms

Effects of petroleum pollution on various environments must be considered since it interferes With the process of coagulation, flocculation, sedimentation, filtration and increased oxygen demand (EPA, 2002). The effects on the ecosystem and its components are devastating. Fish are killed if they ingest petroleum. Petroleum is among one of the most devastating pollutants of water. Most researchers reported that the toxicity of petroleum from nominal dose concentrations rather than measured analytical concentrations of petroleum in the water. The more toxic components of petroleum for aquatic species include benzene, toluene, ethylbenzene, xylene (BTEX) and polycylic aromatic hydrocarbons (PAH); both of which have higher water solubility than heavier oil fractions. These hydrocarbon components are toxic to aquatic organisms during petroleum pollution in coastal environment. The recent studies of petroleum toxicity to aquatic species have reported that water accommodated fraction or chemically–enhanced water–accommodated fraction provide more realistic assessment (Rhoton et al, 2001).

Lethal Concentration (LC_{10}, LC_{50}, LC_{90}) Acute Toxicity

The petroleum exposure to aquatic species determines the acute toxicity and other stresses (Liu, 2003). Rhoton et al (2001) reported that LC_{50} of aquatic species exposed to Alaskan North slope crude oil with a spiked exposure to renewable petroleum was lower and more toxic than with flow– through exposure. The spiked exposure to petroleum spill is more representative of actual field conditions, then the flow-through tests tend to overestimate the toxic effects of a true petroleum spill response (Buikema et al., 2000).

Researchers (McCay et al, 2002) have reported that the acute and chronic toxicities of petroleum with a simulated refinery effluent and its components, was greater to juvenile crawfish than estuarine fish and grass shrimp. They also included that fuel oil has toxic component of the 96 hr LC50 in 0.73 mg/l. The toxicity of the mixture and the chronic exposure to 2.7% artificial refinery mixture formulation resulted in growth inhibition and reproductive impairment (Verhaar et al, 1999). Meer (2002) further reported that long-term exposure to 96 hr LC_{10} of petroleum components tested had deleterious effects on aquatic species growth. O'Connor (2000) reported that the mortality of 50% of the exposed fish like red tilapia *Oreochronics niloticus* to the toxicant of petroleum the concentration and lethal concentration of (LC_{50}) were regressive to the median lethal times and concentrations. Neff and Hightower (2004) also reported that in juvenile crawfish, about 7 to 8 ppm of total hydrocarbons caused 50% mortality in 96 hours, which is equivalent to a nominal dosing level of about 4,400ppm of crude oil concentration. The exposure time effect during the 96 hour tests appeared to supercede the observed difference in total hydrocarbon concentrations associated with differences in the nominal concentrations.

Liu (2003) reported that aquatic species such as Gulfkillfish *Fundulus grandis* and Eastern oysters *Crassostrea virginica* tested with Louisiana crude oil have survival and mortality rate that can be calculated using the equation. Liu (2003) also used variance analyses to determine if differences existed in the mortality as compared to other treatments of the petroleum exposure and organisms. Liu (2003) further reported that Louisiana crude oil concentrations on white shrimp *Penaeus setiferus* change with time at different nominal concentrations in 96-h static tests.

Mortality of Gulf killifish *Fundulus grandis* at different concentrations of Louisiana crude oil in 96h static tests were performed by Liu (2003). These revealed that 96h mortalities began at nominal concentrations of South Louisiana crude oil exceeding 100 ppm and total hydrocarbon levels exceeding 1.11 and Alaska crude oil 1.71 ppm with mortality generally range from 10 to 80%. Welle and Kleinjans (2004) reported that red tilapia (*Oreochronics.niloticus*) and *Sarotherodon melanotheron* fingerlings exposed to concentrations of refinery effluent for 24, 48, and 96 hours lethal concentration (LC_{50}) has no acute toxicity. This showed that tilapia *Sarotherodon melanotheron* is more resistant to oil refinery effluent than red tilapia (*Oreochronics.niloticus*) .

Hicky et al (2003) also reported that crawfish are sensitive to petroleum spill and as such it would be a good species for metal accumulation and detoxification. Previous studies reported that the ability of crawfish to accumulate crude oil was apparent from the fact that tissue concentrations were several times higher than water concentrations of crude oil (Evans, 2000).

Chronic Toxicity: Reproduction, Endocrine Disruption, and Effects on Immune Systems

Chronic toxicity is a function of dose and exposure duration meaning that the more toxin crawfish contact, or the longer the contact is, the more likely toxicity will occur. Lee and Lee (2000) reported that exposure of small amount of petroleum leads to chronic toxicity. Toxic effects also depend on the nature of the toxicant, and environmental conditions. The symptoms of petroleum contamination include the rupture of tissues and enlarging swim bladder, disturbance of circulatory system and other behavioral changes (Olmstead and LeBlanc, 2002). Lee et al (2001) reported that the nature of endocrine disruption seemed to lend itself well to further application as a sub-field of conservation physiology since it has, as one of its goals, elucidation of mechanisms of reproductive and developmental toxicities, which contribute to population declines. Breuel et al (1996) reported that the immune system is a place that attract of toxicant which bring an increasing awareness of the role of environmental pollutants in immune function. Immune suppression constitutes an under appreciated source of acute and chronic impacts on crawfish affected by petroleum pollution. The effects of petroleum pollution on the immune system in crawfish is to determine its timing and chronic relationship to petroleum ingestion (Morrall et al, 2003). It also reported that cell mediated immune mechanisms are more sensitive to the toxic effects of petroleum ingestion.

Toxicity Due to Effect of Coatings on Gills and Respiration

The toxic components in petroleum tend to be lost through oil spill. McLean et al (1996) reported that lethal concentrations of hydrocarbon components that lead to mortality in aquatic species are relatively rare, short lived, and sub-lethal. These effects that impair the ability of aquatic organisms to produce, grow, feed or perform other functions can be caused through prolonged exposure to a concentration of petroleum or oil components far lower than those that will cause death. Petroleum rapidly penetrates into the species through gills and disturbs the body systems such as respiration, nervous system, blood formation and enzyme activity. The occurrence of this disturbance leads to a number of common symptoms like behavioral change and lost of oxygen due to petroleum pollution (Hosmer and Warren, 1998).

Characteristics and Biological Effects on Crustaceans

The biological effects of petroleum on crustaceans influence behavioral performance and largely affect ecosystems. The crustaceans were considerably more sensitive to petroleum pollution. Ma et al (2002) reported that crawfish ability to recover normal behavior after exposure of petroleum decreases with increasing concentration and time. Crawfish *Procambarus clarkii* that were affected by hydrocarbon spill produce fewer eggs and the eggs that produced grew into malformed larvae (Hosmer et al, 1998).

Petroleum pollution often leads to chronic poisoning of crustaceans. Petroleum enters through the gills and disturbs the main functional systems such as respiration, nervous system, blood formation and enzyme activity. The external disturbance includes symptoms mainly of behavioral nature such as excitement, increased activity, and scattering in the water (Stanislav, 2000). The effects of petroleum pollution on crustaceans are largely determined by the proportion of toxic components, the duration of oil exposure as well as the degree of other stresses (NRC, 2002).

Toxicity and Different Hydrocarbon Concentrations in Water

Hydrocarbon concentrations in water change due to dissolution and evaporation, which is measured as a function of time. Hydrocarbon component interactions are important in determining the time dependence of the hydrocarbon component.

Phatarpekar (2000) reported that crude oil has higher total oil–hydrocarbon concentrations and is richer in light aliphatics and single-ring aromatics than the refined oils. The water soluble fractions and oil in dispersant water of refined oils were considerably more toxic to aquatic species, and are commonly found in environmental contaminants (Fernandez and Nuria, 2006). Toxicity associated with small concentrations tends to be larger for crude oil when compared to hydrocarbon concentrations. The more toxic components of crude oil to aquatic species include benzene, toluene, ethylbenzene, Xylene (BTEX) and polycyclic aromatic hydrocarbons (PAH) all of which has higher water solubility than heavier oil fractions. More recent research studies of oil toxicity in aquatic species have reported that the water-accommodated fractions or chemically

– enhanced water-accommodated fractions provide more realistic assessments of hydrocarbon pollution.

Diesel Range Organics

Diesel range organic are all the chromatographic that react between the n-decane (n-C10) that peak the conclusion of n – octacosane (n – C28). Diesel range organics (DRO) are light range petroleum products that include diesel fuel with petroleum hydrocarbon compounds associated to an alkane range (Neff, 2001). Organisms in coastal and estuarine environment exposed to diesel orange organics in higher concentrations are toxic and carcinogenic. Ning et al, (2003) reported that diesel range organics (DRO), free sodium sulfate, and sodium chloride in water are toxic to aquatic species.

Oil Range Organics

The indicators of these organics parameters create volatile organic compounds.

Suzuki et al (2001) reported that oil range organics are light range petroleum product with petroleum hydrocarbon compounds which associated with an alkane range from the first of n – hexane (C6) to first step of n – decane (C10) and with the boiling point ranging between 60 – 170^{0}C. The toxicity exposure of oil range organics potentially lowers the reproductive success and survival of crustaceans, thereby adversely affecting not only biotic communities but also the functioning of the ecosystems.

Polycyclic Aromatic Hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons are found in fuel oils and crude oil. Polycyclic aromatic hydrocarbons form a widespread class of environmental chemical pollutants. Crude oil and refined petroleum products are large variation in the aromatic and polycyclic aromatic hydrocarbons content but the total aromatic hydrocarbons usually in the range from 0.2-7.4% (John et al, 2004). It is associated with crude oil spill and the relative potency, only applied to the assessment of carcinogenic hazards associated with ingestion. Galassi et al (1997) reported that polycyclic aromatic hydrocarbons in some samples are undetectable; the actual levels of these compounds are above the screening levels. Samet and Dominici (2000) also reported that dissolved polycyclic aromatic hydrocarbons are toxic to aquatic species in concentrations usually ranging from 0.1 to 0.5 ppm and stated that results occasionally lie outside the range hydrocarbons have very low solubility in water and are strongly lipophilic. For example, naphthalene which has a water solubility of 30 mg/L decreases with increasing molecular weight of polycyclic aromatic hydrocarbons (Young and Wiley, 1995). Samet and Dominici (2000) reported that polycyclic aromatic hydrocarbons concentration indicates that the polycyclic aromatic hydrocarbons are likely to originate from diffusive sources rather than from a small number of discrete point sources.

OGP Publications (2002) reported that the dissolved aromatic concentration in pore water of the sediments is bio available and causes toxicity and 6 ppb dissolved aromatic concentration could cause effects on sensitive species. Barron et al (1999) stated that PAH toxicity for short-term acute exposures at 48-h LC_{50} concentrations is toxic on crustaceans. The PAH concentrations increasing trend with dose, the exposure develop time to death on crustaceans (Barron et al 1999). McCay (2004) reported that Sensitive species of crustaceans killed against volume exposed to < 1ppb dissolved aromatic concentrations for water column behavior groups.

