8-8-2007

Chemical, Toxicological, and Microbial Characterization of New Orleans Sediments Following Hurricane Katrina

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Chemical, Toxicological, and Microbial Characterization of New Orleans Sediments Following Hurricane Katrina

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Master of Science
in
The Department of Biological Sciences

By

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B.S., Southampton College, 2005

August, 2007

Acknowledgements

I would like to thank my advisor, Dr. Bernard Rees for bringing me onto this project and for his support and patience. I would also like to thank the other members of my committee, Dr. Don Ennis for allowing me to complete most of the research presented here in his laboratory, and Dr. Wendy Schluchter, for helping with the microbiology chapter and her patience in teaching me the "old-school" way of doing things. Lisa Granados was instrumental in the beginning of this project. Thanks are also due to Dr. Gregory Broussard, Dr. Vanessa Madrid, and Laura Zaunbrecher for all their help in fish care and DNA analysis. Funding for this project was provided by the University of New Orleans, and National Science Foundation grant and the National Health Institutes of Health.

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List of Abbreviations

CTAB	hexadecyltrimethyl ammonium bromide
DO	dissolved oxygen
ECP	extracellular proteins
ERS	egg rearing solution
HHMSSL	Human Health Medium Specific Screening Levels
LD	lethal dose
MELA	medaka embryo larval assay
MF	mutant frequency
MTTH	mean time to hatch
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyl
SSC	Sodium chloride, sodium citrate
TE	Tris-EDTA (tris-ethylene diamine tetra acetic acid)
USEPA	United States Environmental Protection Agency

Abstract

On August 29, 2005 Hurricane Katrina struck the Gulf Coast and storm surges breached levees flooding much of New Orleans, Louisiana. One month after the storm, sediment was collected and toxicity was tested using Japanese medaka (*Oryzias latipes*) embryos. Sediments with the highest contaminant levels showed the highest embryonic mortality and most delayed development. However, no sediment caused an increased mutant frequency. When the most contaminated site was resampled in February, 2006 contaminant levels and toxicity decreased. During toxicity testing, approximately 20% of embryos incubated with sediment from one of these sites died and turned red. A red bacterium was isolated that is Gram-negative, cocco-baccilus, non-motile, and most similar to *Hahella chejuensis* based on genetic and metabolic tests. This bacterium caused 100% infection at 10^8 bacterial cells per ml and variable infection at lower doses. This study was the first to examine biological effects of exposure to post-Hurricane Katrina sediments.

Keywords: Japanese medaka, sediment toxicity, Hurricane Katrina, Hahella, bacterial pathogen

Chapter 1-Geographic and temporal variation in toxicity of post-Katrina sediments from New Orleans, LA

Introduction

On August 29, 2005, Hurricane Katrina, a Category 3 hurricane, struck the U.S. Gulf Coast with a force that devastated several major cities including New Orleans, Louisiana. Storm surges breached New Orleans levees and flooded 80% of the city with some areas sustaining as much as three meters of water. Immediately, concern was raised regarding the toxicity of local water-systems and the sediments deposited by the flood water. The research presented in this chapter of my Masters thesis was meant to address the overall concern of post-Katrina sediment contamination and their effects on a biological model, the Japanese medaka (*Oryzias latipes*).

Prior to Hurricane Katrina, soil and sediment samples were taken from the New Orleans metropolitan area was found to have unsafe levels of polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Mielke et al., 2001; Mielke et al., 2004; and Wang et al., 2004). Heterogeneity of contamination was found with areas along busy streets generally being the most contaminated and open areas the least contaminated (Mielke et al., 2004). Mielke et al. (2006) noted that soils throughout the New Orleans area had a higher concentration of lead in close proximity to buildings than open spaces, most likely due to the break down and removal of lead paint. PAH levels in the inner-city were four times higher than those measured in the suburbs. Individual PAHs measured at elevated concentrations before the storm include phenanthrene, fluoranthene, pyrene, benzo(b)fluoranthene, and benzo(a)pyrene (Mielke et al., 2004).

During and following Hurricane Katrina, flood water and sediments were contaminated by the redistribution of existing toxins, industrial spills, and leaks from thousands of small point

sources (e.g. household chemicals and automobiles). After Hurricane Katrina, chemical and microbiological studies have reported contaminant levels found in sediment and water samples taken from New Orleans. Flood water was tested for dissolved oxygen (DO), organic compounds, heavy metals, and fecal coliform bacteria (Pardue et al., 2005). Floodwater was neutral to slightly acidic; DO levels were below saturation at the surface and undetectable at the bottom of the water column. Of the volatile organic compounds found, most were gasoline components such as benzene, toluene, and ethylbenzene. Arsenic was the only metal found to consistently exceed drinking water standards. Fecal coliform bacterial concentrations were found at least one order of magnitude higher than water quality standards dictated by the United States Environmental Protection Agency (USEPA).

Presley et al. (2006) tested both flood water and sediments that had been deposited as flood water receded. In sediments, they found levels of arsenic, iron, and lead that exceeded health standards set by the USEPA's Human Health Median Specific Screening Levels (HHMSSL). Though heptachlor, methoxychlor, DDT, and DDE were the organic compounds most often detected, only benzidine, benz(a)anthracene, benzo(b)flouranthene, and benzo(a)pyrene were detected at levels greater than the USEPA's HHMSSL (Presley et al., 2006). Cobb et al. (2006) confirmed the high concentrations of heavy metals in sediments, reporting levels of arsenic and lead that consistently exceeded USEPA human health levels. All studies conducted on the contamination in New Orleans after Hurricane Katrina concede that more sampling needs to be conducted to accurately assess the toxicity of the city of New Orleans as a whole.

Although Presley et al. (2005) measured heavy metal and pesticide residues in snake and alligator tissues, Hurricane Katrina studies have not, as of yet, measured the direct biological

effects of exposure to post-Hurricane Katrina sediments. In this research, the toxicity of the selected New Orleans sediments was determined using hatch rates, mortality, morphological deformations, and mutagenesis as biological endpoints in the biological model, Japanese medaka (*Oryzias latipes)*. This study was done in conjunction with contaminant measurements done by colleagues from Xavier University of Louisiana, New Orleans, LA (see Appendix). Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organohalogen pesticides and metabolites, and metals were measured in all sediment sites. The purpose of this study was to determine the combined toxicological effects of exposure to post-Katrina New Orleans sediments.

Japanese medaka make an attractive laboratory species due to their small size, cost-effectiveness, ease of culture, year round spawning ability, and extensive toxicology resource information (Kirchen and West, 1976; Iwamatsu, 1994). Medaka embryos are especially useful in developmental assays because of their transparent chorion and well described embryological developmental stages (Iwamatsu, 1994; Winn et al., 2001). In this study, a transgenic strain of medaka engineered by Winn et al. (2000) was used for all experimentation. This strain carries a prokaryotic 45.5 kb λLIZ bacteriophage vector with mutational targets for *in vivo* mutagenesis assays. The transgenic mutational target gene is genetically neutral to the host and since it is prokaryotic DNA, the transgene has more methylation due to its higher G+C content, and greater frequency of methylcytosines at the dinucleotide CpG islands; prokaryotic DNA also lacks transcription coupled repair mechanisms. Mutations are therefore able to accumulate and persist without organismal selection. After exposure to a mutagen, the vector is extracted from the fish's genomic DNA and shuttled into an indicator bacterial species where mutants can easily be quantified (Winn et al., 2000; 2001; Jakubczak et al, 1996).

The bacteriophage λLIZ vector carried by the transgenic medaka has the *cII* and *lacI* mutational targets. Though either the *cII* or the *lacI* gene could be used in mutation assays, the *cII* assay was used in these experiments as it uses less time and reagents, and is less subjective in scoring for mutants with fewer false positive results. The smaller size of the *cII* gene (296 bp) also makes it a more attractive assay (Jakubczak et al., 1996). The *cII* assay was first described by Jakubczak et al. (1996) using the mouse Big Blue system. It is a positive selection system to identify forward mutations in the *cII* gene. In *Escherichia coli* cells, the *cII* protein commits the bacteriophage to the lysogenic cycle. In this assay, a specialized *E. coli* strain (*hfl*-) is used to extend the life of the *cII* protein. Cells with the wild-type *cII* gene on the phage reproduce lysogenically, remaining indistinguishable from other cells in an *E. coli* lawn. *cII* mutants, on the other hand, reproduce lytically, forming plaques. The frequency of spontaneous mutations in the *cII* gene is $2.9 \pm 0.3 \times 10^{-5}$ in DNA obtained from whole fish tissue (Winn et al., 2001). The *cII* assay is capable of detecting point mutations and small insertions and deletions (Jakubczak et al., 1996; Winn et al., 2000; 2001).

In the first chapter of my thesis research, I report an evaluation of the toxicology of sediments collected both one and five months after Hurricane Katrina in New Orleans, Louisiana. Up until this disaster, there has been little research looking at the toxicity of sediments left in the wake of a hurricane or other type of natural disaster. Here, the established Japanese medaka fish model bioassay is used to determine the developmental effects and mutagenesis of toxicants left in New Orleans sediments by Hurricane Katrina. The results of this study may prove to be important in the assessment of the effects of toxicants to human health and wild fish stocks in the surrounding environment, particularly the Mississippi River, Lake Pontchartrain, and the ecologically important wetlands.

Environmental Contaminant Background

Insecticides such as DDT and aldrin and their metabolites are stable solids demonstrating low water solubility and high lipid solubility and non-specific toxicity (demonstrate toxic effects to many vertebrates and invertebrates in addition to target insects). Most pesticides are applied as sprays, granules, or dusts in agricultural and residential areas. They often associate with particles of soil or droplets of water. Organic pollutants are broken down in sediments by hydrolysis, oxidation, isomerization, photochemical breakdown, and can be removed from localized areas in water runoff (Walker et al., 1996). Breakdown usually leads to a decrease in toxicity, however this is not always the case. Aldrin, for example, is metabolized to dieldrin which is a very toxic compound. DDT can also be broken down to DDD and DDE, both of which are highly persistent and toxic compounds (Walker et al., 1996). Hydrophilic compounds do not bind to soil particles and can move easily through sediments making them more readily available to soil-dwelling organisms. This type of compound is less persistent as soil organisms metabolize them rapidly. Compounds are normally less persistent at high temperatures as volatilization, chemical breakdown, and biotransformation are completed faster. In contrast to hydrophilic compounds, lipophilic compounds can be associated with sediment matter for many years (Walker et al., 1996).

Polycyclic aromatic hydrocarbons (PAHs) are very stable and are primarily released into the environment by the incomplete combustion of organic substances such as coal, oil, and wood. PAHs are made up of carbon and hydrogen ions with at least one benzene ring. They have low polarity and low water solubility with a high solubility in lipids (Walker et al., 1996). PAHs are recognized as both mutagenic and carcinogenic to many organisms, including vertebrates (Walker et al., 1996). In fish, unsubstituted PAHs show greater toxicity in embryos

than substituted PAHs (Rhodes et al., 2006) and can increase egg membrane permeability (Strmac et al., 2002).

Polychlorinated biphenyls (PCBs) are commercial mixtures of congeners that are stable, fairly unreactive and ubiquitous in the environment due to their use in hydraulics and as plasticizers (Brouwer et al., 1990). Congeners are listed as numbers with the last two numbers representing the percent of chlorine making up the PCB. PCBs differ in toxicity depending on the number and position of chlorine atoms bound to the biphenyl ring (Brouwer et al., 1990). The most toxic PCBs are the coplanar PCBs which lack chlorine substitution at the ortho position of the biphenyl ring allowing the molecule to assume a planar formation; PCBs of this type are congeners 126, 77, and 81 (Harris et al., 1994a; b). PCBs have limited water solubility but are highly soluble in lipids. In many countries PCB use is either banned or severely restricted (Walker et al., 1996). PCBs reduce embryonic survival and hatching success in many fish species (Mac and Schwartz, 1992; Strmac et al., 2002).

Most metals occur naturally in the environment and have become pollutants due to increased emissions by human activity. A "heavy" metal has been defined in the past as a metal with a specific density ratio relative to water of greater than five. More recently, heavy metals are defined by their chemistry and ability to pollute an area or biological organisms (Walker et al., 1996). Four factors that contribute to the fate of metals in the environment are localization, persistence, bioconcentration, and bioavailability. Though degradation of specific compounds can occur resulting in organometallic compounds (e.g. methylmercury) that are generally more bioavailable and toxic than inorganic metals, metals largely cannot biodegrade and have long residence times in sediment (Bryan and Langston, 1992; Walker et al., 1996). The mobility of most metals in sediment is mostly dictated by the clay content, organic matter, and pH of the

sediment in that the more clay, organic matter, and the higher the pH, the more tightly bound metals will be to sediment particles (Walker et al., 1996). Metal concentrations in sediments are usually three to five orders of magnitude higher than those found in the overlying water column. Metal toxicity is usually a result of several bioaccumulated metals acting synergistically. Embryonic stages of development are more sensitive to metals than other stages, especially adults, often by orders of magnitude (Bryan and Langston, 1991).

Lead is found in the environment at high levels from gasoline and the break down of lead paint. Lead is usually associated with particulates where it can become alkylated, either chemically or biologically. Trimethyl lead, formed from inorganic lead in the environment, is the most toxic form of lead to most biological organisms (Bryan and Langston, 1991). Arsenic, another toxic metal, is released through mining and smelting, but also from the use of pesticides, wood preservatives, and in the production of glass, alloys, and medicines (Bryan and Langston, 1991). Though arsenic is not considered to be as harmful to aquatic organisms as other heavy metals, studies have reported fish that ingested arsenic have reduced growth as compared to normal fish (Bryan and Langston, 1991). The USEPA also lists inorganic arsenic's cancer endpoint as low as 0.39 μg/g (USEPA, 2007).