Aliphatics

Aliphatic compounds have higher hydrogen to carbon ratios and are obtained from the crude oil degradation and also a non-aromatic organic compound (Lindgren, 1996). Branislav et al (2001) reported that aliphatic depends on the group on the chain. If it is hydrophobic groups, it will avoid water and if it is hydrophilic group it will interact with water and also reported that aliphatics toxicity depend on the concentration levels.

Aromatics

The concentrations of aromatic hydrocarbons in water are forced rapidly in the sea by dilution.

Varanasi (1999) reported that the range of concentration of aromatic substances in water depends on the nature of the reservoir and whether oil or gas condensate with high concentrations of substances in the BTEX group, which are more soluble in water than other hydrocarbons. More recent studies reported that the environmental effects of aromatic substances have been researched and a number of toxic mechanisms were obtained, mechanisms which support carcinogenicity and mutagencity. Attributes to polycyclic aromatic hydrocarbons in the aquatic habitat are limited. The experience of aquatic species by exposure of aromatic substance is low and aromatic compounds from one specific field over a period of one week identify that there is little variation in composition during time (Neff, 2001).

Other Potential Hydrocarbon/ Crude Oil Contaminants

Contamination is an exposure of toxic substance into water. A discharge of petroleum or petroleum products onto surface waters or upon the land in quantities may result in violations and affect the ecosystem. Other chemical contamination in the water are, sulfur, barium, metals and surfactants.

Sulfur

Sulfur is a major water quality problem in its role in microbial sulfate reduction and the methylation of mercury. Bates and Spiker (1999) reported that the lower redox potentials in sulfur contaminated areas, values more reducing than natural, and may adversely impact macrophyte by limiting oxygen penetration. High concentration of sulfur has two differential effects with respect to petroleum production; the stimulation through increased sulfate reduction and inhibition through buildup of excess sulfide in the water (Hayes et al., 1998).

Sulfur caused chemical changes in water in acidic sensitive ecosystems. Water affected by sulfur diminished ability to neutralize the time needed for terrestrial and aquatic ecosystems. It also degrades water quality by lowering pH levels, for example, increasing acidity.

Barium

Barium is a silvery – white metal that can be found in the environment. Barium is light and its density is half of one that oxidizes in air. It reacts vigorously with water to form hydroxide, and liberating hydrogen. If combined with other chemicals such as sulfur, carbon and oxygen and it reacts with almost all the non-metals it often forms poisonous compounds (Jaworska, et al, 2002).

Peterson et al (2000) reported that barium compounds have water-soluble qualities and can spread over a great distances in the water, affecting aquatic species that absorb it. Thus accumulating in the bodies of the aquatic species. Barium forms insoluble salts with other common components such as carbonate and sulfate.

Morrall et al (2003) reported that when barium compounds are polluted in the water and absorbed by aquatic species (crustaceans) it accumulates in their bodies and probably kills the aquatic species (crustaceans). They further stated that it is found in most land soils at low levels and these levels may be higher in coastal environment. Researchers reported that barium is a toxic compound.

Metals

Water is the largest area of exposure to chemicals. Recent studies reported that dietary exposure to metals could be major, especially in aquatic areas where metal contaminations in the water column are low, but are high in the food chain due to past contamination of sediments.

Niyogi and Wood (2003) reported that metals penetrate aquatic species in a chemical form. In the water, the actual bioavailability and toxicity depends on the form and interactions with biological receptor sites on sensitive aquatic species, for example juvenile crustaceans. Competition with natural substances in the water may minimize or eliminate bioavailability and toxicity of the metals. The acute toxicity of LC_{50} of metals to crustaceans over 96-h exposure exhibited an increased sensitivity. The rate of metals uptake in crustaceans related to concentration increases mortality with higher metal concentrations (Gale et al, 1998).

Stephen et al (2005) stated that accumulation of metals in crustacean tissues in a wetland environment contaminated by metal pollution such as zinc, cadmium and lead reflects the concentrations of metals in the sediments.

Surfactants

Surfactants are ingredients in detergents. These chemicals reduce surface tension in water and allow aqueous solutions to spread and penetrate more easily to aquatic species.

Swedmark and Granmo (2000) reported that surfactants affects aquatic species adversely, such as by altering the properties of aquatic species gills and changing the way the aquatic species takes in other substances. More recent studies reported that some surfactants are biodegradable under aerobic conditions, but many are not biodegradable under the anaerobic conditions such as those found in sewage sludge and river sediments (Swedmark and Granmo, 2000) further reported that fish react to acutely toxic concentrations of surfactants with a sequential pattern of increased activity, inactivation and immobilization. If not removed from the exposure, the fish will die. The cause of death is suffocation as a result of physical and chemical disruption of the gill epithelium.

Physicochemical Parameters

Parameters are used to measure physical, chemical, and biological properties in the water. Water quality should be related to the anticipated beneficial use of the water such as fish and wildlife protection, water should be managed so that no contamination at one location will be detrimental to its use at another location (Clesceri and Greenberg, 1998). Parameters are essential to monitor during hydrocarbon pollution in ecosystem. The organisms used as biomonitoring uses parameters for survive.

Temperature

The rate of biological and chemical processes depends on temperature. Haarstad et al (1999) reported that temperature affects the oxygen content of water. Oxygen levels become lower as temperature increases therefore photosynthesis in aquatic plants. And the metabolic rates of crustacean and the sensitivity of organism decrease. However more recent researchers reported that temperature is a fundamental factor in water quality and exerts an

enormous influence over crustacean and if the temperature of a crustacean is altered, a shift in community composition will be destroyed. Van der Oost et al. (1996) reported that cold water fish such as trout and salmon are very sensitive to temperature change when temperature increases above 20^0C, they suffer physiological stress.

Dissolved Oxygen

Dissolved oxygen is a basic requirement needed for healthy crustacean. Crustaceans suffer if dissolved oxygen concentrations are below 3 to 4 mg/l. Larvae and juvenile crawfish are more sensitive and require even higher concentrations of dissolved oxygen (Van der Oost et al, 2003).

Varanka et al (2001) reported that further exposure to low dissolved oxygen conditions can suffocate adult crustacean or reduce their productive survival by suffocating sensitive eggs and larvae. Also low dissolved oxygen concentrations favor anaerobic bacteria activity that produces noxious gases or foul odors that are associated with polluted water bodies. With the increase in temperature, the water cannot hold as much of each type of gas for oxygen concentration, the approximate saturation level at 50^0F is 11.5mg/l at 70^0F; 9mg/l and at 90^0F, 7.5mg/l, impurities added to the water further decrease these saturation levels (Williams and Iatropoulos, 2002). Williams and Iatropoulos (2002) further state that crustacean is well adapted for extracting oxygen from low concentrations found in water.

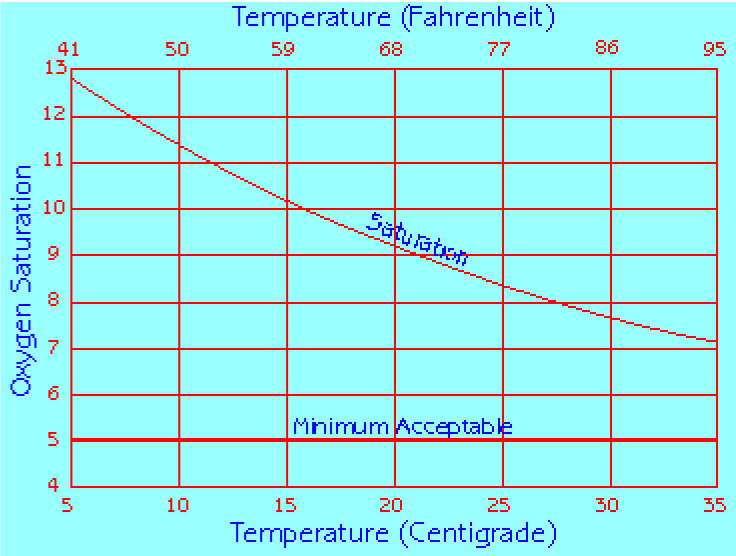


Figure 2.1: Oxygen from Temperature (Williams and Iatropoulos, 2002).

The rate of oxygen consumption is closely related to the water temperature. Crustacean metabolic activities are basically enzyme-catalyzed chemical reactions that are temperature dependent.

PH

The concentration of hydrogen and hydroxyl ions in water cause many chemical reactions to be absorbed in crustacean. The level of acidity of the water is important to the aquatic species why most aquatic species adapted to neutral conditions.

Ammonia (NH_3)

Ammonia is a nutrient required for life. One form of nitrogen found in water is ammonia. However, researchers reported that above certain concentrations it can be toxic to crustacean. At high concentrations, fish deaths occur, some fish are more sensitive to ammonia concentration, such as juvenile fish. Lawson et al (1995) reported that ammonia in water is complicated; toxicity depends on the proportion of the molecule which is un-ionized. This in turn is related to the pH and temperature of the water. Ammonia in water includes runoff of animal wastes and fertilizers from farm land, the discharge of sewage effluents, and food processing wastes, such as those from freezing works. Ammonia also comes from the bacterial breakdown and organic matter in water contains nitrogenous material (www.isb.lk/wings/clean/water_ quality, water quality, 2004).

Salinity

Salinity is a concentration of dissolved salts in the water; aquatic animals are adapted to a range at which they live. Some fish, such as sunfish cannot survive in salt water or salty conditions (Water Awareness Workshop for Colombia CLEAN Environment Team members, 2004). Salinity is measured as a ratio of salts to water and is expressed in parts per thousand (ppt), which means unit parts of salts per thousand units of water.

Tidal Influence

Tidal influence the degree of salt water intrusion and contaminant transport in unconfined coastal environment. Mao et al (2004) reported that high tidal range will reduce complicated effects on the distribution and the compositional characteristics of hydrocarbons pollution. Kauppila (2006) reported that tidal fluctuations significantly affect the hydrodynamics which potentially puts aquatic species at risk with pollution. The stimulus for migration of juveniles at the tidal limit is water temperature with weaker influences related to seasonal temperature increases.

Vegetation Influence

Aquatic species depend on vegetation for food, spawning and shelter. Vegetation is a vital moderating effect on the aquatic species. The influence of vegetation is seasonally dependent with greater impacts during petroleum pollution, when availability of surface moisture is limited (Wagner et al., 2004)

Pfeifer et al (2003) reported that vegetation physically slows runoff enhances infiltration, and takes up dissolved nutrient. The changes of parameters impact vegetation by altering the partitioning between fast and slow runoff processes.

Gerhardt et al (1999) reported that annual water balance is insensitive to total vegetation shown below. The grass versus wood composition of vegetation and the uncharacteristically low vegetation cover provided exceptions to this finding.

Niger Delta of Nigeria constitutes over 90% of Nigeria foreign exchange earning. Petroleum is refined, store and transported through these region. It is characterized by extensive interconnectivity of creeks, mangrove swamps and coastal ecosystems. Petroleum exploration and exploitation have deleterious effects on the ecosystem and biodiversity in Niger Delta areas (Odiete, 1999). Dublin-Green et al (1998) reported that overall effects of oil on ecosystem interferes with the functioning of various organs systems of plants and animals and also stated that oil on the water surface forms a layer that prevents oxygen from dissolving in water. Almost 100% of Nigeria crude oil production is located in Niger Delta region. Niger Delta of Nigeria is also a leader in commercial and recreational fisheries production and its coastal wetlands contribute 90% to the total volume of domestic fisheries harvest. These region is one of the most important oil producing areas in the country, is contaminated by crude oil spill. The effect of crude oil spill in this area has destroyed the ecosystem. Okerentugba and Ezeronye (2003) reported that Niger Delta crude oil range from 0 to 200ppm is highly toxic to crustaceans.

The testing of peanut and mineral oil represent biofuel and biodiesel as a group area of energy industry (ASTM, 1997) specification.

Chapter 3

Acute Toxicity (LC50) of South Louisiana Crude, Peanut and Mineral Oils to Juvenile Crawfish (Procambarus clarkii).

Objectives

The objectives of this study were to determine the acute toxicity (LC50) of south Louisiana crude oil (SLC), peanut oil and mineral oil to juvenile crawfish, and to assess if the oils also would cause mortality due to smothering.

Materials and Methods

Crude Oil

The juvenile crawfish *Procambarus clarkii* species also known as red swamp crawfish were tested for toxicity. They were obtained between January and May, 2007 from the Louisiana State University (LSU) Aquaculture Research Station, Baton Rouge, Louisiana, USA. The crawfish used were 30mm±6mm in total length with a mean weight of (mean ± SD) 0.82 ±0.50grams. The crawfish were randomly distributed in the tanks. The experiment was carried out at the Aquaculture Engineering Laboratory in the Department of Biological and Agricultural Engineering. The South Louisiana crude oil had high aromatic compounds with some aliphatic compounds including molecular weight of alkenes (Delaune, 2000).

The test organisms were acclimated to ambient laboratory conditions held prior to use in the exposure tests for 4 days in aerated tanks. The crawfish were randomly distributed in the tanks. They were not fed. Two liters of de-ionized water were added to two liters of crawfish pond production water (aerated for one day) before the experiment. The water quality parameters were approximately: Ammonia < 4.0ppm, pH= 7.8±0.5, dissolved oxygen = 6.5±0.5ppm and temperature = 21±5^0C and were measure according to ASTM 8010 E (1992) regulations. Mortality was recorded morning and evening and mortalities were removed and examined after 96-h of exposure. The water environmental conditions of pH, dissolved oxygen (DO), ammonia and temperature were maintained within the constant range recommended by EPA.

Water quality parameters during the toxicity test were tested by colorimetric analysis except temperature, using mercury in glass thermometer and DO, using Orion 5 star DO bench top. The temperature was controlled by using aquarium heater for constant temperature of 21 ± 5^{0}C. The data below represent the dose. Each tank has replicates and the percentage mortality was based on 10 organisms per tank with a total of 40 organisms.

Peanut Oil

Peanut oil was used as a test chemical to assess if oil viscosity is a determinant of acute toxicity. The peanut oil primarily consists of aliphatic compounds and lacks aromatic compounds. A major component of peanut oil is fatty acids, including palmitic, oleic, and linoleci. The oil also contains 6-8% arachidic, arachidonic, behenic, lignoceric and other fatty acids (Goodrum et al, 2006). It was obtained from a Wal-Mart store. The same organisms were used with 30±5mm total lengths and a mean weight of 0.63±3grams. The water parameters were ranged as the same of crude oil exposure conditions according to ASTM 8010 E, 1992 regulations. The test organisms were held for 28 or 48h for acclimation before transporting them to the aquarium tanks, which were well aerated. The experiment was monitored morning and evening. The tanks were covered with black cloth and pipe was hooked to the fan for free flow of air.

Mineral Oil

Mineral oil is a by- product in the distillation of petroleum. It is a transparent, colorless, oily liquid that is insoluble in water. It primarily contains aliphatic compounds and cyclic paraffin's which are related to white petrolatum (De Schrijver at el,1997) . This oil has been shown to in absorption of some vitamins and minerals that include Beta-carotene, calcium, phosphorus, potassium and vitamins D, E, and K(Henrik et al, 2002). Mineral oil may vary in actual composition. Red crawfish (*Procambarus clarkii*) used were measured 30±5 total lengths and a mean weight of 0.65±3mm grams.

Statistical Analysis

All Statistical analyses were conducted using descriptive analysis system to estimate the 96-h LC_{50} with 95% confidence limits for each of the three chemical toxicants for each species using both nominal dosed and nominal concentrations of the oils, peanut oil, mineral oil, and analytical measured concentration of south Louisiana crude oil, peanut oil and mineral oil. The three chemical toxicants for each graph of organisms using different nominal dosed concentrations of the oils and analytical measured concentrations of crude, peanut and mineral oils changed during 96 hour tests were weighted to the mean of the measured three chemical concentrations at 0, 24, 48,72 and 96 hours was used in the statistical analyses.

The 96 hours LC_{50} of each toxicant of the species were calculated for each definitive test separately. The standard deviation, average and cumulative average was used to determine if mortality, as determine from the 96 h LC_{50}, differed among the species, and among different chemicals within a species. The concentration levels of this study were arranged differently based on the toxicity of the oils. Water sample of each the oil were collected after adding the oil with de-ionized water and store in the refrigerator for no more than 48-hours before the analysis at environmental studies laboratory, Louisiana State University, Baton Rouge, Louisiana.

Water Analysis

De-ionized water was used and two liters of de-ionized plus two liters of pond water were added to each aquarium. Water samples were collected to determine the ammonia, dissolved oxygen, pH and concentration of hydrocarbon was analyzed. Freshwater master test kit, API was used to determine the level of ammonia and pH.

Exposure Chemicals and Test Solution

The South Louisiana crude oil was obtained from the Exxon Mobil Company, Baton Rouge, Louisiana, and was collected directly from the production lines. The oil was stored in tightly covered containers at $< 20^0$C to minimize evaporation of volatile components. The peanut and mineral oil has slight aliphatic compounds with absent of aromatic compounds. Crude oil, peanut and mineral oils were separately mixed with four liters de-ionized water and aerated for 30minutes before the animals were introduced. The oil was distributed and stirred with glass rod in their storage container prior to each use of the tank to dispense the oil components evenly. The difference in analytical results was attributed to sampling variability within the tank system. The concentration units of the analysis from aliphatic and aromatic are ug/l. Sample of water were from tanks where the concentration were as shown

Table 3.1: Aliphatic compounds, 96 hour South Louisiana Crude oil experiment of different concentration.

Aliphatic compounds	50 ppm	100 ppm	200 ppm	400 ppm	600 ppm
nC-10 Decane	285	446	503	345	685
nC-11 Undecane	385	4783	5874	6150	3735
nC-12 Dodecane	765	11606	19349	15645	11718
nC-13 Tridecane	3009	15440	24809	19180	18465
nC-14 Tetradecane	6538	15117	26042	19455	21919
nC-15 Pentadecane	12635	21195	31577	21898	27053
nC-16 Hexadecane	11592	17516	27965	20545	23538
nC-17 Heptadecane	11503	17693	29408	21814	23713
Pristane	9001	12044	16714	15033	15744
nC-18 Octadecane	11235	17077	29680	20927	23150
Phytane	7669	11120	16995	13517	16696
nC-19 Nonadecane	9172	14366	25052	17949	22479
nC-20 Eicosane	9797	14845	26276	17769	22020
nC-21 Heneicosane	8374	12573	18237	15365	16842
nC-22 Docosane	6820	10236	16488	12611	13633
nC-23 Tricosane	5468	8393	12573	10453	11484
nC-24 Tetracosane	4412	6704	10645	8142	8984
nC-25 Pentacosane	5036	7959	13187	10184	11319
nC-26 Hexacosane	4091	6656	12157	8626	9742
nC-27 Heptacosane	2568	4242	8483	5567	6119
nC-28 Octacosane	2512	3908	7698	5438	6546
nC-29 Nonacosane	1821	3333	6541	4462	5303
nC-30 Triacontane	1509	2776	5799	4118	4673
nC-31 Hentriacontane	1274	2783	5711	3757	4538
nC-32 Dotriacontane	733	1531	3026	2822	2816
nC-33 Tritriacontane	0	0	0	0	0
nC-34 Tetratriacontane	0	0	0	0	0
nC-35 Pentatriacontane	0	0	0	0	0
ALIPHATIC	138206	244340	400790	301775	332914

Table 3.2 Aromatic compounds, 96 hour South Louisiana crude oil experiment of different concentration.

Aromatic Compounds	50 ppm	100 ppm	200 ppm	400 ppm	600 ppm
Naphthalene	20	810	954	1023	575
C-1 Naphthalene	286	6132	8953	7869	6160
C-2 Naphthalene	766	12253	19504	15002	15519
C-3 Naphthalene	632	11097	18113	13446	14672
C-4 Naphthalene	289	6045	10298	6263	6139
Fluorene	282	659	874	709	821
C-1 Fluorene	338	1558	2697	1898	2125
C-2 Fluorene	306	2592	3550	2802	2950
C-3 Fluorene	151	1843	2754	2123	2335
Dibenzothiophene	452	804	1333	1019	1158
C-1 Dibenzothiophene	302	1663	2972	2026	2420
C-2 Dibenzothiophene	179	1810	3314	2084	2796
C-3 Dibenzothiophene	140	1165	1841	1184	1456
Phenanthrene	910	1743	2870	2061	2308
C-1 Phenanthrene	627	3870	6609	4613	5570
C-2 Phenanthrene	530	3399	6139	4206	4945
C-3 Phenanthrene	195	1725	2812	2066	2183
C-4 Phenanthrene	30	571	686	437	542
Anthracene	952	1824	2989	2191	2553
Fluoranthene	83	167	248	148	187
Pyrene	127	248	318	313	371
C-1 Pyrene	42	409	754	559	621
C-2 Pyrene	23	324	674	444	516
C-3 Pyrene	18	127	67	248	308
C-4 Pyrene	5	5	23	14	23
Napthobenzothiophene	58	99	179	127	226
C-1 NBT	29	64	173	43	33
C-2 NBT	10	54	173	82	49
C-3 NBT	7	9	64	14	18
Benzo (a) Anthracene	48	70	153	94	117
Chrysene	48	70	153	94	117
C-1 Chrysene	44	146	244	195	140
C-2 Chrysene	22	88	182	115	71
C-3 Chrysene	3	4	10	13	6
C-4 Chrysene	0	0	4	1	2
Benzo (b) Fluoranthene	16	19	32	16	24
Benzo (k) Fluoranthene	7	14	11	8	21
Benzo (e) Pyrene	31	51	96	79	92
Benzo (a) Pyrene	36	60	112	92	108
Perylene	33	55	104	85	100
Indeno (1,2,3 - cd) Pyrene	0	0	1	0	1
Dibenzo (a,h) anthracene	2	0	1	1	2
Benzo (g,h,i) perylene	1	2	1	0	1
AROMATIC	8080	63647	103037	75805	80379

Acute Toxicity Testing

Ninety- six hour static tests were conducted to determine the LC_{50} of South Louisiana crude oil. Lower concentrations were then tested to allow identification of sublethal concentrations. The experiments were conducted in a system of six aquarium tanks. Each tank was filled with 4 liters of de-ionized water with 2 liters of crawfish pond water. Ten crawfish were randomly assigned to each tank over an exposure period at the beginning of the experiment according to ASTM 726-96 specification. The activity patterns of the crawfish in the treatment groups relative to the control were observed in 72-hours. A 125ml of mixed solution was collected from each tank to analyze the components of the crude oil and 5ml of API test tube were used to measured the ammonia and pH level, thermometer sensor were used to measured the temperature and over an exposure period.