In a mixture of compounds, toxicity is usually additive, however, sometimes toxins can act in a more than additive fashion. It is possible that one compound inhibits the detoxifying mechanism of and organism for another compound, leaving the affected organism more susceptible to the second compound. It is also possible for one compound to activate another compound or to act as a cofactor needed by another compound to demonstrate toxicity (Walker et al., 1996). Since the exact mechanism of action is not known for many compounds, it is impossible to predict the toxicity of a mixture of compounds solely from a measurement of the

concentration of compounds in water or sediment; contaminant concentration measurement experiments should be done in conjunction with a biological model to truly determine the effects of contaminant mixtures.

In fish, pollutants are most often taken up through the gills and skin by diffusion or into the digestive tract with ingested food. Many pollutants can be transported to other tissues through blood and lymph fluids where they can interfere with DNA, proteins, cell membranes, or metabolic pathways. Lipophilic toxicants can be biotansformed by oxidation, hydrolysis, or reduction leading to metabolites with hydroxyl groups which exhibit decreased toxicity and increased water solubility for easier excretion (Walker et al., 1996). In one experiment, for instance, medaka embryos were seen to metabolize the pesticide benzo(a)pyrene (McElroy et al., 2006). Medaka (Winn et al., 2000; Chikae et al., 2004; Rhodes et al., 2005; McElroy et al., 2006) as well as various other fish species have been implicated as susceptible to the compounds reported in this study (e.g. Yang et al., 2006; Henshel et al., 2006; Lamai, et al., 1999; Weis and Weis, 1977).

MATERIALS AND METHODS

Sediment Collection. Surface sediment was collected from five New Orleans locations (Figure 1.1) on September 30, 2005, one month after Hurricane Katrina. No effort was taken to distinguish between sediment and soil. Sample A was collected from the mouth of the London Avenue Canal near Lake Pontchartrain; sample B was taken from the beach at the mouth of the Industrial Canal; sample C was collected from Pratt Drive, south of Robert E. Lee Boulevard, where the London Avenue Canal levee broke (water was still seeping through the repaired levee at the time of collection); sample D was dried sediment scraped off the pavement of the 5500

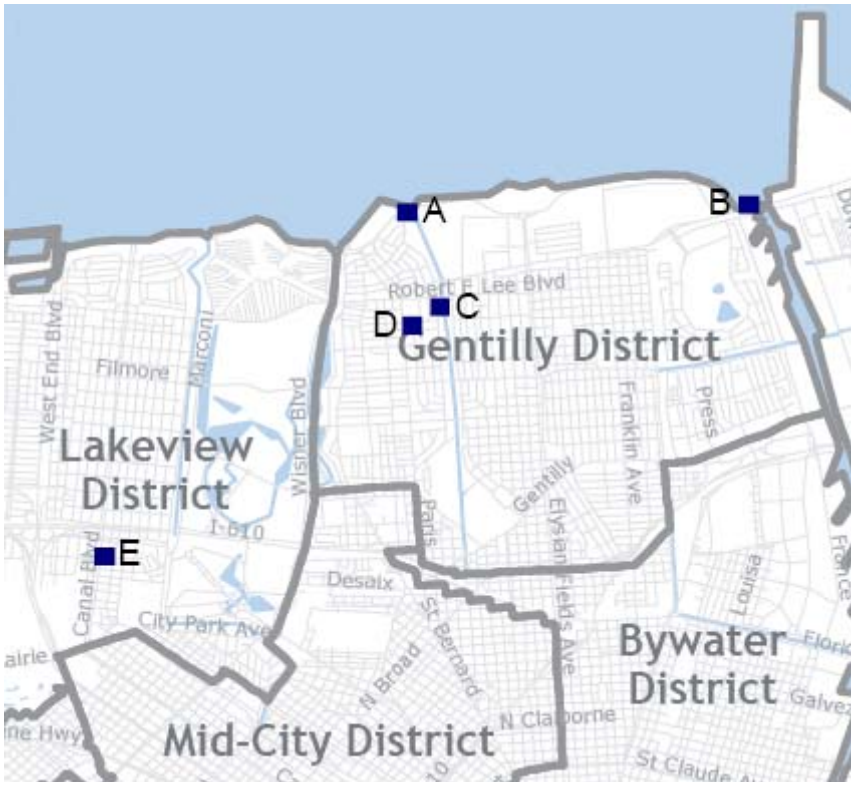

Figure 1.1. Sediment sampling site locations (A-E) within New Orleans, LA marked with blue squares. Lake Pontchartrain is shown at the top of the figure.

block of Chamberlain Drive, in a neighborhood that had been flooded by 8-10 feet of water; and sample E was collected from the median of Canal Boulevard near Pontalba Street near a railroad underpass. Multiple samples from each site were collected and homogenized before being distributed for storage. As site D was found to be the most toxic site (see Results), sediment was re-sampled on February 12, 2006 to evaluate temporal changes in the toxicity of those sediments. After a day at ambient temperature, all samples were stored in the dark at 4°C for the remainder of the experiment. A sub-sample from each site was used to determine the dry weight of each sample. Sediments were analyzed by colleagues at the Xavier University of Louisiana for PAHs, PCBs, organohalogen pesticides and metabolites, and metals using procedures described in Mielke et al (2004) and Wang et al. (2005).

Animal Care. Inbred homozygous transgenic Japanese medaka (*Oryzias latipes*) embryos from an established colony at the University of Louisiana at Lafayette were used for all experiments. Adult fish were maintained at 27 ± 2°C in 40 liter tanks containing filtered, dechlorinated water on a 16:8 hour light:dark photoperiod and were fed high-protein Tetra flake food twice daily and brine shrimp once daily. Bi-monthly water changes replaced approximately 20% tank water with fresh dechlorinated water. Eggs were collected directly off the abdomen of female medaka 2-4 hours after fertilization. The eggs were rubbed across a nylon screen removing the connecting fibers and rinsed twice with an 18% (weight/volume) NaCl solution. Each egg was examined under a dissecting microscope to confirm fertilization (presence of a perivitelline space). Embryos were maintained in 1 x egg rearing solution (ERS: 10 ml solution containing 10% NaCl, 0.3% KCl, 0.4% CaCl2, 1.6% MgSO4, and deionized water up to 1 liter with additional NaCl to 4 g/L) in a 25°C incubator kept on a 16:8 hour light:dark photo-period until hatched. Experimental exposures were done in ERS incubated with sediments as described

below. After hatching, fry were maintained in 2 L plastic bins containing dechlorinated water at 27 ± 2°C on a 16:8 hour light:dark photoperiod and fed powdered high-protein Tetra flake food twice daily. After approximately one month in the plastic bins, fish were transferred to 40 L aquariums and maintained as stated above.

Medaka Embryo-Larval Assay (MELA). MELA was performed under the supervision of Lisa Granados at the University of Louisiana at Lafayette. The toxicology of all sediments was tested as per guidelines of established MELA protocols for detecting adverse effects of toxicants from sediments on medaka embryos (Winn, 2001). To do this, fully aerated ERS was shaken with sediment samples (5 ml ERS mixed with an amount of sediment equivalent to 1 g dry weight sediment) overnight at room temperature. The mixture was centrifuged and the sediment-treated ERS was decanted through a nylon screen to remove large particulates. Sediment-treated ERS was dispersed into twenty 12 x 32 mm glass screw-cap vials with 1 ml treated ERS and one fertilized embryo in each. A control for normal embryo development was included using ERS that had not been incubated with sediment.

Each embryo was examined daily using a dissecting microscope. Developmental milestones (e.g., organ development) were noted as well as any deviation from normal embryo development. The assay was terminated when all embryos had either hatched or died. Hatched fry were removed from sediment-treated ERS and were grown for three months in untreated water, as described above. Winn et al. (2001) recommended a 15-30 day grow out period for fish for accurate mutagenesis results while Heddle et al. (1995) suggested longer expression times for multiple chemicals; the 3 month growth time used in this study was sufficient for the incorporation of DNA mutations. This study consisted of two trials with 20 embryos per study site for September sediments and one trial of 40 embryos for the February sediment.

Mutagenesis Assay. Surviving embryos were grown up to adulthood (3-4 months) and DNA was extracted from whole fish tissues to determine mutant frequency (MF) (Winn et al., 2000). Briefly, three fish per trial per site (n=6 per site) were placed in ice-cold water and then decapitated. Fish were individually homogenized and the resulting homogenate was digested with proteinase K (0.6 mg/ml proteinase K in 0.15 mol/L NaCl, 0.05 mol/L sodium citrate, 1% sodium dodecyl sulfate, pH 7.0) for three hours at 37°C. DNA was extracted three times with an equal volume of phenol:chloroform (50:50). Potassium acetate was added to a final concentration of 1M and a final chloroform extraction was performed. DNA was precipitated with 100% ethanol and re-suspended in 10 mM Tris-1 mM EDTA (TE), pH 7.5 overnight. DNA concentrations were at least 2 μg/μl.

The *cII* assay was used to determine the MF of each fish. The bacteriophage lambda (λ) LIZ transgenic shuttle vector was excised from about 10 μl genomic fish DNA using packaging extracts from Dr. Richard Winn's laboratory (University of Georgia). DNA was incubated in packaging mixes for 3 hours at 30°C before being diluted with suspension medium to a final volume of 1 ml. *E. coli* strain G1250 (*hfl*-) was grown in maltose and $MgSO_4$-supplemented TB-1 broth until 0.5 optical density (OD_{600}) was obtained. The cells were pelleted and re-suspended in an equal volume of 10 mM $MgSO_4$. To determine the packaging efficiency, an aliquot of packaged phage was diluted 1000 fold, mixed with G1250 cells in 10 mM $MgSO_4$, incubated at room temperature for 30 minutes, and plated in triplicate on TB-1 agar plates using TB-1 top agar. The plates were incubated at 37°C overnight and all plaques were counted to determine the titre of packaged phage. The remaining undiluted packaged phage was divided and mixed with G1250 *E. coli*, incubated at room temperature for 30 minutes and then plated on TB-1 agar plates using TB-1 top agar. These plates were incubated at 24˚C for 40 hours to select

for *cII* mutant phage. All mutant plaques were counted for each sample (Jakubczak et al, 1996; Winn et al., 2000).

The *cII* MF was calculated by dividing the total number of mutant plaques isolated at 24˚C by the titre of each packaged phage. Mutant frequencies were averaged for sediment treatments and compared among samples and to the previously established spontaneous mutation rate of the λLIZ bacteriophage (3×10^{-5}; Winn et al, 2000).

Statistical Analyses. All statistics were performed using SYSTAT 10.2. Averages are given as values ± standard deviation. Significant differences were considered to have p values of ≤ 0.05. Survival analysis (Dixon and Newman, 1991) was used evaluate the medaka hatching data, represented as the cumulative proportion hatched versus days post-fertilization. A log-normal model fit the hatching data better than alternative models. Preliminary analyses showed no significant differences among replicate trials run with September sediments for any treatment site, and data from the two trials were pooled, resulting in a total of 40 embryos per sediment sample. Using these data, the median time to hatch (MTTH) of embryos exposed to each sediment sample was modeled using the following equation:

$$\ln(\text{MTTH})=\mu+\beta\ (\text{treatment})+\sigma W$$

where μ= location, β=treatment effects (where 0=control and 1=treated with sediment), σ= scale, and W=shape (=0) for the log-normal model.

Results

Sediment analyses. Pesticide, PCB, PAH, and heavy metal concentrations were measured by colleagues at Xavier University of sediments collected in September, 2005 from 5 New Orleans sites (A-E) and from one of these sites in February, 2006 (D2). Sediments were

tested for 29 pesticides and pesticide metabolites, 27 PCB congeners, 19 PAHs, and 12 heavy metals (see Appendix A). There was variation in contaminant concentration among individual contaminants as well as among sampling sites. The total concentrations of each contaminant class are shown in Table 1.1. Site D was consistently one of the most contained sites with the highest total concentration of pesticides (104.3 μg/g), PCBs (0.367 μg/g), and heavy metals (952 μg/g) and site C, another heavily contaminated site, was found to contain the highest concentration of total PAHs (4.41 μg/g); site B was the least contaminated site for all contaminants. When site D was re-sampled in February, 2006, total pesticide concentrations decreased to about 4% of the previously measured September concentration and total PCB concentrations dropped by about half; total PAH concentrations declined only slightly from their previous levels (Table 1.1).

Ten organic and one inorganic compound were found at concentrations above the USEPA Region 6 Human Health Medium Specific Screening Levels (HHMSSL) for residential soils: site D had high concentrations for all eleven compounds (Table 1.2). Arsenic was the only heavy metal found in concentrations greater than the HHMSSL for cancer risk (0.39 μg/g), and it was elevated above this level at all sites except for site B. Lead concentrations ranged from 7 μg/g to 127 μg/g, lower than HHMSSL for non-cancer risk (400 μg/g) (no HHMSSL for cancer risk from lead exposure is tabulated for Region 6). Though no single PCB congener was above HHMSSL for cancer risk (0.220 μg/g), the total PCB concentration in site D did exceed this level (Table 1.1). Pesticides that were elevated at site D in September, 2005 had uniformly decreased by February, 2006 whereas PAH contaminants did not decrease to such an extent.