Each tank, the temperature was maintained at 21^0 C ±5 with one aquarium heater. The exposure concentration was randomly assigned to the tank array. The dissolved oxygen was measured with thermo Orion 5 star DO bench top equipment.

The aquarium tanks were made of glass of ten-liter volume capacity. The body size of the crawfish was consistent in the experiment and the experiment was conducted only 96-hours.

A preliminary range finding test was conducted to determine the range of oil to be used in the test. PVC pipes were constructed and hooked with fan to avoid odors of the chemicals in the laboratory.

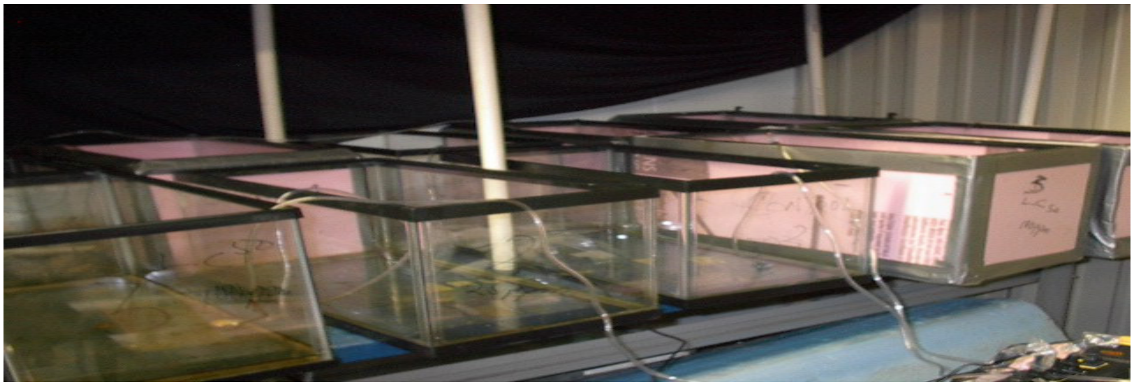


Figure 3.1 Aquarium tanks for exposing crawfish to oil. Free air was supplied to maintain sufficient dissolved oxygen for the test.

The lethal concentrations (LC_{50}) of crude oil were estimated at 96 hours acute toxicity test. Deceased animals were counted at the same time each day. Nominal doses of crude oil at 0, 25, 50. 67, 84 and 100 ppm were used for determining the LC_{50} crude oil toxicity.

Figure 3.2 PVC pipes constructed with black cloth and hooked with fan to avoid odors of the chemicals in the laboratory.

The mortality of all 10 crawfish per tank was observed at concentrations of 25ppm and no sub lethal behavioral effects were observed below 3 days. At concentrations of 25 to 100ppm, individuals exhibited lethargic behavior, 21 were severely stressed and 19 died.

The results of the crude oil exposure experiments are significantly different between tanks. Crawfish mortality was recorded after four-day period.

Water Conditions of SLC, Peanut and Mineral Oil

The water parameters, temperature, pH, ammonia and dissolved oxygen were maintained in a constant range for the entire experiment. Water quality parameters were held near constant levels as shown. Desire levels were for Ammonia 4.0mg/l, Dissolved oxygen 6 - 6.5mg/l, pH 7.6 – 7.8, Temperature 21- 22^0C for all experiment.

Table 3.3 Typical data of water quality parameters of South Louisiana crude oil experiment

Time, Days	Ammonia	DO	pH	Temp.^{o}C
0	4.0	6.5	7.8	22
1	4.0	6.5	7.6	22
1.5	4.0	6.5	7.8	22
2	4.0	6.	7.8	22.5
2.5	4.0	6.5	7.6	22
3	4.0	6.5	7.8	21.6
3.5	4.0	6.	7.7	21.5
4	4.0	6.5	7.8	22

Behavioral Effects

The effects of crude oil, peanut and mineral oil on crawfish behavior was also investigated in this experiment. The crawfish demonstrated abnormal reaction within 48-h of crude oil exposure which continued for the duration of the experiment. The crawfish exhibited behavior related to attempting to escape such as climbing the aquarium wall.

Results

Crude Oil

The 96-h LC_{50} was effectively determined for crawfish with South Louisiana Crude Oil (SLC). The parameters were nearly constant and no mortalities occurred in the control tank. The organisms began to die at crude oil concentrations of 25ppm from the second day. Mortality generally ranged from 40 to 90% at concentrations of 50 to 600ppm tested which the lethal concentration (LC_{50}) was between 100 and 200 ppm. The second experiment mortality ranged from 20 to 60% at concentrations of 25 to 100ppm. Based on nominal dose concentrations, crude oil appeared to be toxic to crawfish. The lethal concentration (LC_{50}) of 0, 25, 50, 67, 84 and 100 ppm at 96 hours is between 84 and 100 ppm. Most of the mortalities occurred within 24 – 72 hours of exposure and up to 80% mortality had occurred in 100 ppm crude oil after 72 hours exposure. So, the tolerance of crawfish to crude oil was low.

The concentration of south Louisiana Crude oil dissolved in water increased over time. The termination of the test at 96-hour, generally, the ranged was 25 to 100ppm HC. The mortality of the crawfish was higher in 72 hours.

The data below represent the dose. Each tank has replicates and the percentage mortality was based on 10 organisms per tank with total of 40 organisms.

Table 3.4 Concentration and mortality data of South Louisiana Crude Oil

Conc ppm	0	25	50	67	84	100	200	400	600
T1	0	2	3	3	5	6	8	8	9
T2	0	1	4	4	6	6	7	9	8

(Continue)

T3	0		3			5			
T4	0		4			7			
Average	0	1.5	3.5	3.5	5.5	6	7.5	8.5	8.5
Mortality %	0	15	35	35	55	60	75	85	85
Stdev	0	0.707	0.577	0.7077	0.707	0.816	0.707	0.707	0.707
Stdev %	0	7.071	5.773	7.071	7.071	8.164	7.071	7.071	7.071

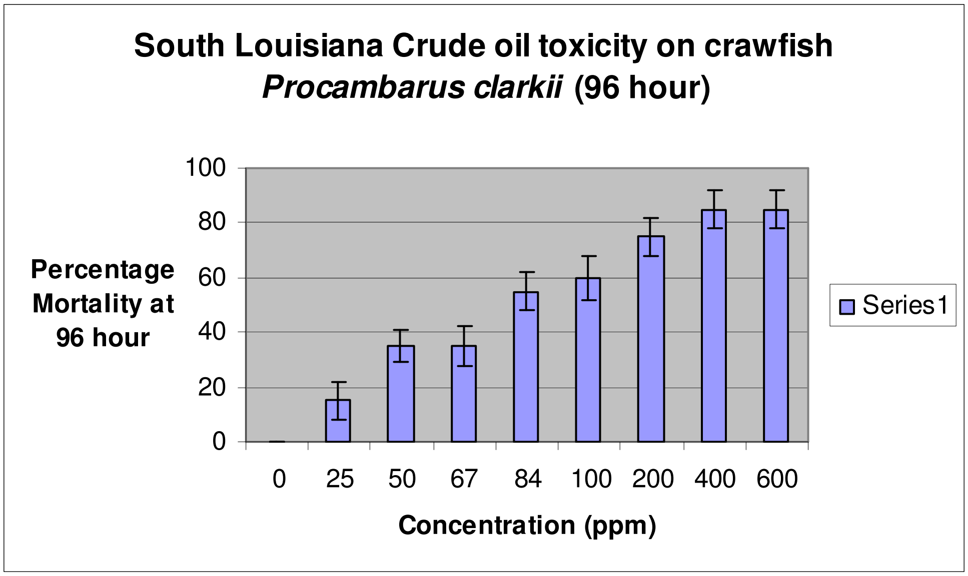


Figure 3.3 South Louisiana Crude oil toxicity on crawfish (*Procambarus clarkii*) at 96 hr

Peanut Oil

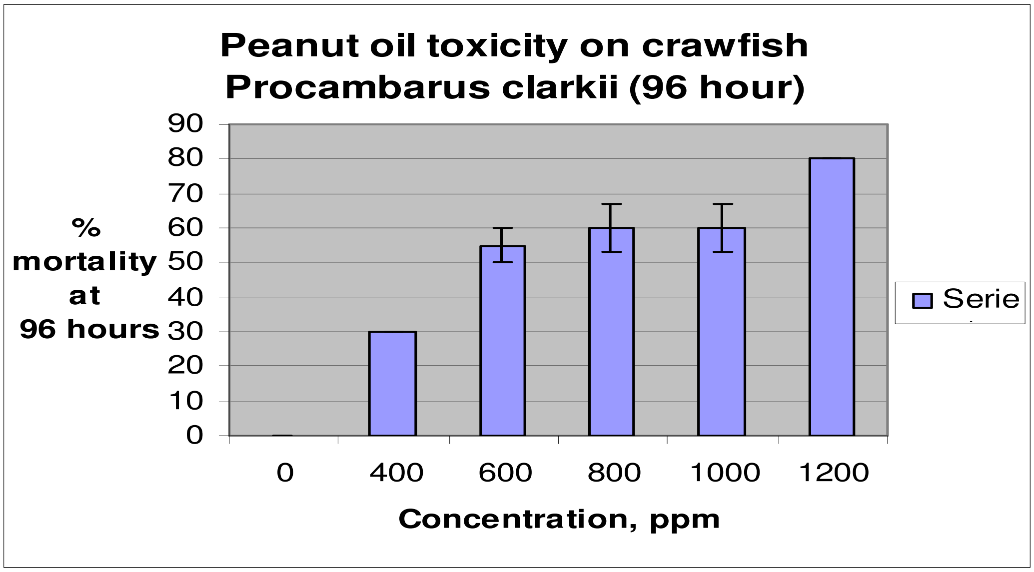
Peanut oil acute toxicity was tested at 0, 400, 600, 800, 1000 and 1200ppm. The LC_{50} mortality depends more on increasing concentrations (depending on the initial concentration) than on the length of exposure. The water quality parameters were held constant with slight variations in the parameters caused by cold weather.

The LC_{50} was approximately 600 ppm, the mortality was significantly due to suffocation. Peanut oil analysis (Appendix Table 4) shows significant low molecular weight components which could lodge in gills resulting in suffocation. However, peanuts are known to have toxic effects in some individuals, and thus, some of this effect could be due to acute toxicity. The ability of the peanut oil to react with the crawfish depends on many physical and chemical properties, rather than viscosity alone.

In this study, the observed toxic effect may have been due to the oil coating the gills and causing suffocation in the oil. In conclusion, the peanut oil is slightly toxic to P. clarkii and further studies are needed to clarify this effect.

Table 3.5 Concentration and mortality data of Peanut oil

Peanut Oil Conc ppm	0	400	600	800	1000	1200
Tank 1	0	3	5	6	6	8
Tank 2	0	3	6	6	6	8
Tank 3	0			5	5	
Tank 4	0			7	7	
Average	0	3	5.5	6	6	8
Mortality %	0	30	55	60	60	80
Stdev/2	0	0	0.5	0.707107	0.707107	0
Stdev %	0	0	5	7.071068	7.071068	0



Figure, 3.4. Peanut oil toxicity on crawfish (*Procambarus clarkii*) at 96 hr.

Mineral Oil

In this study, mineral oil did not have adverse effects on juvenile crawfish in a experiment at concentrations of 0, 400, 600, 800, 1000 and 1200ppm. In a second experiment, mortality was determined at concentrations of 0, 2000, 4000, 6000, 8000 and 10000ppm. This is the estimated concentrations of oil within a range defined by the highest concentrations tested at which no significant mortality. Nacci et al (1998) reported that acute toxicity tests do not necessarily mean that oil is not toxic to the test organisms. Exposure of the test organisms continues until the toxic response manifested at 96-h. The acute test should be allowed to continue until acute toxicity mortality or defined lethal effect will occur.

Table 3.6 Concentration and mortality data of Mineral oil

Mineral Oil						
Conc ppm	0	2000	4000	6000	8000	10000
Tank 1	0	0	1	2	2	3
T2	0	0	1	1	3	3
T3	0					
T4	0					
Average	0	0	1	1.5	2.5	3
Mortality %	0	0	10	15	25	30
Stdev/2	0	0	0	0.707107	0.707107	0
Stdev %	0	0	0	7.071068	7.071068	0

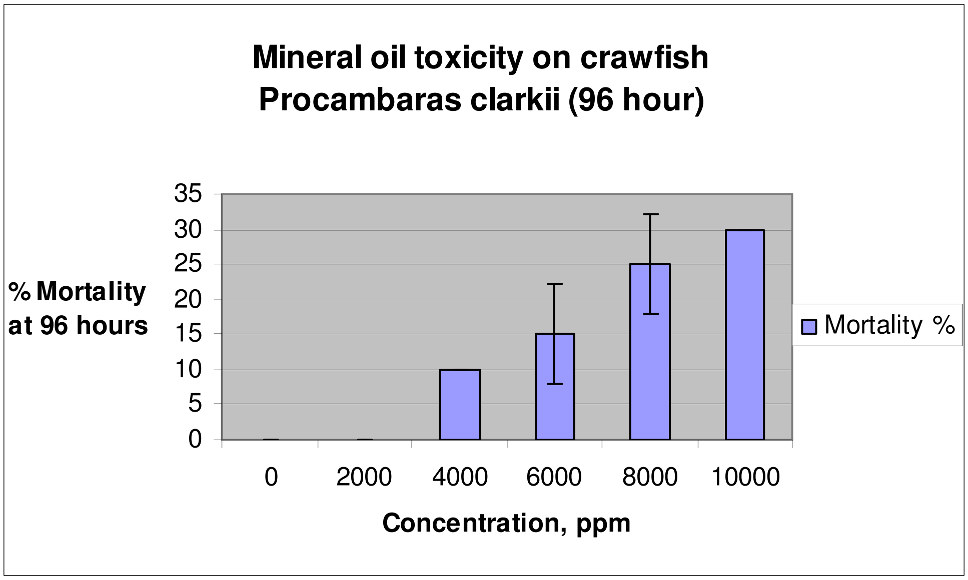


Figure 3.5. Mineral oil toxicity on crawfish (*Procambarus clarkii*) at 96 hr

Discussion

Hydrocarbon concentrations from typical Louisiana Crude Oil were determined and used in the acute toxicity analysis. The hydrocarbon concentration was linked to the toxic effect. This is because toxicity of crude oil has significantly higher concentrations of soluble hydrocarbons in solution (McKenna, 2001). South Louisiana Crude oil (LC_{50} = 84 ppm) in coastal environment is considered more toxic to juvenile crawfish than peanut (LC_{50} = 600 ppm) and mineral oil (LC_{50} = > 10000 ppm). South Louisiana Crude oil potentially exposes aquatic organisms to higher levels of toxic aromatic hydrocarbons. The levels of soluble hydrocarbons in South Louisiana crude oil within 24-h indicated higher levels of volatile and soluble hydrocarbons (Rhoton et al, 2001). Toxicity estimates from other research studies were compared with an index of toxicity to estimate 24-h LC_{50} values (Boehm et al, 2001). The LC_{50} values of SLC in these studies, crawfish generally reflect relatively high toxicity of this chemical. Red swamp crawfish are most likely to be exposed to lethal concentrations only in the event of a localized spill in the environment. Most studies reported that water solubility fraction of hydrocarbon was less than 10ppm for heavy oils (Gerhardt, A., 1999), while concentration tested in this study ranged from 0 to 1200 ppm. Previous work suggested that LC_{50} should be used to identify the effects of low concentrations and dosing levels of chemical components. The toxicity of material that expresses a loading value has little value expect for the different kinds of preparations that can be identified (Singer et al, 1998).

In this study, it was observed that crude oil was toxic. When the crude oil was exposed to the aquarium tank, much of the oil was floating on the surface of the water. One hypothesis was that toxicity may have been confounded by oxygen reduction. The significance of hydrocarbon levels in SLC was likely attributed to the evaporation of volatile components. High levels of SLC potentially expose aquatic organisms to higher levels of toxic aromatic hydrocarbons.

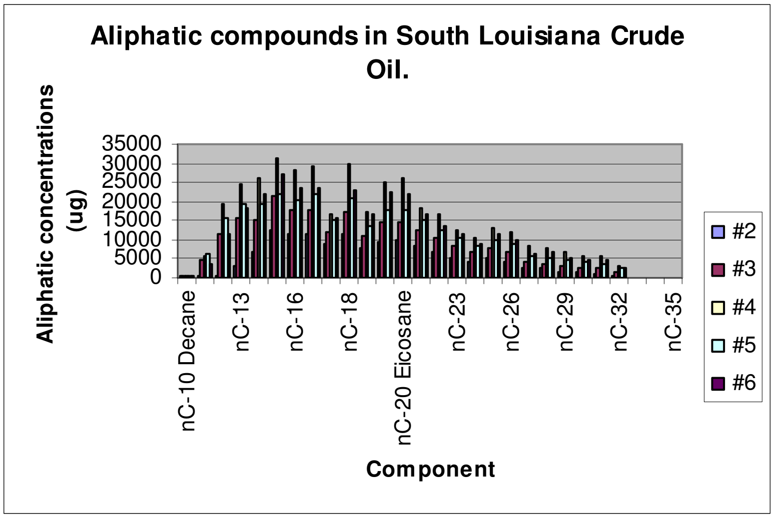


Figure 3.6 Aliphatic compounds concentrations in South Louisiana Crude oil.

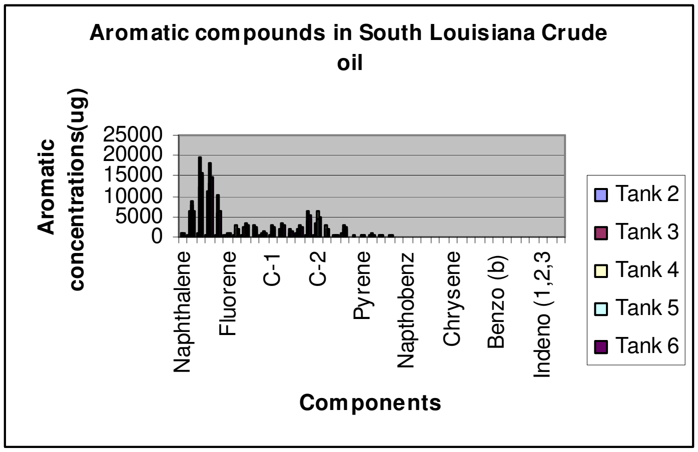


Figure 3.7 Aromatic compounds concentration in South Louisiana Crude oil.

Peanut oil is predominantly used in food applications. It is less toxic and eliminates emissions and solvent waste. Goodrum, 2000, reported that peanut oil extracted with supercritical CO_2 could be used as a sustainable alternative to diesel engine fuel. The oil is an organic oil derived from peanuts noted to have a slight aroma and taste of its parents legume (Muir et al, 1998). Kritchevsky et al (1998) reported that oil is sensitive to contamination by ferrous, rust particles and water. Peanut oil is insoluble in water. Hence contact of water give rise to soluble lower fatty acids and glycerol which causes rancidity together with changes in color and odor that may cause mortality to test organisms. The laboratory analysis (Appendix table 4) indicated that peanut oil lack aromatic compounds that cause toxicity to organisms. Most recent research reported that peanut oil belongs to the class which respiration processes are suspended to test organisms in a higher dose concentration. Peanut oil has a particular density (0.9 g/cm^3) with a rise in temperature which density diminishes by leading the same time increase in volume.

In this experiment, mineral oil has considered to be less toxic and higher. The oil acts as a lipid solvent; it interfering with absorption of essential fat- soluble substances (Raj et al, 2000). Singer et al (1998) reported that possible hazards associated with mineral oil include impurities such as polycyclic aromatic hydrocarbons and poly nuclear aromatic compounds which have been detected in samples of mineral oil for scientific research. Eversole et al (1997) reported that mineral oil was absorbed to a limited extent from the intestinal tract and stable emulsions of oil that penetrates more effectively than doe's non-emulsified oil.

In this study, the toxicity of mineral oil to juvenile crawfish increased with higher concentration level but had low effects in this study compared with other oils tested. The test organism tolerated the maximum test dose of the mineral oil. Since there was no sign of toxicity or mortality of the test organisms at lowest concentrations dose of 0, 400, 600, 800 1000 and 1200ppm no LC_{50} values could determined. The test organisms were given repeated higher doses of 0, 2000, 4000, 8000 and 10000 of LC_{50} of 96-h also suggested the LC_{50} at 96 h is above 10000 ppm according to ASTM 726-96 specification. See appendix for peanut and mineral oil analysis.

Chapter 4

Conclusions

Louisiana has 40% of the nation's coastal wetlands making it number one in the USA for coastal ecosystems, riverine and estuarine. There is a large amount of petroleum production which is refined, stored and transported through the state. Louisiana contributed a significant percentage of USA crude oil production and most of the crude oil from other areas of the country passes through the coastal area of Louisiana.

The objective of this study was to determine the toxicity of South Louisiana crude, peanut and mineral oil of (LC_{50}) mortality to juvenile crawfish *Procambarus clarkii* and record the lethal effects of the crude, peanut and mineral oil, also to determine the levels of ammonia, pH, temperature and dissolved oxygen in water affected by pollution of oils. The south Louisiana crude oil was found to be highly toxic in freshwater (LC_{50} = 84 ppm).

The acute effects on red swamp crawfish *Procambarus clarkii* introduced in South Louisiana Crude, peanut oil (LC_{50} = 600 ppm) and mineral oil(LC_{50} > 10000 ppm) were evaluated using toxicity tests.