Medaka Embryo Larval Assay (MELA). Medaka embryos incubated in non-treated control ERS displayed low percent mortality (7.5%) and hatched between days 9 and 15 post-

Table 1.1. Total concentrations of organohalogen pesticides, PAHs, PCBs and metals in Post-Hurricane Katrina New Orleans sediments from five collection sites measured by colleagues at Xavier University of Louisiana. Sediments were collected in September, 2005, except for D2 which was collected in February, 2006. Concentrations are given in µg/g (ppm).

Contaminant Class	A	B	C	D	E	D2
Organohalogen Pesticides	9.128	1.083	29.809	104.289	10.585	4.215
PAHs	0.986	0.97	4.412	2.430	1.382	2.030
PCBs	0.005	0.002	0.062	0.367	0.007	0.180
Metals	305	48	178	952	373	ND

ND Not determined

Table 1.2. Concentrations of specific contaminants exceeding USEPA's Human Health Median Specific Screening Levels (HHMSSL) measured in Post-Hurricane Katrina New Orleans sediments from five collection sites by colleagues at Xavier University of Louisiana. Sediments were collected in September, 2005, except for D2 which was collected in February, 2006. Concentrations are given in μg/g and values exceeding HHMSSL for that compound are in bold.

Pesticides		HHMSSL	A	B	C	D	E	D2
	Aldrin	0.029 (C)	BD	BD	BD	**1.93**	BD	BD
	Chlorobenzilate	1.800 (C)	BD	BD	BD	**7.745**	BD	0.029
	4, 4' DDE	1.700 (C)	BD	BD	0.149	**1.720**	BD	1.08
	Dieldrin	0.030 (C)	BD	BD	**0.069**	**4.885**	BD	BD
	HCH (alpha)	0.090 (C)	0.026	BD	**0.431**	**0.720**	**0.205**	**0.123**
	HCH (beta)	0.320 (C)	BD	BD	0.197	**0.329**	BD	BD
	HCH (gamma)	0.440 (C)	BD	BD	0.243	**1.138**	BD	BD
PAHs	Benz(a)anthracene	0.150 (C)	0.064	0.006	**0.251**	**0.157**	0.080	0.132
	Benzo(a)pyrene	0.015 (C)	**0.036**	0.003	**0.111**	**0.079**	**0.040**	**0.131**
	Dibenz(a,h)anthracene	0.015 (C)	0.013	BD	**0.038**	**0.022**	**0.020**	**0.090**
Metals	As	0.39 (C)	**2**	BD	**1**	**9**	**19**	ND

BD Below limit of detection.
ND Not determined.

fertilization. Embryos in sediment-treated ERS, however, suffered higher mortality and delayed hatching (Figure 2a; Table 1.3). The embryos treated with sediment from the most toxic site, site D, suffered a 65% mortality with delayed hatching occurring up until day 28 post-fertilization. Survival analysis revealed a highly significant effect of treatment site on hatch rate ($p<0.01$). Median time to hatch (MTTH) varied among treatments in the following order (from shortest to longest time to hatch: untreated ERS control < E ≈ B ≈ C < A << D (Figure 1.2a; Table 1.3). Mortality in embryos exposed to ERS incubated with sediment collected from site D in February, 2006 was half that observed with September sediments (32.5%) and had a shorter MTTH (16.4 days) which was more consistent with MTTH for other sediments sampled during the September collection (Figure 1.2b).

Developmental anomalies were noted in a small percentage of embryos incubated in sediment-treated ERS (7%). The most common defects were abnormal organ development, curved spines, and blood pools and aneurisms (Figure 1.3). Some of these defects resolved during development (e.g. blood pools and aneurisms) while other deformations persisted to adulthood (e.g. bent spines). None of these defects were seen in the 60 embryos incubated in control ERS (<1.7%).

Mutagenesis Assay. Embryos exposed to sediment-treated ERS were grown to adulthood (three months), and the *cII* MF was determined for the λLIZ mutational shuttle vector. In this experiment, the average MF ranged from $2 \pm 1 \times 10^{-5}$ to $6 \pm 5 \times 10^{-5}$ for sediment-treated fish (Table 1.3). Sites did not vary significantly from one another (ANOVA, $p=0.128$), nor were they significantly different from the spontaneous MF of reference bacteriophage (3×10^{-5}). Power analysis indicates that for the given sample size, the experimental MF would need to be at least three times the value of the spontaneous MF in order to be considered significantly different.

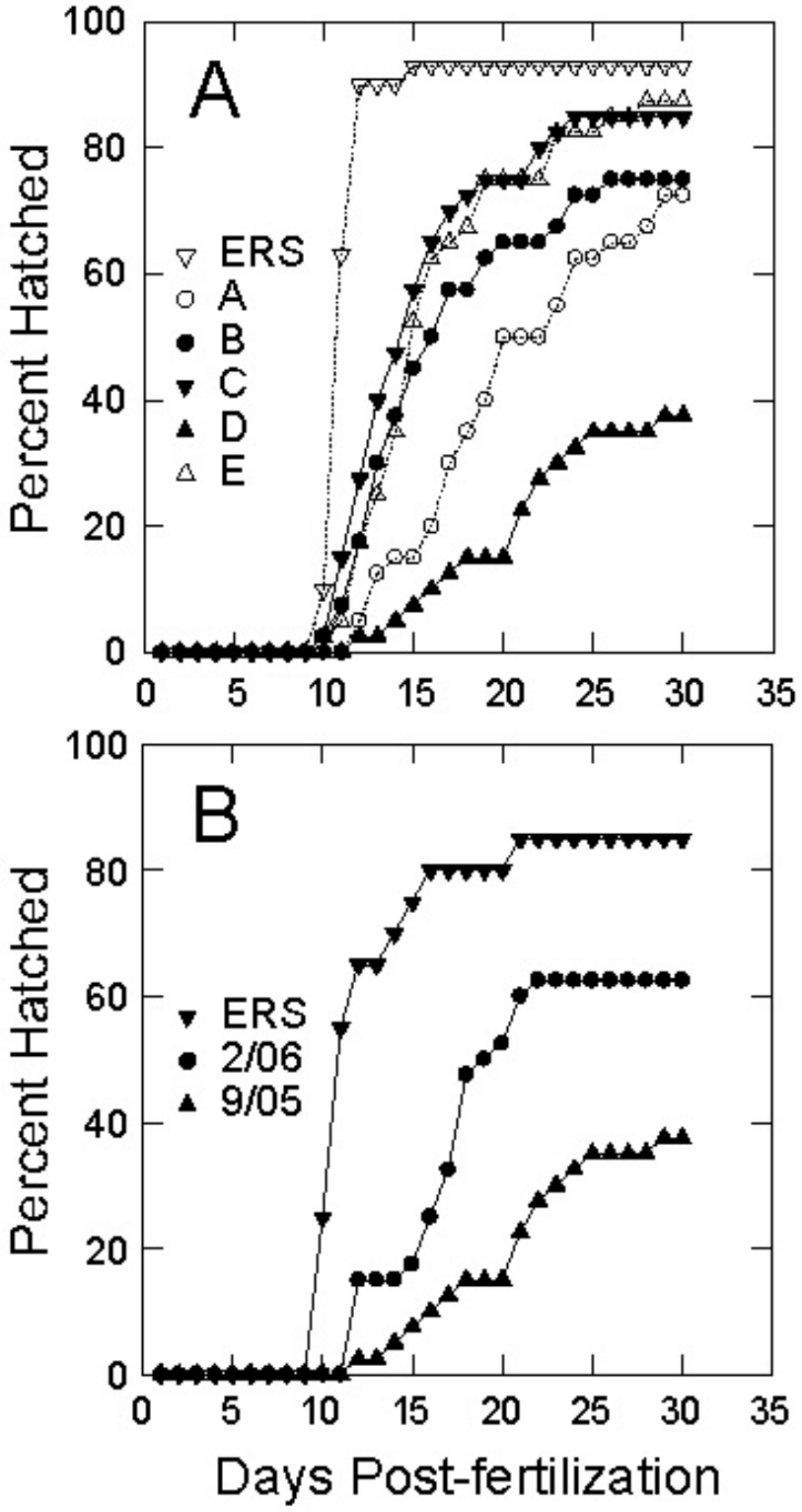

Figure 1.2.
a. Average hatch rate for embryos incubated in ERS treated with sediment from each site from September collection (n= 2 trials of 20 embryos each). Figure 2b. Hatching rates for embryos incubated in ERS treated with September and February collections from site D. Independent ERS controls were performed in parallel with incubations in February, 2006 sediments. Curve for September, 2005 is from Fig. 2a and shown here for comparison.

Table 1.3. Summary of biological data for medaka exposed to Post-Hurricane Katrina New Orleans sediments from five collection sites. Sediments were collected in September, 2005, except for D2 which was collected in February, 2006. Mean time to hatch (MTTH) is given in days; mortality is measured at 30 days post-fertilization. Mutant frequency (MF) of the transgenic *cII* gene was measured 3 months post-exposure (mean ± s.d; n=6).

	MTTH	Percent mortality after 30 days	MF
Control	10.8	7.5%	3×10^{-5} [a]
A	18.7	27.5%	$4 \pm 3 \times 10^{-5}$
B	15.2	25%	$5 \pm 2 \times 10^{-5}$
C	15.5	12.5%	$6 \pm 5 \times 10^{-5}$
D	30.3	62.5%	$2 \pm 1 \times 10^{-5}$
E	14.4	15%	$2 \pm 2 \times 10^{-5}$
D 2	16.4	37.5%	ND

ND Not determined.
a. Spontaneous MF of the *cII* λLIZ gene (Winn, 2000; 2001)

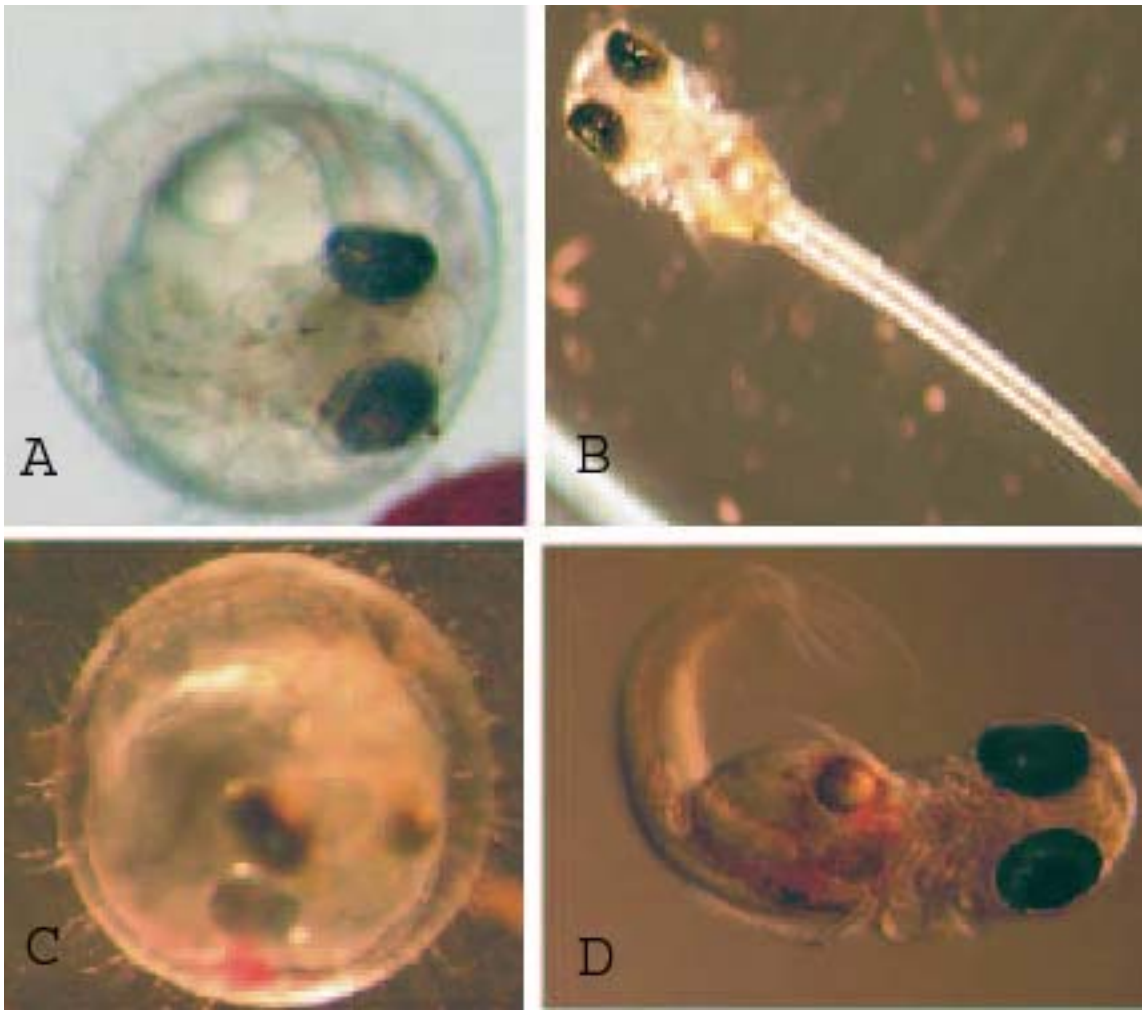

Figure 1.3. A. Normal embryo 8 days post-fertilization. B. Normal fry on day of hatching C. Underdeveloped embryo- note blood pool near bottom of yolk sac. D. Newly hatched fry- note the bent spine and small tail.

Discussion

Prior to Hurricane Katrina, there was little available information regarding the environmental toxicity of sediments following a hurricane. In this study, in conjunction with measurements of sediment contaminant concentrations, a toxicology model was used to determine the adverse effects of exposure to post-Katrina New Orleans sediments. Though the contaminant concentration results presented here are consistent with other analytical studies on the contaminants of New Orleans sediments following Hurricane Katrina (Pardue et al., 2005; Presley et al., 2006; Cobb et al. 2006) this study further reveals that exposure to sediments containing these contaminants demonstrates toxic effects.

Before Hurricane Katrina, soils from around the New Orleans metropolitan area were significantly contaminated by organic and inorganic pollutants with higher levels associated with inner city and heavily trafficked areas (Mielke et al., 2001; Mielke et al., 2004; and Wang et al., 2004). Mielke et al. (2004) reported the median concentration of total PAHs in urban New Orleans soils as 2.93 μg/g with a high PAH concentration of 7.28 μg/g. The total PAH levels measured in the current study (0.097 μg/g- 4.41 μg/g; Table 1) were within this range of previously measured PAHs.

Metal concentrations measured before Hurricane Katrina by Mielke et al. (2004) were between 289 μg/g and 646 μg/g, with lead concentrations ranging from 86 μg/g in open areas to 196 μg/g near busy streets. Metal concentrations measured for the current study after Katrina were between 48 μg/g to 952 μg/g with site D being the only site to exceed metal concentrations measured before the storm (Table 1); though As, Hg, and Ti were not measured in the Mielke et al. (2004) study, the concentrations of these metals (9 μg/g, 0.331 μg/g, and 33 μg/g respectively) do not completely account for the higher metal concentration found at site D (see

Appendix A). Though lead levels were measured at or below previously published values (7 – 127 μg/g) in this experiment, Presley et al. (2006) and Cobb et al. (2006) both measured lead at much higher concentrations (10 μg/g to 1880 μg/g) in post-Katrina sediments. Despite the fact that As levels in this study were not measured at concentrations as high as in other post-Katrina studies (28 μg/g; Cobb et al., 2006), four out of the five sites tested did display concentrations exceeding HHMSSL's cancer endpoint (0.39 μg/g). Seven of the individual pesticides measured in this study exceeded USEPA's HHMSSL in site D. All seven pesticides had decreased to concentrations below HHMSSL when resampled 4.5 months later in February, 2006. At least two PCBs were detected that the USEPA considers to be of the "highest toxicity and abundance" (congeners 77, 138, and 170) while three others detected are listed as "high toxicity and abundance" (101, 180, 183) (PCBs: Cancer Dose-Response Assessment and Application to Environmental Mixtures, EPA, 1996).

As in other studies (Presley et al., 2006; Cobb et al., 2006) contaminants measured for this study were non-uniformly distributed among New Orleans sediments. Sites outside the city's levee system, in close proximity to Lake Pontchartrain (sites A and B) had consistently lower contaminant levels than residential sites within the levee system (sites C, D, and E). This was not surprising as outside the levee system, water levels returned to normal quickly after the storm, little sediment was deposited, and these sediments remained in constant exchange with lake water. Within the levee system, however, sampling sites were exposed to floodwater for extended periods of time, with greater potential for leaching, redistribution, and deposition of sediments and contaminants. Even among seemingly similar sites, contaminant levels were heterogeneous; sites C and D were both residential areas less than 1 km apart, but the concentrations of many of the various contaminants (see Appendix), were different. Factors that

could have contributed to the observed heterogeneity include the number of and proximity to point sources of pollutants, the amount of water received by the area, vicinity to drainage canals, and the length of time flood waters stood.

In the current experiment, there was also a toxic effect of sediment on embryo development and mortality. Van der Gaag et al. (1983) said that one of the first effects of chronic toxicity is retarded growth, a cumulative effect of many small adverse effects. MTTH, used as biological endpoint in this study, was variable between sites. While all sites hatched later than the ERS controls, embryos treated with sediment from site D had the longest MTTH and the highest percent mortality. The most dramatic biological effect correlated with the highest concentrations of organohalogens, PCBs, and heavy metals (see Appendix). Interestingly, when sediment was resampled 4.5 months later, deleterious developmental effects and mortality decreased with a corresponding dramatic decrease in pesticides. Organic compounds can be removed from sediments by natural mechanisms such as hydrolysis, oxidation, isomerization, photochemical breakdown, microbial breakdown, and movement by water runoff (Walker et al., 1996). Any or all of these mechanisms could have been responsible for the reduction of toxicants seen in this experiment.

While embryonic development was adversely affected by exposure to post-Katrina sediments, no significantly elevated mutant frequencies were found using the *cII* mutagenesis assay. Increased mutation is frequently observed in conjunction with exposure to elevated concentrations of PAHs (Walker et al., 1996; McElroy et al., 2006). Though PAHs were not as elevated as the organohalogen pesticides in these samples, it is interesting to note that the site with the highest PAH levels (site C) was also the site with the highest MF (Table 1.1; see Appendix).

Though compound concentrations were not exceptionally higher than they were before the hurricane, an obvious sediment toxicity effect was observed. I propose that the observed mortality and morphological effects observed are a result of the cumulative toxicity of multiple contaminants rather than a single compound. Many compounds have been shown to have synergistic effects in regards to toxicity of both organisms and the environment (Walker et al., 1996). Toxicity of PAH mixtures, in particular, are regarded as more acutely toxic that individual PAHs at similar concentrations (Rhodes et al., 2005). Strmac et al. (2002) also suggested synergistic interaction of compounds to explain toxicity displayed in their experiment using river sediments.

In the first study to use *in vivo* toxicity testing in conjunction with sediment contaminant measurements, Detroit River sediments were measured for PAHs, PCBs and organochlorine insecticides (Metcalfe et al., 2000). Negative embryological effects were correlated with the concentrations of PAHs. This study demonstrated the strength of using early developmental assays with medaka to show toxic effects of sediment contaminants (Metcalfe et al., 2000).

PAH toxicity has been more extensively studied in medaka than other compound types. McElroy et al. (2006) suggested that while fairly tolerant of high does of PAHs, a low induction of mortality and increased frequency of developmental abnormalities were observed in medaka when incubated with high doses of PAHs. Embryos have been observed to have delayed hatch times (15-17 days) and decreased hatching success in the presence PAHs (Chikae et al., 2004; Rhodes et al., 2005). Also, PAHs can induce morphological abnormalities such as yolk sac edema, depressed circulation, and small sizes (Rhodes et al., 2005). Incardona et al. (2004) studied morphological abnormalities and cardiac dysfunction in zebrafish embryos exposed to

non-alkylated PAHs and noted that embryos treated with PAHs demonstrated higher incidences of curved spines and a significant reduction of growth.

This study is by no means a complete representation of the toxicity of New Orleans post-Katrina. This study, in conjunction with others like it, reveals contaminant heterogeneity of sites around the city. A complete analysis of sediment toxicity and risk could only be possible if all sediments were sampled in New Orleans after Hurricane Katrina. Instead, the sites we have sampled should be viewed as a snap-shot to make more generalized conclusions regarding the toxicity of New Orleans sediments. This study has important implications. Not only does it generally address the question of toxicity in New Orleans following Hurricane Katrina, but it also suggests that contamination levels, and therefore contamination risk, may decrease over time. Though it would be difficult to extrapolate this information to other urban areas given the variance of samples in New Orleans, we did see a higher toxic effect than would be expected by the contaminant concentrations. These results also indicate that certain chemical compounds can and will decrease in concentration with a corresponding decrease in toxicity over time. This study demonstrates the importance of using a biological model in exotoxicology studies.

Chapter 2-The Characterization and Pathogenecity of a Strain of Bacteria similar to *Hahella chejuensis* found in New Orleans, LA

Introduction

Bacterial pathogens of fish are a diverse group of organisms distributed ubiquitously in the environment. There is no general mode of infection or life cycle for fish pathogens. Pathogens can be transmitted vertically, from mother to offspring, or horizontally from fish to fish, fish to embryo, or from water to fish or embryo; this horizontal transmission can occur by diffusion from the water, contact with other infected organisms, or by ingestion of food or dead infected animals. With some diseases, fish act as carriers, having an infection with no outward symptoms of the disease. It is possible that only stressed carriers release and transmit pathogens to other fish (Hunter et al., 1980). The infectiousness of a pathogen is determined using an LD_{50}: the dose at which mortality occurs in half of the population.

Bacterial adhesion to host surfaces is the first step in microbial pathogenesis (Dawson et al., 1981). Many bacterial pathogens exploit host cell functions to enter non-phagocytic cells by rearranging the microtubules and microfilaments of the cytoskeleton (Gonzalez et al., 1990). Santos et al. (1991) reported that of the *Aeromonas, Vibrio,* and *Yersinia* bacterial species treated with heat, none had decreased hydrophobicity nor did any of the species lose the ability to adhere to cultured fish cells; with this information, the authors suggested that proteins may not be responsible for initial adherence to host cells. In addition, Santos et al. (1991) suggested that surface adherence is mediated by specific mechanisms of the bacterial cell wall. Biofilms are another mechanism that can help bacterial pathogens attach to a host cell (Southey-Pillig et al., 2005). Motility can also important for some pathogens to gain access to a cell; flagellin A protein is necessary for virulence in *Vibrio anguillarum* because when mutated, virulence

decreases by 500-fold (Baya et al., 1992). For many pathogens cytotoxicity is associated with proteases, phospholipases, or both (Baya et al., 1992).

Virulence of many bacterial species has been proposed to be regulated, in part, by quorum sensing genes. These genes allow individual cells to communicate and work together in a coordinated fashion. *Pseudomonas aeruginosa*, a Gram-negative γ-Proteobacteria, has an extensively studied quorum sensing system that has been shown to regulate the expression of extra-cellular virulence factors such as elastases, proteases, and oxidative enzymes, all of which are needed for virulence. Mutants defective in quorum sensing genes are less pathogenic and less virulent than strains with normal quorum sensing genes. (Miller and Bassler, 2001; Juhas et al., 2005).

In fish, there are some general conditions indicative of bacterial pathogens before mortality occurs. Behavioral changes include cessation of feeding, inactivity, and erratic movement such as twirling and spiraling (characteristic of neurological damage). Other physical characteristics include faded or darkened pigment of the skin, hemorrhaging around the eyes, mouth, gills, or fins, distended abdomen, and ulcers and abscesses found throughout the body. Post-mortem inspection also often reveals skeletal deformities, muscle and organ opaqueness and hemorrhaging, swollen organs, and tumors (Austin and Austin, 1999).

The group, *Aeromonadaceae* contains soil-dwelling Gram-negative bacteria. A representative fish pathogen in this family is *Aeromonas salmonicida. A. salmonicida* is considered to be one of the most important fish pathogens due to its widespread distribution and host range (Austin and Austin, 1999). *A. salmonicida* is a non-motile, fermentative, Gram-negative rod that produces a brown, water-soluble pigment. Though initially suspected to only infect salmonid species, *A. salmonicida* has a much broader host range. Traditionally, *A.*

salmonicida causes furunculosis (lesions in muscle) and ulcerative dermatitis. Acute infections usually cause mortality in 2-3 days with bacteria found in the blood and tissue of the animal (Austin and Austin, 1999). Though fish play a major role in the transmission of the disease and many have believed that *A. salmonicida* was an obligate fish pathogen, it has been found to survive in seawater. Though live fish can transmit the bacteria and carriers do occur, infected dead fish have received more attention as the pathogen spreaders (Austin and Austin, 1999). Most infection is believed to come from lateral infection, from fish to fish or water to fish; vertical transmission is not a likely mode of transmission for this particular pathogen. The pathogen may gain entry to a fish host through the gills, lateral line, mouth, anus, a surface injury, or into the gastrointestinal tract associated with food (Austin and Austin, 1999). *A. salmonicida* produces an A-layer that is a prerequisite for virulence as it is associated with adhesion to the host by increasing the hydrophobicity of the bacterium; it also acts to protect the bacteria from host defenses such as macrophages. *A. salmonicida* may also develop capsules to increase adherence and invasion of fish cells (Austin and Austin, 1999).

The *Vibrio spp.* make up the largest group of fish pathogens (Austin and Austin, 1999). Among some of the more notable *Vibrio* pathogenic species are *V. anguillarum, V. harveyi, V. cachariae, V. salmonicida,* and *V. splendidus. V. anguillarum* was the first bacterial fish disease to be described in European literature in the eighteenth century (Bonaveri, 1761; Drouin de Bouville, 1907). It is characterized by red necrotic lesions in abdominal muscle, at the base of fins, and around the vent and mouth with the greatest concentration of bacteria found in the blood. It is a normal part of the microflora in many aquatic environments and can survive in seawater for prolonged periods of time. *V. harveyi* and *carchariae* cause hosts (particularly sharks) to become lethargic, stop eating, and form necrotic cysts and ulcers in the kidneys, liver,

and spleen. *V. salmonicida* was discovered in 1979 and since then has become a serious problem for salmon farms in the Northern hemisphere. Hemorrhaging indicative of infection can be seen around the abdominal organs and swim bladder of infected fish. *V. slendidus* causes swelling of the abdomen, stomach, and intestines with a mucoid liquid and hemorrhaging in the peritoneal cavity (Austin and Austin, 1999). *Vibrio damsela* is a halophilic bacterium that causes disease in both cold and temperate water fish and has been recovered from fish, humans, and directly from seawater (Fouz et al., 1993).