The south Louisiana crude oil was more toxic than peanut oil and mineral oil. The static non-renewal exposure was used to evaluate the lethal effect of three chemicals: South Louisiana Crude, peanut and Mineral oil to each of the test organisms over an exposure of 96 –h in the laboratory. Control was used without oil for exposure of organisms (De-ionized water was used only) and five toxicant concentrations for each test were used. The solutions were made by exposing the chemicals directly in the exposure water. The water quality was monitored morning and evening. The crude oil, peanut oil and mineral oil were analyzed at Environmental studies laboratory in coastal energy building, Louisiana state university. Baton Rouge, Louisiana. The 96-h LC_{50} were determined for each three chemical toxicants for each of the test organisms. The mean LC_{50} of Hydrocarbon SLC was 67 and 84 ppm. The results of this study showed that sub lethal exposure to small concentrations of South Louisiana crude oil can greatly reduce the performance of the test organisms.

In peanut oil and mineral oil mortality was positively correlated with increasing levels of concentrations to determine the LC_{50} of the chemicals. The LC_{50} values of mineral oil in this study on crawfish generally reflect the relatively low toxicity (LC_{50} >10000 ppm) , while peanut oil, possibly due to lower molecular weight components was moderately toxic (LC_{50} = 600 ppm). Changes in behavior have implications for chronic exposure. The average mortalities in all control experiments were less than 5% which showed that the short-term environmental condition were suitable for testing organisms during the toxicity test. The findings of these laboratory studies showed that short term exposure of South Louisiana crude oil has an acute toxic effect on crawfish but the peanut and mineral oil have lower acute toxicity on crawfish. Future work should differentiate toxicity from suffocation; develop techniques to reduce toxicity, study mechanisms of toxicity/asphyxiation and long-term effects.

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Appendix: **Raw Data**

Appendix Table 1, Mortality of Crawfish (*Procambarus clarkii*) at different concentrations (ppm) of South Louisiana Crude oil (SLC) in 96-h non- renewal tests.

	No.Org.			Cumulative Mortality			
Conc(ppm)	0hr	12hr	24hr	48hr	72hr	96hr	% Mortality
0	0	0	0	0	0	0	0
25	0	1	1	0	0	0	20
50	0	0	0	0	3	0	30
67	0	0	1	0	2	0	30
84	0	0	2	0	1	2	50
100	0	1	2	0	3	0	60

		No. Org.			Cumulative Mortality		
Conc.(ppm)	0hr	12hr	24hr	48hr	72hr	96hr	% Mortality
0	0	0	0	0	0	0	0
50	0	1	2	0	1	0	40
100	0	0	2	2	2	0	60
200	0	1	2	1	4	0	80
400	0	1	3	2	2	0	80
600	0	2	5	1	0	1	90

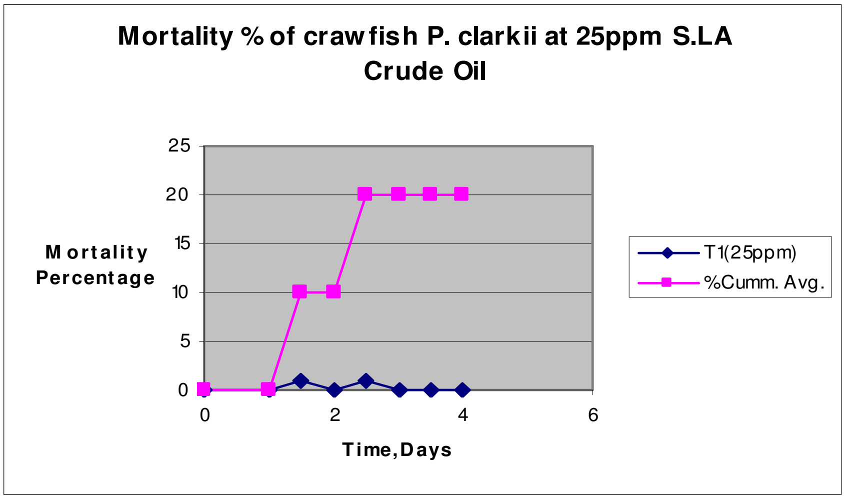
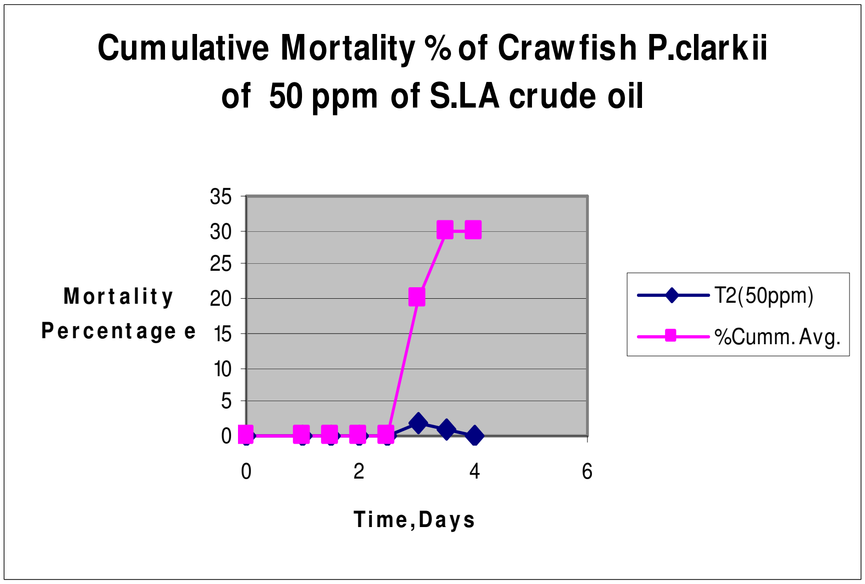
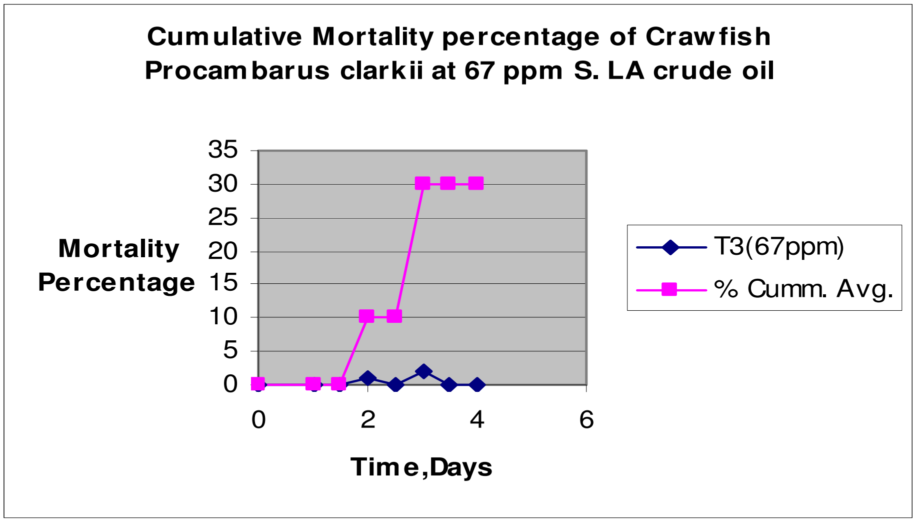


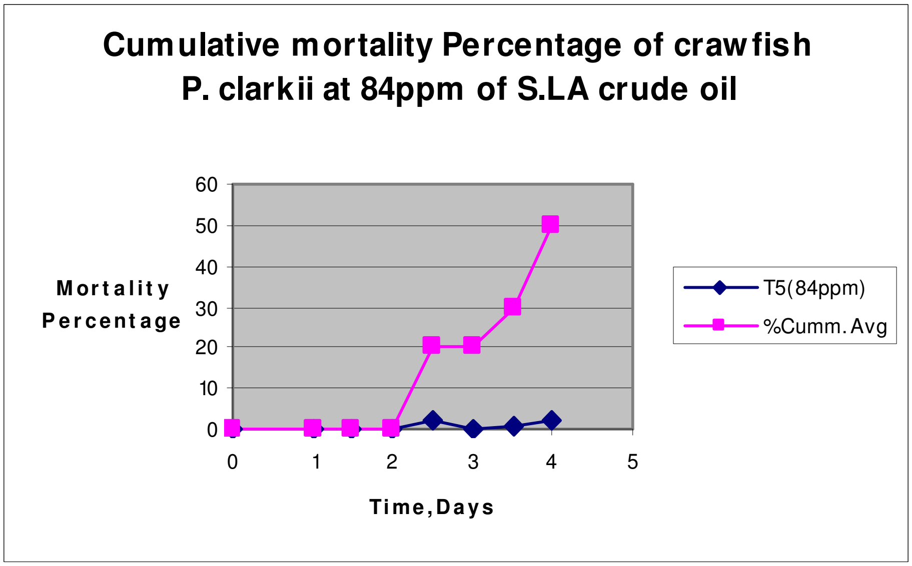
Figure Cumulative mortality percentage (%) of South Louisiana crude oil of 25ppm at 96 hr



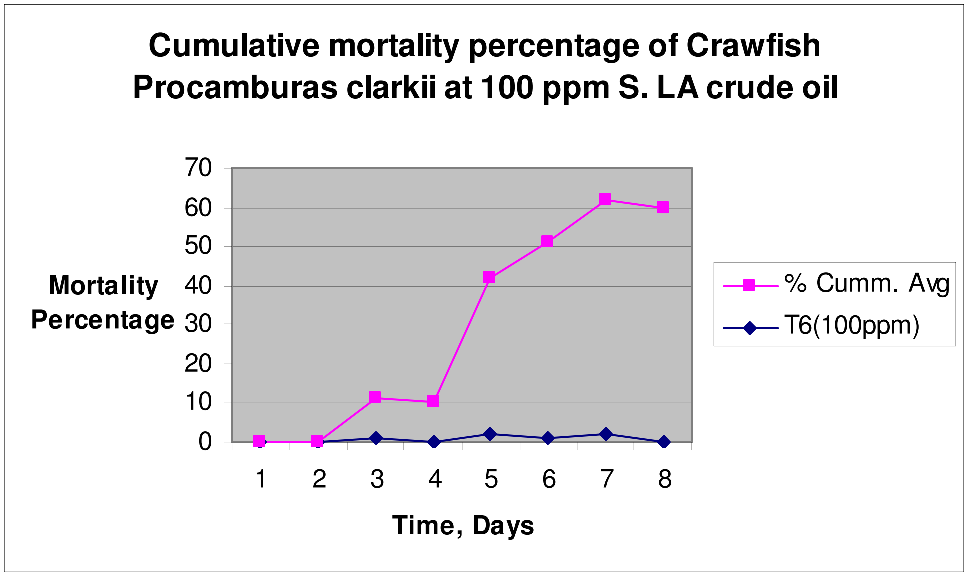
Appendix figure 1: Cumulative mortality percentage (%) of South Louisiana crude oil of 50ppm at 96 hr.



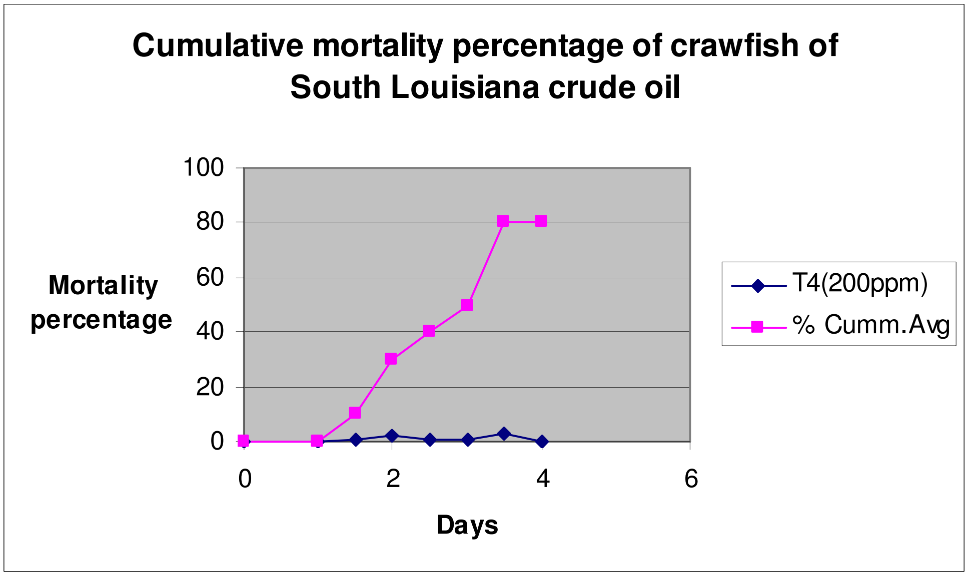
Appendix figure 2: Cumulative mortality Percentage (%) of South Louisiana crude oil of 67ppm at 96 hr



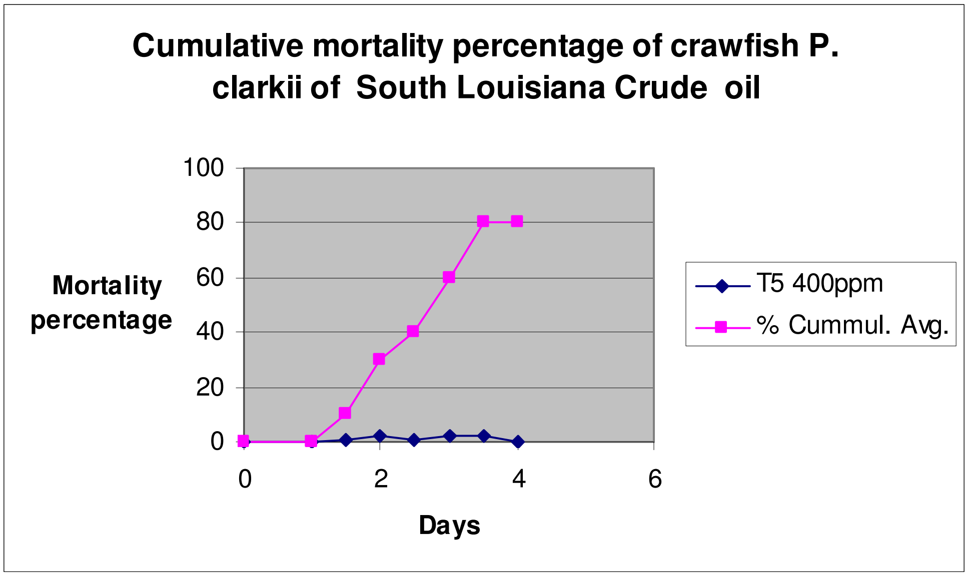
Appendix figure 3: Cumulative mortality percentage (%) of South Louisiana Crude oil of 84ppm at 96 hr



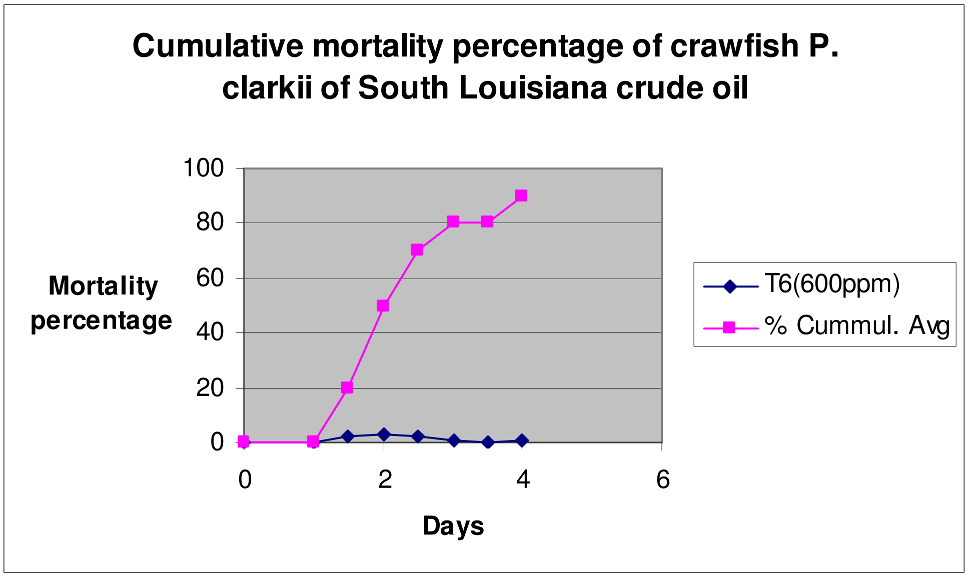
Appendix figure 4: Cumulative mortality percentage (%) of South Louisiana crude oil of 100ppm at 96 hr.



Appendix figure 5: Cumulative mortality percentage (%) of South Louisiana crude oil of 200ppm at 96 hr



Appendix figure 6: Cumulative mortality percentage (%) of South Louisiana crude oil of 400ppm at 96 hr.

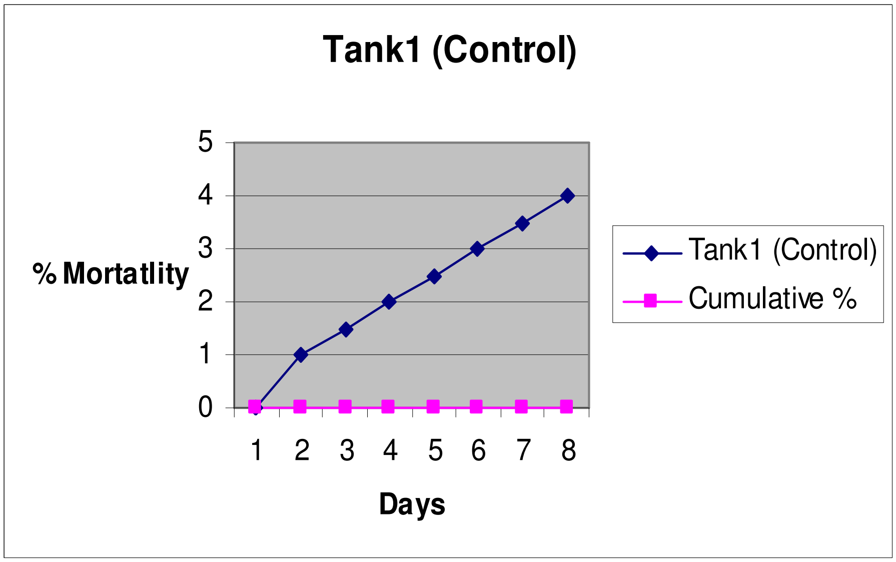


Appendix figure 7: Cumulative mortality percentage (%) of South Louisiana crude oil of 600ppm at 96 hr

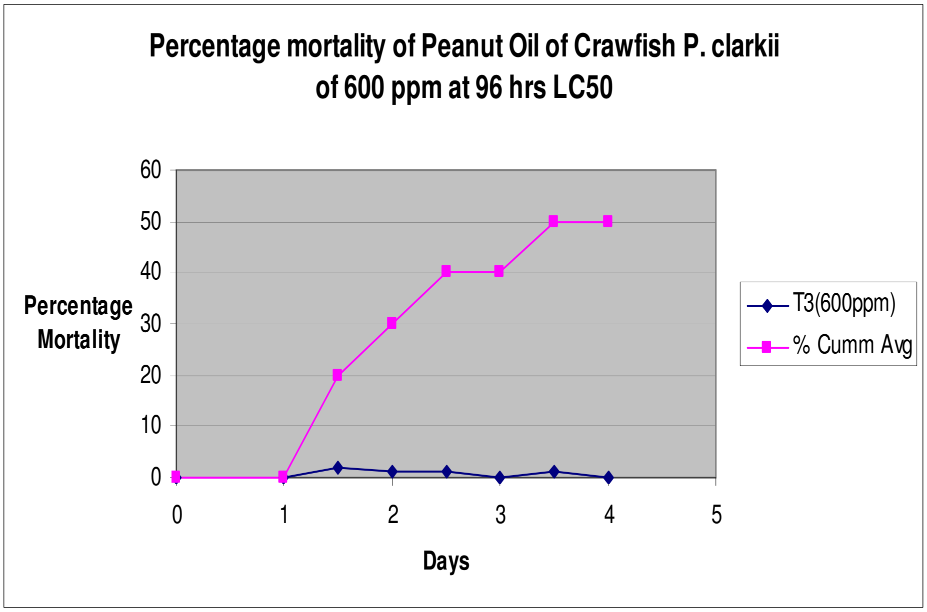
Appendix table 2, Mortality of crawfish <u>Procambarus clarkii</u> at different concentrations (ppm) of peanut oil in 96-h non renewal tests.

	No. Org.				Cumulative Mortality		
Conc. (ppm)	0hr	12hr	24hr	48hr	72hr	96hr	% Mortality
0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0
400	0	0	1	1	1	0	30

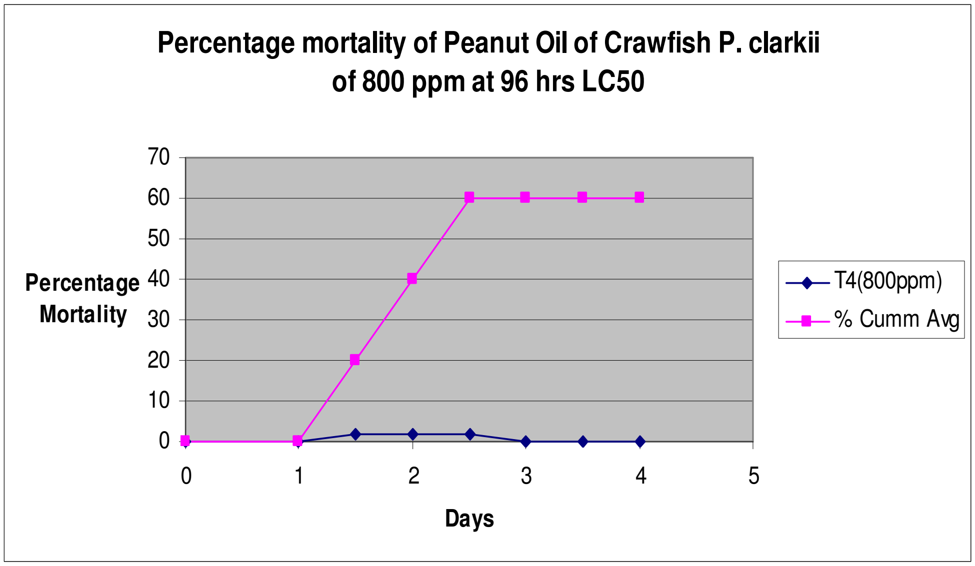
	No. Org.				Cumulative Mortality		
Conc. (ppm)	0hr	12hr	24hr	48hr	72hr	96hr	% Mortality
0	0	0	0	0	0	0	0
400	0	0	1	1	1	1	30
600	0	2	2	1	1	0	50
800	0	2	4	0	0	0	60
1000	0	3	2	1	1	0	60
1200	0	3	4	1	1	0	80