The *Enterobacter* is another family well known for their pathogenic properties. Fish pathogens in this group include *Edwardsiella spp, Serratia spp.,* and *Yersinia spp. Edwardsiella spp.* cause characteristic infections of lesions and abscesses with internal nodules filled with bacteria in many types of fish species around the world. Intra-peritoneal injection of bacteria caused mortality in 80% of fish within 10 days of infection (Meyer and Bullock, 1973). The LD_{50} is approximately 10^6 bacterial cells per fish. *E. tarda* infects by immersion, orally, intra-peritoneally and intra-muscularly (Austin and Austin, 1999). *Serratia spp.* display few to no external characteristics of disease but may cause swelling of organs with the formation of bacterial nodules (Austin and Austin, 1999). *S. marcescens*, a red pigmented bacterium, is a known opportunistic pathogen that has occasionally been associated with fish bacterial infections. *S. marcescens* was shown to be pathogenic to white perch, striped bass, and rainbow trout; intraperatoneal injection was associated with LD_{50} values in the range of 10^3 to 10^4 bacterial cells per fish. Dead fish demonstrated necrosis of muscular tissues and a reddish color along the lateral line of the body and head (Baya et al., 1992). *Yersinia ruckeri*, a bacterium causing Enteric Redmouth, affects mostly salmonid fish, especially rainbow trout. Enteric Redmouth is symptomatic of reddening of the mouth and throat from subcutaneous

hemorrhaging and inflammation of organs and muscles tissue (Austin and Austin, 1999). Though considered to be of "high virulence" (an LD_{50} of between 10^3 to 10^5 cells per fish) *Y. ruckeri* does not display the classical virulence factors (e.g. proteases or toxins) normally found in other pathogenic bacteria (Romalde and Toranzo, 1993).

Several *Pseudomonas sp.* have been implicated in causing fish diseases including *P. angiulliseptica* and *P. fluorescens*. *P. anguilliseptica* infects with few outward physical symptoms, but may cause small hemorrhages around the mouth and ventral area of the body. *P. fluorescens* can cause disease in many types of fish and is mostly associated with "fin and tail rot" and high mortality; symptoms are often associated with stress including a change in water temperature or salinity (Austin and Austin 1999). Most *Pseudomonas* species are thought to be spread through water.

Most virulence factors are produced extracellularly and it is possible for virulence to be associated solely with extra-cellular products (ECP). It is often difficult to pinpoint a specific part of the extracelluar matrix that causes virulence as the ECP contains a combination of proteases, phospholipases, hemolysins, and lipopolysaccharides, all of which have been implicated in pathogeneity of at least one bacterium. *Pseudomonas spp., Vibrio spp., A. salmonicida, Y. ruckeri,* and *S. marcesans* plus many other pathogens produce extracellular products. Injections of ECP from *A. salmonicida, V. damsela, Y. ruckeri,* and *S. marcesans* are lethal, and the virulence of these products decreased with all ECP when heat-treated (Austin and Austin, 1999; Fouz et al., 1993; Romalde and Toranzo, 1993; Baya et al., 1992). Though the exact mechanism of virulence of these ECP for most species has not yet been determined, many consider exotoxins such as proteases and lipases the most important ECP factor for the pathogenicity of bacteria (Austin and Austin, 1999; Romalde and Toranzo, 1993; Baya et al.,

1992). The degree of phospholipase, hemolytic, and cytotoxic activity have been directly correlated with the degree of pathogenesis of many species (Fouz et al., 1993). Lipopolysaccharides, however, are not considered to be the cause of pathogenicity for many pathogens (Fouz et al., 1993; Romalde and Toranzo, 1993).

While describing the toxicity of New Orleans post-Katrina sediments, I observed between 20 and 25% of the embryos becoming red and dying when incubated with sediments from one of these sites (the mouth of the London Avenue Canal). The following is the second chapter of my thesis research; here I report the pathogenesis and characterization of a pigment producing bacterial pathogen found in sediments collected from the shores of Lake Pontchartrain, New Orleans, LA. The bacterium discussed here most closely resembles the Korean mudflat species, *Hahella chejuensis.* This genus has not previously been described as a pathogen. Its distribution and rate of infection in sediments may prove to be important to the significant seafood industry in Southern Louisiana.

Materials and Methods

Bacterial Collection. Samples of surface soils and sediments were collected from the mouth of the London Avenue canal near Lake Pontchartrain, New Orleans, Louisiana in September, 2005 (site A, Figure 1.1). Sediment was shaken overnight with egg rearing solution (ERS: 10 ml solution containing 10% NaCl, 0.3% KCl, 0.4% CaCl2, 1.6% MgSO4, and deionized water up to 1 liter with additional NaCl to 4 g/L) before sediments were removed by centrifugation. A newly fertilized Japanese medaka (*Oryzias latipes*) embryo was added per 1 ml of the treated ERS. Infected eggs (identified by mortality and the aborted embryo turning red in color) were removed, surface sterilized with a 0.03% bleach solution to remove membrane

associated bacteria, rinsed twice with sterile water, and homogenized in 1 ml broth. Broth (100 µl) was spread on marine agar (Difco Marine Agar, 2216) and incubated at 27°C for 2 days. Red pigmented bacteria were isolated, designated New Orleans strain DE4272, and maintained in a glycerol suspension (11% w/v) at -80°C. Liquid cultures were made using broth (marine broth (Difco), tryptic soy broth (1.5% casein digest, 0.5% soytone, and 0.5% NaCl), and Luria-Bertani broth (1% tryptone, 0.5% yeast, 0.5% sodium chloride)) by inoculating broth from a single colony from an agar plate; broth was incubated at 25°C for 2 days while shaking.

Pathogenesis. To confirm pathogenecity of the bacteria, Koch's postulate was confirmed. To do this, medaka embryos were exposed to sediment suspected to be contaminated with bacteria. Infected, red eggs were removed from solution and bacteria were recovered as described above. ERS with healthy medaka embryos was then inoculated with recovered red bacteria and allowed to incubate for 12 days or until infection ensued; the same red bacterium was reisolated from the newly infected medaka embryo.

To determine the lethal dose of the bacteria, bacteria were grown in marine broth at 27°C shaking for 2 days or until an optical density (OD_{500}) of 1.0 was reached. The bacterial solution was vortexed and diluted to expected concentrations of between 10^3 and 10^7 bacteria per ml. Newly fertilized healthy embryos were added to each dilution in 6-well polystyrene plates. Aliquots of each dilution were spread on marine agar plates and each plate was incubated at 27°C. After 2 days, all colonies were counted to determine the actual dose of each dilution used to dose embryos. Dosing was repeated 3 to 8 times per bacterial concentration; for each experiment, non-treated ERS was used as an infection control.

DNA Extraction. DNA was extracted from bacterial colonies grown on marine agar (Marmur, 1969): several colonies were added to 450 µl Tris-EDTA (50 mM Tris, 5 mM EDTA,

pH 8) and 50 µl 5M NaCl and vortexed until a fine cell suspension was reached. Lysozyme (50 µl of a 4mg/ml solution) was added and the mixture was incubated at 37°C for 20 minutes. Sodium dodecyl sulfate solution (10%) was added and tubes were incubated at 55°C for 15 minutes. Proteinase K (50 µl of a 0.2 mg/ml solution) was added and the solution continued to incubate at 55°C until complete lysis was achieved (0.5-3 hours). Three equal volume chloroform extractions were performed to remove protein before 50 µl of 3M sodium acetate and 1 ml of 95% ethanol was added to precipitate the DNA. DNA was removed from solution by spooling with a sealed glass Pasteur pipette. DNA was washed with 70% ethanol and dissolved in high quality distilled water. A hexadecyltrimethyl ammonium bromide-NaCl (10% CTAB in 0.7 M NaCl) extraction was done by adjusting the NaCl concentration to 0.7 M and adding 0.1 volume CTAB-NaCl solution before extracting with chloroform-isoamyl alcohol. A final phenol-chloroform extraction was done to remove any remaining protein. DNA was checked for RNA contamination by electrophoresis. RNase A treatment was added if necessary. DNA purity was determined by 260:280 ratio and DNA concentration was determined by absorbance at 260 nm.

16S rDNA analysis. PCR amplification was done using extracted DNA to amplify the 16S rDNA gene using Borneman et al.'s (1996) procedure. Two separate PCR reactions were done with the following primers: 530F (TGACTGACTGAGTGCCAGCMGCCGCGG) and 1494R (TGACTGACTGAGGYTACCTTGTTACGACTT) or AC181F(AGGTTGATCHTGGTYAG) and AC22R (ACGGNTACCTTGTTACGACTT) (where M = A or C; Y = C or T; H = A, C, or T; and N = A, C, T, or G). DNA (5-20 ng) and primers (20 pmol each) were added to a PuReTaq$_{pm}$ Ready-to-Go PCR bead (GE Healthcare) dissolved in 21 µl nuclease-free water. PCR reactions were placed in a thermocycler using the following

program: 94°C for 2 minutes; 30 cycles at 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; followed by 72°C for 5 minutes. PCR products were purified using Wizard Purification Systems Kit for PCR Preps (Promega). Purified PCR products were then cloned using DH5α *Escherichia coli* cells into the pGEM-T-Easy Vector System which has hanging T ligation ends and a SP6 RNA polymerase promoter (Promega). Plasmid DNA was recovered and digested with NotI to remove the 1500 bp fragment from the plasmid to confirm the rDNA insert.

The purified sequence of the 16S rRNA PCR amplified gene was determined using Big Dye sequencing mix with an ABI3100 Genetic Sequencer. Chromatograms were compared and manually edited where necessary using the Sequencher 4.7 software package (GeneCodes Corporation). Completed 16S rDNA sequence results were compared to known nucleic acid sequences using GenBank's BLAST for nucleic acids feature (Altschul et al., 1997). Using the most related species, an alignment was made with Sequencher 4.7. The most similar sequences found using GenBank's BLAST were used to make a maximum likelihood phylogenic tree using CLUSTALX and Paup software programs (Thompson et al., 1997; Swofford, 2002) with *Aquifex sp.* as an outgroup. Bootstrap values were determined using 100 replicates.

Morphology. Colony morphology was determined using 2-day-old cells grown on marine agar at 27°C. Cell morphology was determined with cells grown in marine broth (Difco) using a Nikon eclipse E800 microscope with oil immersion.

Phenotypic tests. Optimum growth temperature was determined using a range of 15°-50°C with marine agar plates. Salt tolerance was tested with tryptic soy agar (1.5% casein digest, 0.5% soytone, and 1.5% agar) with varying NaCl concentrations (0.5 to 10%). All other metabolic and biochemical tests (see Table 2.1 for list) were performed as described by Leboffe

Table 2.1. Results of metabolic and biochemical tests for New Orleans strain DE4272, *H.chejuensis,* and *H. ganghwensis* determined in a side by side experiment.

	New Orleans DE4272	*H. chej*	*H. gang*
Gram	-	-	-
T_{opt} [a]	27-32°	20-45 °	35°
Optimum Salt	0.5-3%	2%	4-6%
Ab^{r} [b]	amp, pen, sulfa	pen, sulfa	strepto, pen, sulfa
Pigment	red	red	cream
Catalase	+	+	+
Oxidase	-	ND	ND
Citrate	+	+	-
Glucose	-	-/-	+/-
Lactose	-/-	-/-	+/-
Sucrose	-/-	-/-	+/-
Maltose	-/-	-	ND
Treholose	-/-	-/-	+/-
Fructose	-/-	-/-	ND
Galactose	-/-	-/-	+/-
Starch	-	-	+
Decarb. orn.	+	-	+
Decarb. lysine	-	-	ND
Blood agar	-	ND	ND

H2S	-	-	-
indole	-	-	-
Gelatin	-	+	-
Nitrate	No growth	ND	ND
Methyl red	No growth	-	+
Motility	-	-	+

a optimum temperature for growth
b antibiotic resistance

- is a negative test
+ is a positive test
-/- fermentation and gas production
ND is not determined.

and Pierce (2002). Carbohydrates (glucose, lactose, sucrose, trehalose, maltose, fructose, and galactose) were tested using purple broth (1% peptone, 0.1% beef extract, 0.5% NaCl, 0.002% Bromcresol purple, and 1% carbohydrate) and Durham tubes with bacteria grown in broth. To determine the metabolism of citrate, bacteria was spread on a slant of Simmons Citrate Agar (Leboffe and Pierce, 2002). The catalase test was performed using colonies and 3% Hydrogen peroxide. Decarboxylation tests (lysine and ornithine) were conducted using bacteria from broth culture and Decarboxylase medium (0.5% peptone, 0.5% beef extract, 0.05% glucose, and 1% L-lysine or L-ornithine). Bacteria were tested for antibiotic resistance using ampicillin (10 μg), gentamicin (10 μg), tetracycline (30 μg), streptomycin (10 μg), sulfonamide (300 μg), and penicillin (10 μg) disks.

Pigment. Red pigment was extracted from cells by growing bacteria for 5 days on marine agar at 27°C. Colonies were removed from agar and added to methanol-HCl (4 ml 1M HCl per 96 ml methanol) and the solution was vortexed until a fine cell suspension was reached (about 3-5 minutes). The methanol-HCl solution was left on ice for 20 minutes before the cells were removed by centrifugation (Allen et al., 1983). The absorbance spectrum of pigment extracts was determined using a Perkin Elmer Lambda 35 UV/Visible light spectrophotometer.

Determination of Percent G+C. The percent G+C content in the genome was determined using a fluorescence assay modified from Xu et al. (2000). Genomic DNA was diluted to 5 μg/ml and dialyzed overnight in 0.1 x SSC. Between 50 and 100 ng of DNA was added to 2 μl SYBR green PCR master mix dye and the final volume was brought to 20 μl with 0.1 x SSC. Melting temperature (T_m) was determined using a 96 well plate in a real time PCR machine (BioRad MyiQ) using the following program: 1 minute at 70°C, and then temperature increased 0.2°C for 141 cycles with a 10 second delay time at each temperature until 98°C was

reached. Melting temperature was also determined for *Hahella chejuensis* and *Hahella ganhwensis* for comparison. Melting temperature was determined at least three times for each species from the first derivative of relative fluorescing units/temperature (-d rfu/d T). For each trial, all peaks within 10% of the highest peak were averaged to give the T_m. G+C was determined using the T_m in the following equation (Xu et al., 2000):

$$(\%\ G+C)\ x = (\%\ G+C)\ r + 1.4652\ (Tm_x - Tm_r) + 0.0063\ (Tm_x^2 - Tm_r^2)$$

with published values for T_m (85.5) and G+C content (50.5) of *E. coli* used as the reference species (r).

Geographic distribution. To determine the geographic distribution of this bacterium, sediment was collected from the following five sites in Louisiana and Mississippi in June of 2007: the mouth of the London Avenue Canal, near Lake Pontchartrain, New Orleans, LA; Pointe aux Herbes near Bayou Sauvage National Wildlife Refuge, New Orleans, LA which opens to the east shore of Lake Pontchartrain; Buccaneer State Park on the Gulf of Mexico in Waveland, MS; Big Branch Marsh National Wildlife Refuge, Lacombe, LA on the north shore of Lake Pontchartrain; and Port Fourchon, at the southern tip of LA near the Gulf of Mexico (Figure 2.1). Water temperature and salinity were measured at each site at the time of collection. Sediments were collected on the shore near the water's edge and distributed into two conical tubes using a trowel that was disinfected with 70% ethanol between sampling sites; samples were kept in the dark at 4°C for the remainder of the experiment. To determine the presence of bacteria, sediment was distributed into the bottom of a 6 well polystyrene plate and covered with screen. Enough ERS was added to fill the well and 20 embryos were added to each well. Five replicates were completed for each site (n=100 embryos per site). Ten embryos per replicate

Figure 2.1. Map of Southern Louisiana and Mississippi designating where samples were taken to test for bacteria distribution. Sampling sites are indicated with red diamonds.

were incubated in non-treated ERS as a control for bacterial infection. The experiment was terminated 12 days after fertilization.

Results

Pathogenesis. When incubated with sediment from site A (mouth of the London Avenue Canal), approximately 20-25% of medaka embryos turned red and died within 12 days of inoculation (Figure 2.2). Confirmation of Koch's postulate confirmed the pathogenesis of New Orleans Strain DE4272. Bacteria from sediments and bacteria extracted from other infected medaka embryos were both pathogenic to healthy Japanese medaka embryos. Infection was associated with mortality for all embryos.

In dosing experiments, doses determined by colony count were grouped by rounding doses to the nearest order of magnitude. Doses of 10^8 bacteria per ml were infectious 100% of the time whereas doses of 10^3 bacteria per ml were never observed to be infectious; infections at bacterial concentrations in between (10^4-10^7) were much more variable. Infection at doses of 10^7 was highly variable (17%-100%) but generally exceeded 50% (Figure 2.3a). Infection variability was not a result of fish embryos or bacterial differences as in one dosing experiment, using the same clutch of embryos and bacteria grown from a single cell, three doses at 10^7 bacteria per ml had infection frequencies between 10 and 66%. Actual doses (as determined by colony counts) did not always agree with the expected dose (as determined by with a spectrophotometer at OD_{500}). For example, a day at which dosing was done with doses varying between 10^5 and 10^7 bacteria per ml according to the OD_{500}, actual doses were all within the 10^7 magnitude. When the expected doses as determined by OD were used for comparison, infection frequency was just

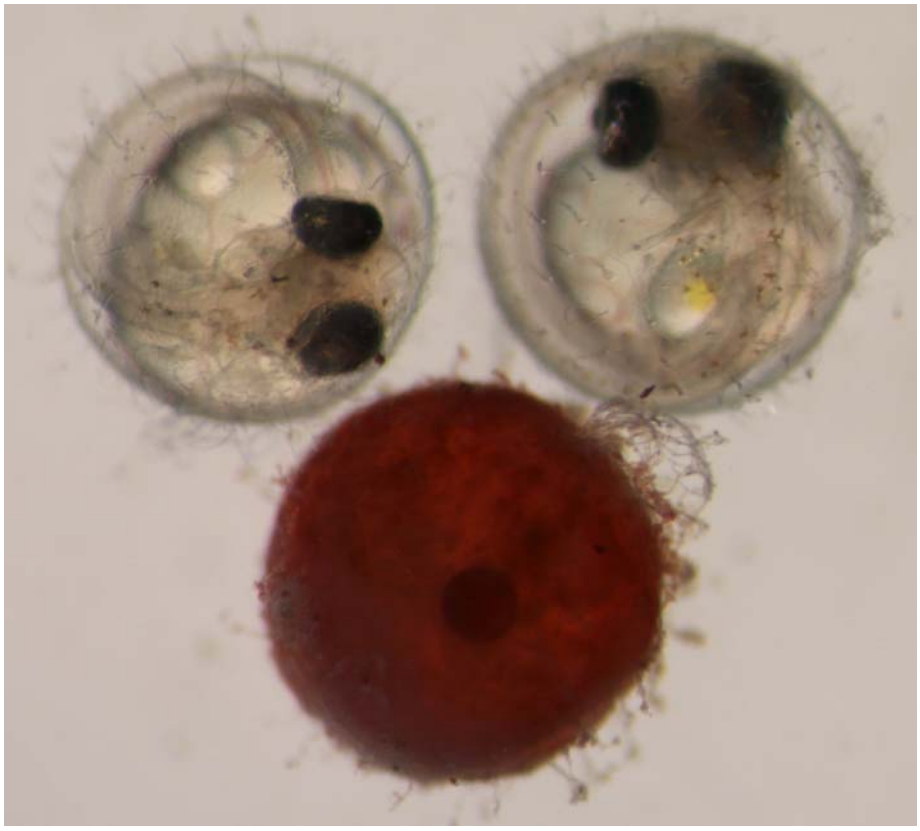

Figure 2.2. Embryo infected with red bacterium, New Orleans strain DE4272 (bottom). Live embryos at the top of the figure are for comparison of normal Japanese medaka embryos.

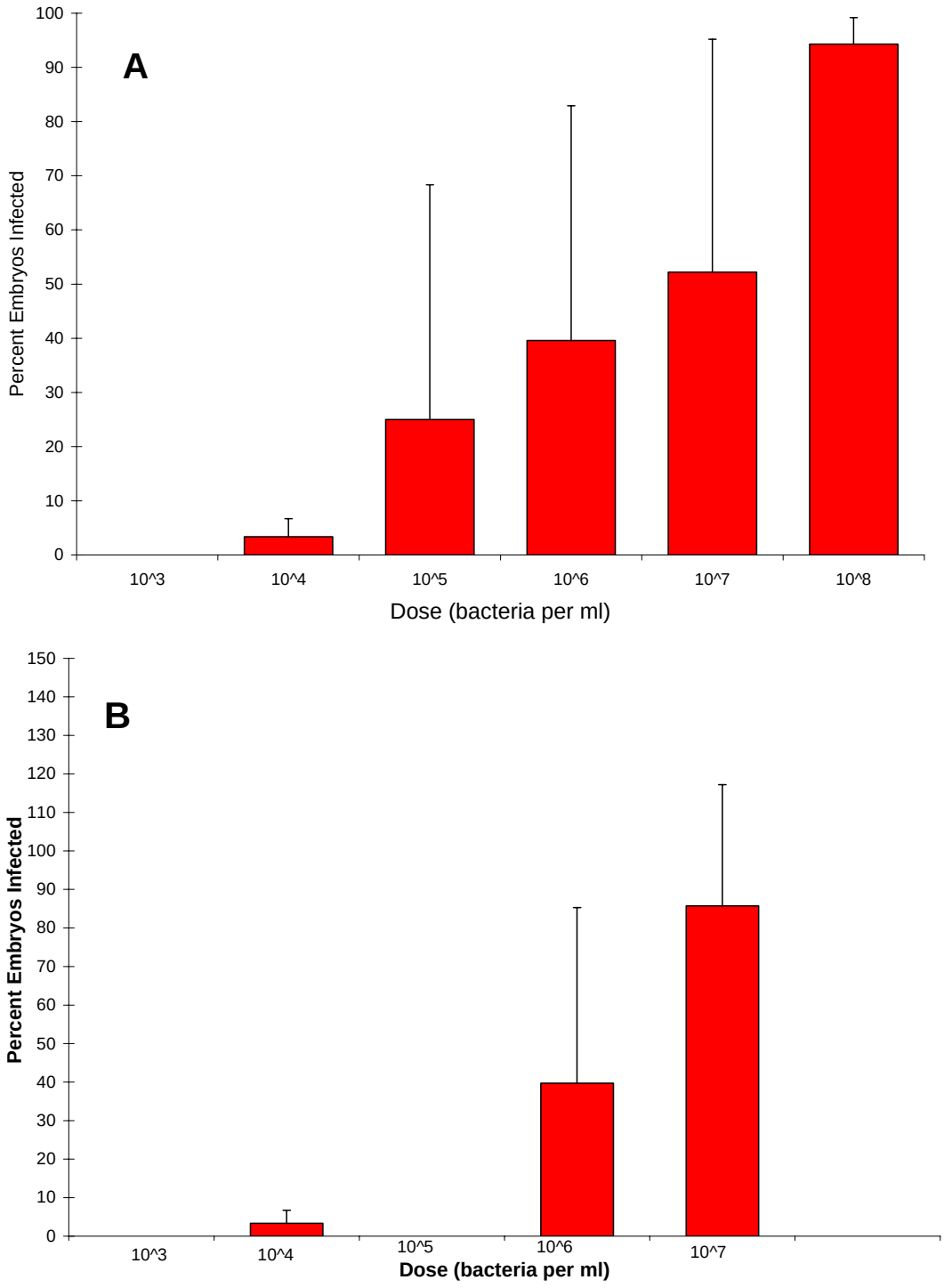

Figure 2.3. A. Percent embryos becoming infected by red bacteria by day 12 after fertilization. Doses were determined by colony counts and were then rounded to the nearest order of magnitude. Standard deviation bars show the high variability of dosing. B. Percent embryos infected by 12 days after fertilization when dose concentrations were determined by OD.

as variable as with colony counts (Figure 2.3b). Based on either approach for determining bacterial concentration, the LD50 appear to be between 10^6 and 10^7 cells per ml.

DNA. Sequencing successfully identified 1445 bp of the 16S rDNA gene (96% of the 1500 bp gene) (Appendix B). Preliminary comparisons indicated the bacterium is part of the γ-Proteobacter group, and based on the 16S rDNA similarity, strain DE4272 is most closely related to *Hahella chejuensis* (99% sequence identity); the 1% base differences all constituted of clearly distinguishable differences when using the chromatogram (Sequencer 4.7). Other closely related species include *Hahella ganghwensis* (94%), *Marinobacter taiwanensis* (90%), an *Alteromonadaceae sp.* (90%), and a *Pseudomonas sp.* (89%). The maximum likelihood phylogenetic tree is shown in Figure 2.4 with bootstrap values taken from 100 replicates shown at each branch.

Morphology. New Orleans strain DE4272 forms round colonies about 2 mm in diameter after 2 days of growth on marine agar at 27°C. Colonies are shiny and red in color and developed undulations after several days of growth (Figure 2.5). When bacteria are grown in broth without shaking, the bacteria form a thick pellicle at the surface of the liquid with very little dispersion throughout the broth. When broths were shaken, however, bacteria dispersed throughout broth and stained the broth red (as it remained red even after cells were removed by centrifugation). Individual cells are Gram-negative, cocco-bacilli shaped, and are non-motile (Figure 2.6).