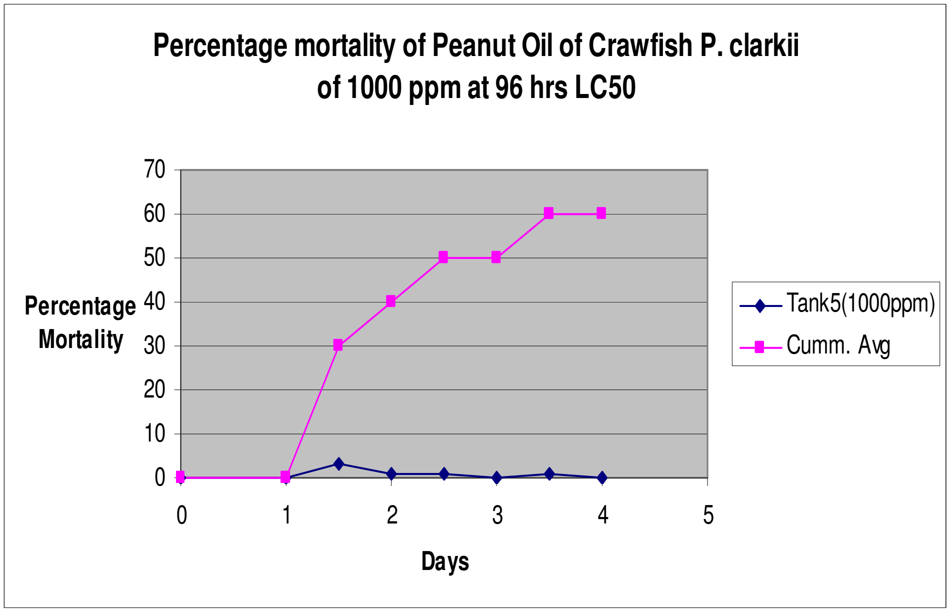
Appendix figure 8: Cumulative mortality percentage (%) of peanut oil of 0ppm at 96 hr



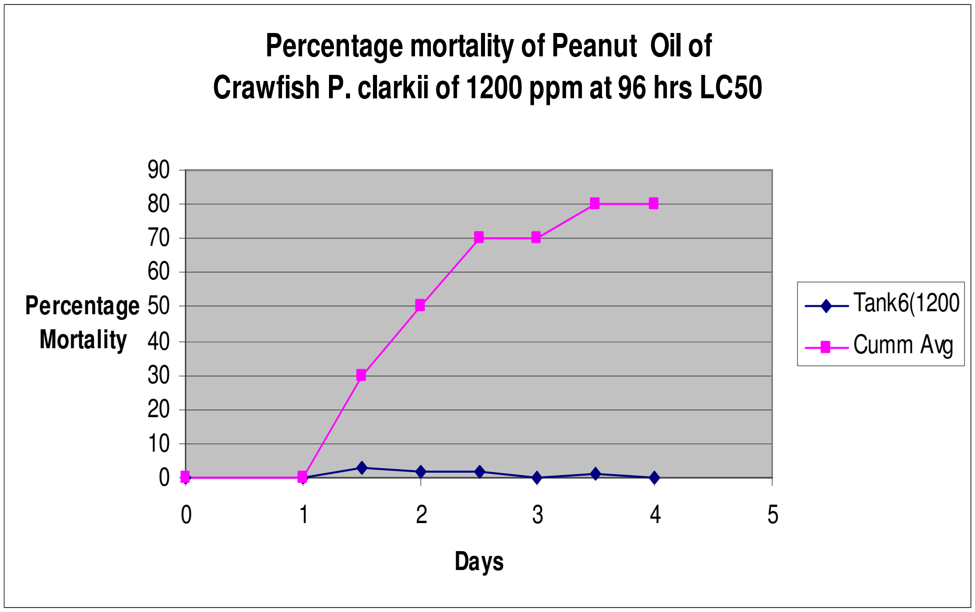
Appendix figure 9: Cumulative mortality percentage (%) of peanut of 600ppm at 96 hr.



Appendix figure 10: Cumulative mortality percentage (%) of peanut oil of 800ppm at 96 hr



Appendix figure 11: Cumulative mortality percentage (%) of peanut oil of 1000ppm

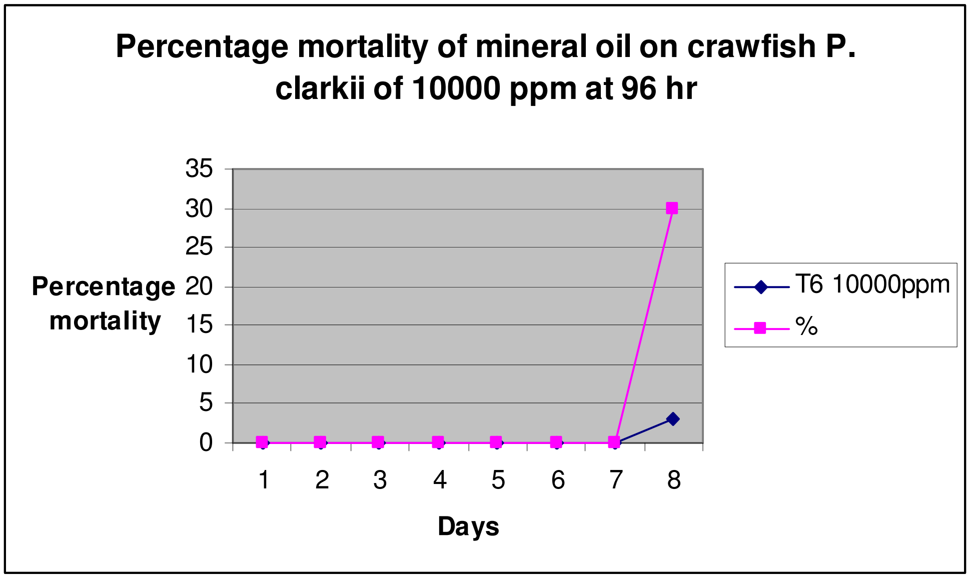


Appendix figure 12: Cumulative mortality percentage (%) of peanut oil of 1200ppm

Appendix table 3 mortality of crawfish Procambarus clarkii at different concentrations (ppm) of mineral oil in 96-h non-renewal.

No. Org.			Cumulative mortality				
Conc. (ppm)	0hr	12hr	24hr	48hr	72hr	96hr	% Mortality
0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0
800	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0
1200	0	0	0	0	0	0	0

No. Org.	Cumulative Mortality						
Conc. (ppm)	0hr	12hr	24hr	48hr	72hr	96hr	% Mortality
0	0	0	0	0	0	0	0
2000	0	0	0	0	0	0	0
4000	0	0	0	0	0	0	0
6000	0	0	1	0	0	1	20
8000	0	0	0	0	2	0	20
10000	0	0	0	1	2	0	30



Appendix figure 13: Cumulative percentage mortality (%) of mineral oil of 2000 4000, 6000, 8000 and 10000 ppm at 96 hr.

Appendix Table 4, analysis of peanut and mineral oil

	GRM7143B.D	GRM7143C.D
	Okie Mineral Oil	Okie Peanut Oil
Name	**Conc (ng/mg)**	**Conc (ng/mg)**
nC-10 Decane	0	0
nC-11 Undecane	8	4
nC-12 Dodecane	21	6
nC-13 Tridecane	38	6
nC-14 Tetradecane	65	6
Naphthalene	0	0
C-1 Naphthalene	5	0
C-2 Naphthalene	17	0
C-3 Naphthalene	15	0
C-4 Naphthalene	7	0
nC-15 Pentadecane	116	4
nC-16 Hexadecane	151	3
nC-17 Heptadecane	186	0
Pristane	104	0
nC-18 Octadecane	232	0
Phytane	133	0
nC-19 Nonadecane	200	0
nC-20 Eicosane	145	0
nC-21 Heneicosane	125	0
nC-22 Docosane	69	0
nC-23 Tricosane	76	0
nC-24 Tetracosane	86	0
Fluorene	1	0
C-1 Fluorene	0	0
C-2 Fluorene	0	0
C-3 Fluorene	0	0
Dibenzothiophene	0	0
C-1 Dibenzothiophene	0	0
C-2 Dibenzothiophene	0	0
C-3 Dibenzothiophene	0	0
Phenanthrene	0	0
C-1 Phenanthrene	0	0
C-2 Phenanthrene	0	0
C-3 Phenanthrene	0	0
C-4 Phenanthrene	0	0
Anthracene	0	0
Phenanthrene-d10 SS #1	0	0
5-alpha Androstane SS #2	0	0
nC-25 Pentacosane	0	0
nC-26 Hexacosane	0	0
nC-27 Heptacosane	0	0
nC-28 Octacosane	0	0
nC-29 Nonacosane	0	0
Fluoranthene	0	0
Pyrene	0	0

C-1 Pyrene	0	0
C-2 Pyrene	0	0
C-3 Pyrene	0	0
C-4 Pyrene	0	0
Napthobenzothiophene	0	0
C-1 NBT	0	0
C-2 NBT	0	0
C-3 NBT	0	0
Benzo (a) Anthracene	0	0
Chrysene	0	0
C-1 Chrysene	0	0
C-2 Chrysene	0	0
C-3 Chrysene	0	0
C-4 Chrysene	0	0
nC-30 Triacontane	0	0
nC-31 Hentriacontane	0	0
nC-32 Dotriacontane	0	0
nC-33 Tritriacontane	0	0
nC-34 Tetratriacontane	0	0
nC-35 Pentatriacontane	0	0
Benzo (b) Fluoranthene	0	0
Benzo (k) Fluoranthene	0	0
Benzo (e) Pyrene	0	0
Benzo (a) Pyrene	0	0
Perylene	0	0
Indeno (1,2,3 - cd) Pyrene	0	0
Dibenzo (a,h) anthracene	0	0
Benzo (g,h,i) perylene	0	0

VITA

Okey Umejuru was born in Oboburu Town, Port Harcourt, Nigeria. He completed his high school education at Government Secondary School Ahoada, Rivers State, Nigeria. He enrolled at Rivers State College of Education, Port Harcourt, in 1995 for a Bachelor of Agricultural Science Program. He graduated with second class honors degree in 1999. After graduating, he worked with Weatherford Oilfield Services as a Service Technician between 2000 and 2002. He later joined Elf Petroleum Nigeria Limited as a Production Operator between 2002 and 2004. He enrolled at Louisiana State University for the Master of Science in Biological and Agricultural Engineering in 2005. He expects to receive that degree in December, 2007.