Metabolic Tests. The results of the metabolic and biochemical tests are summarized in Table 2.1. Results are given in comparison to the two closest related species, *Hahella spp.* as determined in a side-by-side experiment. New Orleans strain DE4272 grew best between 25° and 32°C, but growth was observed up to 40°C; the bacteria grew best between 0.5% and 3%

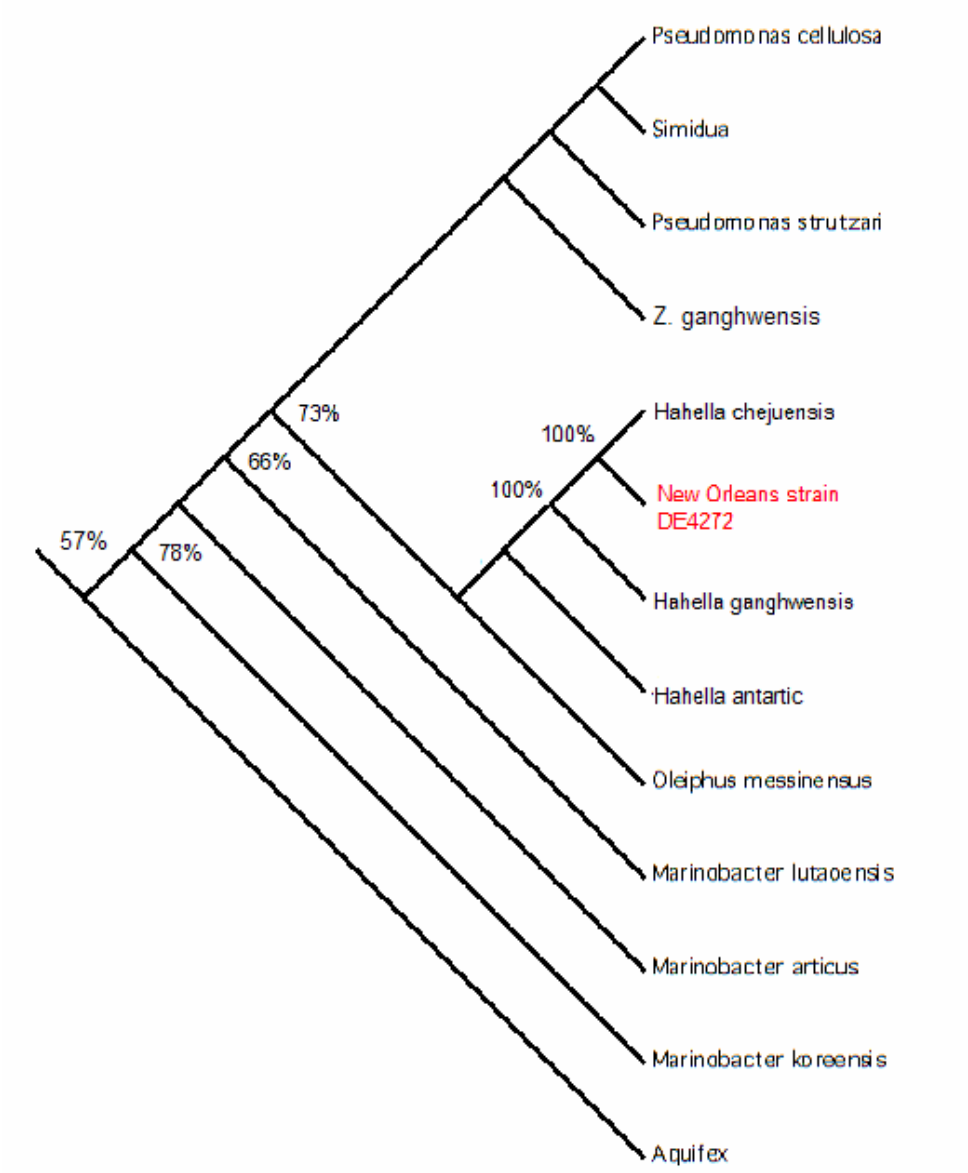

Figure 2.4. Phylogenetic tree with New Orleans strain DE4272 in red. The tree shows that New Orleans strain DE4272 most closely identifies with *H. chejuensis.* Bootstrap values were made using 100 trials.

Figure 2.5. Colony morphology on marine agar after 2 days of growth at 27°C. Colonies, about 2 mm in diameter, are red, shiny and have distinct undulations on the surface of the colony indicating the formation of biofilm.

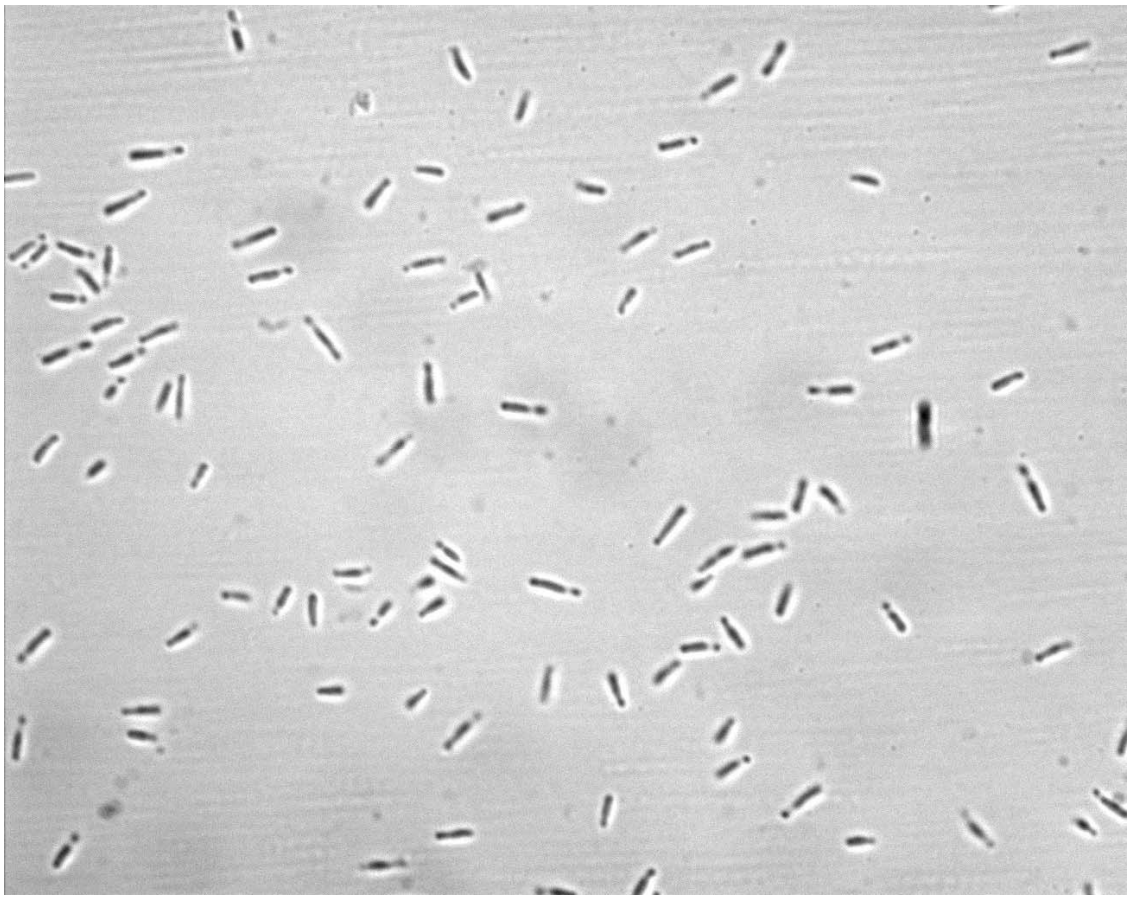

Figure 2.6. Cell morphology seen at 100x on a compound microscope. Individual cells are cocco-bacillus shaped, Gram-negative, and non-motile.

salinity, though growth was observed up to 5% NaCl. Bacteria were resistant to ampicillin, penicillin, and sulfonamide. The red pigment produced by strain DE4272 absorbed maximally at 536 nm (Figure 2.7).

G+C Content. The melting temperatures were determined for the genomic DNA of New Orleans strain DE4272 and two other *Hahella spp* (Table 2.2). The average melting temperature for the New Orleans strain was 87.5°C ± 0.6° (n = 3 trials), with a resulting G+C content of 55.8%. *H. chejuensis* had a melting temperature of 87.5°C ± 0.7° (n= 5 trials) and a G+C content of 55.8%. The average T_m determined for *H. ganghwensis* was 83.8°C ± 1.1° (n = 4 trials) with a G+C content of 46.5%. G+C content values for both *H. chejuensis* and *H. ganhwensis* match published values (Lee et al., 2001; Baik et al., 2005).

Bacterial Distribution. Table 2.3 summarizes the data concerning geographic distribution of the bacteria. Water temperature ranged from 29°-33°C on the day of collection. Areas sampled from Lake Pontchartrain had salinities of less than 5 ppt (<0.5%) and water sampled from Buccanear State Park in Waveland, MS had a salinity of over 10 ppt (1%). Bacteria were recovered from the three sediment samples taken from the shores of Lake Pontchartrain. Bacteria were consistently found in sediments collected at the mouth of the London Avenue Canal, New Orleans, LA between 20 and 30% of embryos incubated with sediment. Bacteria were also found in sediments from Bayou Sauvage and Big Branch Marsh where 2% of total eggs incubated in sediments from each site became infected. Neither Gulf of Mexico sediment samples demonstrated infection of red bacteria.

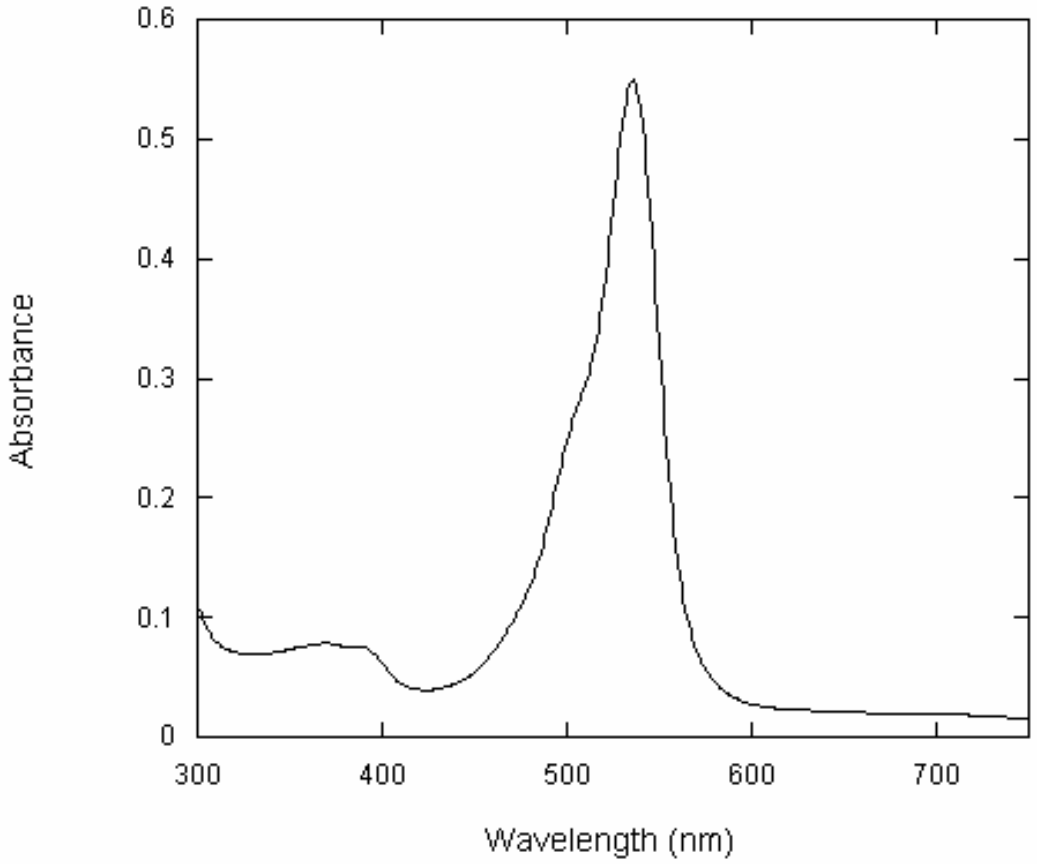

Figure 2.7. Absorbance spectrum of pigment which was methanol-HCl extracted from New Orleans strain DE4272. Maximum absorbance (536 nm) indicates the pigment is prodigiosin.

Table 2.2. Average melting temperature of genomic DNA (taken from 3 trials for NO strain DE4272; 5 trials for *H. chejuensis*; and 4 trials for *H. ganghwensis*) shown with standard deviation.

Species	**Tm**	**G+C content**	**Published G+C**
NO Strain DE4272	87.47° ± 0.6°	55.8 %	NA
H. chejuensis	87.46° ± 0.7°	55.8 %	55[a]
H. ganghwensis	83.8° ± 1.1°	45.5%	44[b]

a Lee et al., 2001
b Baik et al., 2005

Table 2.3. Distribution of New Orleans strain DE4272 in Lake Pontchartrain and the Gulf of Mexico as determined using sediment and 100 healthy Japanese medaka embryos. Water quality data that was determined at time of collection is also included.

	Water Temp (°C)	Water Salinity[a]	% Red Embryos[b]
Mouth of London	29.1	2.9	27
Avenue Canal			
Pointe aux Herbes	32.2	4.9	2
Buccaneer State park	32.8	11.4	0
Big Branch Marsh	31.5	3.3	2
Fourchon, LA	NA	NA	0

a parts per thousand
b on day 12

Discussion

The phylum subclass with which New Orleans strain DE4272 identifies, γ-Proteobacteria, is a large and physiologically diverse group of Gram-negative bacteria. This phylum also includes many well-known and well-studied pathogens such as *Entero, Vibrio, Serratia*, and *Pseudomonas* species. The genus *Hahella* belongs to the family *Hahellaceae* and the order *Oceanospirillales*. The genus *Hahella,* was proposed by Lee et al. (2001) to describe a red-pigmented marine bacterial strain in the phylum subclass γ-Proteobacteria. In 2005, Baik et al. discovered a genetically related species lacking the red pigment, and the genus *Hahella* was modified to include *H. ganhwensis*, a cream-pigmented bacterium. Both species were isolated from marine mud-flat sediment samples in the Republic of Korea. Both are Gram-negative, oxidase and catalase positive, and halophilic. More recently (2007), a third *Hahella* species was identified in the Antarctic (Lee, unpublished, NCBI accession # EF495227).

Based on genetic, morphological, and metabolic tests, New Orleans strain DE4272 most closely resembles *H. chejuensis*. The 16S rDNA gene sequence identity (99%) indicates the bacteria are at least very closely related, if not strains of the same species. DNA hybridization should also be done to confirm if New Orleans strain DE4272 is truly a sub-strain of *Hahella chejuensis*. *H. chejuensis* is a polysaccharide producing, red pigmented, Gram-negative bacteria, as is New Orleans strain DE4272. Most metabolic test results were the same for *H. chejuensis* and the New Orleans strain when tested in the side by side experiment; those tests that differed (Methyl-red, gelatin, and ornithine decarboxylase) may just represent strain differences. Though many of the metabolic results for *H. chejuensis* determined in this study did not match published data (Lee et al., 2001), this could be because of experimental differences such as salinity, temperature, and changes formed in laboratory bacteria. It is also possible that Lee et al. (2001)

characterized many of their metabolic tests as being able to metabolize a substrate, whereas in this study, metabolism of carbohydrates was characterized by the ability of the bacterium to ferment the substrate and to produce gas.

As demonstrated in this study, New Orleans strain DE4272 is pathogenic to Japanese medaka embryos. While doses of 10^8 were infectious to nearly 100% of embryos, serially decreasing the dose did not decrease the toxicity of the pathogen in a predictable manner; instead, infection was sporadic at concentrations between 10^4 and 10^7. Though quorum sensing genes have not been described in *Hahella spp.*, quorum sensing is one explanation as to how and why infection variability may occur. With quorum sensing, virulence is not expressed until a certain minimum number of cells is reached; it is possible that the New Orleans strain DE4272 cannot enter and become pathogenic to a fish cell until some critical number of cells is reached. Quorum sensing has also been associated with biofilm formation. When grown in broth, New Orleans strain DE4272 forms a thick pellicle at the surface of the liquid. Also, during dosing experiments, a thin pink film was left behind on the surface of the polystyrene plates used. These data suggest that this strain is a biofilm-forming bacterium. Biofilm may aid with attachment to the fish embryonic chorions providing a source of entry for the bacteria. Biofilm formation may help explain the divergence of dosing amounts between the OD dose and actual dose: if even a few cells were clumped together with a biofilm (as it may have been impossible to completely separate each cell by vortexing) the OD estimate may have been an overestimation of what was actually in the broth.

Maximum absorbance of methanol-HCl extracted pigment, along with mass spectrometry data (personal communication, Kan Chen) indicates the pigment produced by New Orleans strain DE4272 is prodigiosin or an analog thereof. Prodigiosin has previously been extracted from

other red bacteria such as *Serratia marcescens*, and *H. chejuensis* (Cruz-Camarillo and Sanchez-Zuniga, 1968; Jeong et al., 2005) and thought to be under quorum control in *S. marcescens* (Thomson et al., 2000). Prodigiosin may have antibacterial, immunosuppressive, and cytotoxic capabilities (Han et al., 1998). If prodigiosin does in fact have cytotoxic activity, this is another possible mechanism for the pathogenesis of New Orleans strain DE4272 demonstrated toward Japanese medaka embryos. The mechanism of pathogenesis would also be interesting to know, as well as if the extra-cellular products produced by New Orleans strain DE4272 are virulent and if so, if the pathogenicity is removed after heat treatment.

H. chejuensis as well as *H. ganghwensis* was discovered in mudflats of Korea. Areas in which the New Orleans strain DE4272 was found are also mudflat-like habitats, defined as sheltered coastal areas near estuaries, bayous, or canals. New Orleans strain DE4272 was found in sediments from three areas around Lake Pontchartrain, LA, including both the north and south shores. In a limited sampling experiment, bacteria were not found in areas on the Gulf coast, outside of the lake system (Buccaneer State Park, MS or Fourchon Port, LA). Though the bacterium was only found in sediments from sites associated with the lower (0.5%) salinities, higher salinity is probably not the cause for the lack of recovery of the bacteria from Buccaneer State Park, MS or Fourchon, LA (1+% NaCl) since *in vivo*, the bacteria grew well at salinities exceeding this value. It is possible that the New Orleans strain DE4272 does occur in these areas, but was just not recovered in these sampling efforts.

It is clear that New Orleans strain DE4272 is a pathogen for Japanese medaka. In other experiments (Don Ennis, personal communication) New Orleans strain DE4272 was also seen to be pathogenic to zebrafish *in vivo*; there is no reason to suspect that the bacteria would not also be pathogenic to many other, local fish species that lay eggs in Lake Pontchartrain. The

frequency of infection is likely attributed to several factors, including virulence of the bacterial strain as well as the susceptibility of the target species. More work needs to be done to describe the pathogenicity of New Orleans strain DE4272 toward other fish species and life stages, particularly those living in Lake Pontchartrain.

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Appendix A

Appendix Table A.1. Organohalogen pesticide concentrations in Post-Katrina New Orleans sediments from five collection sites. Concentrations are in ng/g (ppb). Values that exceed EPA Region 6 Human Health Medium Specific Screening Levels (HHMSSL) are shown in bold.

Compound[a]	CAS No.[b]	HHMSSL[c]	A	B	C	D	E	D2
Aldrin	309-00-2	29(C)	ND	ND	ND	**1930**	ND	ND
1-bromo-2-nitrobenzene	577-19-5	NA	920	515	924	886	1813	ND
Chlordane (alpha)	5103-71-9	1600(C)[d]	ND	ND	126	471	51	52
Chlordane (gamma)	5566-34-7	1600(C)[d]	35	14	212	ND	110	287
Chlordene	3734-48-3	NA	ND	ND	260	1264	ND	ND
Chlorobenzilate	510-15-6	1800(C)	ND	ND	ND	**7745**	ND	29
Cis-nonachlor	5103-73-1	NA	ND	ND	192	1224	81	ND
4,4' DDD	72-54-8	2400(C)	29	ND	466	494	ND	ND
4,4' DDE	72-55-9	1700(C)	ND	ND	149	**1720**	ND	1079
4,4' DDT	50-29-3	1700(C)	ND	ND	1294	1084	ND	ND
Diallate	2303-16-4	NA	ND	ND	33	177	ND	ND
Dieldrin	60-57-1	30(C)	ND	ND	**69**	**4885**	ND	ND
Endosulfan I	959-98-8	$3.5x10^5$(N)[e]	233	ND	ND	2367	1099	625
Endosulfan II	33213-65-9	$3.5x10^5$(N)[e]	ND	ND	456	5270	ND	105
Endosulfan sulfate	1031-07-8	NA	4094	ND	1300	9931	ND	ND
Endrin	72-20-8	$1.8x10^4$(N)	ND	ND	ND	8242	ND	261
Endrin aldehyde	7421-93-4	NA	ND	ND	2152	4562	55	ND
Endrin keton	53494-70-5	NA	3468	ND	16552	13793	5933	301
HCH (alpha)	319-84-6	90(C)	26	ND	**431**	**720**	**205**	**123**
HCH (beta)	319-85-7	320(C)	ND	ND	197	**329**	ND	ND
HCH (delta)	319-86-8	NA	ND	ND	97	462	ND	1260
HCH (gamma)	58-89-9	440(C)	ND	ND	243	**1138**	ND	ND

Heptachlor epoxide	1024-57-3	53(C)	ND	16	ND	ND	ND	ND
isomer B)								
Hexachlorobenzene	118-74-1	300(C)	ND	ND	ND	54	ND	ND
Isodrin	465-73-6	NA	ND	ND	438	1583	ND	ND
Methoxychlor	72-43-5	$3.1x10^5$(N)	276	538	3989	29317	1192	ND
Trans-nonachlor	39765-80-5	NA	47	ND	229	4641	46	93
Total			9128	1083	29809	104289	10585	4215

ND Below the limit of detection (10-100 ng/g).
NA Data not available.
a Compounds that were below limit of detection in all sediment samples were 1,2-dibromo-3-chloropropane (CAS No. 96-12-8) and heptachlor (CAS No. 76-44-8).
b Chemical Abstracts Service Registry Number.
c EPA Region 6 Human Health Medium Specific Screening Levels (updated May 2007) for residential soils. Values in ng/g dry sediment followed by C for cancer risk or N for non-cancer risk.
d HHMSSL for total chlordane (alpha and beta isomers).
e HHMSSL for total endosulfan (I and II).

Appendix Table A.2. PCB congener concentrations in Post-Katrina New Orleans sediments from five collection sites. Concentrations are in ng/g (ppb).

PCB congener[a]	CAS No.[b]	A	B	C	D	E	D2
1	2051-60-7	BD	BD	BD	BD	BD	2
5	16605-91-7	5	2	13	51	7	1
18	37680-65-2	BD	BD	BD	22	BD	BD
29	15862-07-4	BD	BD	1	18	BD	2
31, 50, 77	16606-02-3	BD	BD	2	60	BD	3
	62796-65-0						
	32598-13-3						
44	41464-39-5	BD	BD	2	3	BD	BD
52	35693-99-3	BD	BD	1	3	BD	BD
66	32598-10-0	BD	BD	13	4	BD	BD
101	37680-73-2	BD	BD	2	BD	BD	2
138[c]	35694-06-5	BD	BD	BD	13	BD	38
141	52712-04-6	BD	BD	12	56	BD	BD
151	52663-63-5	BD	BD	8	BD	BD	BD
170	35065-30-6	BD	BD	8	54	BD	BD
180	35065-29-3	BD	BD	BD	BD	BD	35
183	52663-69-1	BD	BD	BD	18	BD	19
187	52663-68-0	BD	BD	BD	13	BD	BD
188	74487-85-7	BD	BD	BD	35	BD	BD
208	52663-77-1	BD	BD	BD	17	BD	78
Total		5	2	62	367	7	180

BD Below the limits of detection (<1 ng/g).

a PCB congeners that were below limit of detection in all sediment samples were PCB-87 (CAS No. 38380-02-8); PCB-104 (CAS No. 56558-16-8); PCB-110 (CAS No. 38380-03-9); PCB-154 (CAS No. 60145-22-4); PCB-153 (CAS No. 35065-27-1); PCB-201 (CAS No. 40186-71-8); and PCB-206 (CAS No. 40186-72-9).

b Chemical Abstracts Service Registry Number.

c synonymous with PCB-137

Appendix Table A.3. PAH concentrations in Post-Katrina New Orleans sediments from five collection sites. Concentrations are in ng/g dry sediment (ppb). Values that exceed EPA Region 6 Human Health Medium Specific Screening Levels (HHMSSL) are shown in bold.

Compound[a]	CAS No.[b]	HHMSSL[c]	A	B	C	D	E	D2
Acenaphthene	83-32-9	$3.7x10^6$(N)	12	BD	84	40	6	5
Acenaphthylene	208-96-8	NA	30	BD	55	69	49	26
Anthracene	120-12-7	$2.2 x10^7$(N)	57	5	214	181	50	43
Benz(a)anthracene	56-55-3	150(C)	64	6	**251**	**157**	80	132
Benzo(a)pyrene	50-32-8	15(C)	**36**	3	**111**	**79**	**40**	**131**
Benzo(b)fluoranthene	205-99-2	150(C)	BD	4	140	96	65	BD
Benzo(g,h,i)perylene	191-24-2	NA	13	1	37	26	21	94
Benzo(k)fluoranthene	207-08-9	1500(C)	39	4	145	BD	64	215
Benzo(e)pyrene	192-97-2	NA	33	3	107	BD	52	129
Chrysene	218-01-9	15000(C)	82	9	327	212	143	174
4H-Cyclopenta(def)-phenanthrene	203-64-5	NA	9	BD	63	234	8	117
Dibenz(a,h)anthracene	53-70-3	15(C)	13	BD	**38**	**22**	**20**	**90**
Fluoranthene	206-44-0	$2.3x10^6$(N)	206	25	979	591	340	197
Fluorene	86-73-7	$2.6x10^6$(N)	BD	BD	87	48	5	BD
1-Methylphenanthrene	832-69-9	NA	11	BD	65	245	8	112
Naphthalene	91-20-3	$1.2x10^5$(N)	68	3	161	139	37	108
Phenanthrene	85-01-8	NA	114	11	632	280	81	147
Pyrene	129-00-0	$2.3x10^6$(N)	199	23	916	11	313	310
Total			986	97	4412	2430	1382	2030

BD Below the limits of detection (1-5 ng/g).
NA Data not available.
a Indeno(1,2,3-cd)pyrene (CAS No. 193-39-5) was below limit of detection in all sediment samples.
b Chemical Abstracts Service Registry Number.
c EPA Region 6 Human Health Medium Specific Screening Levels (updated May 2007) for residential soils. Values in ng/g dry sediment followed by C for cancer risk or N for non-cancer risk.

Appendix Table A.4. Heavy metal concentrations in Post-Katrina New Orleans sediments from five collection sites. Concentrations are in μg/g dry sediment (ppm). Values that exceed EPA Region 6 Human Health Medium Specific Screening Levels (HHMSSL) are shown in bold.

Metal	CAS No.[a]	HHMSSL[b]	A	B	C	D	E
As	7440-38-2	0.39(C), 22(N)	**2**	BD[c]	**1**	**9**	**19**
Cd	7440-43-9	39(N)	1	BD	BD	3	BD
Co	7440-48-4	900(C)	2	BD	1	5	2
Cr	7440-47-3	210(C)	3	BD	1	5	2
Cu	7440-50-8	2900(N)	18	3	12	37	15
Hg	7487-94-7	23(N)	0.062	0.006	0.100	0.331	0.042
Mn	7439-96-5	3200(N)	168	25	38	235	191
Ni	7440-02-0	1600(N)	8	BD	3	10	4
Pb	7439-92-1	400(N)	37	7	53	127	62
Ti	7440-32-6	NA[e]	22	2	9	33	10
V	7440-62-2	390(N)	4	BD	2	11	4
Zn	7440-66-6	$2.3x10^4$ (N)	40	11	58	477	64
Total			305	48	178	952	373

BD Below the limits of detection (1 μg/g except for Hg, where the detection limit was 1 ng/g).

NA Data not available.

a Chemical Abstracts Service Registry Number.

b EPA Region 6 Human Health Medium Specific Screening Levels (updated May 2007) for residential soils. Values in ng/g dry sediment followed by C for cancer risk or N for non-cancer risk.

Appendix B

Sequence data from 16S rDNA from New Orleans strain DE4272.

AGATTGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGGTAACAGTCCTTC
GGGAGCTGACGAGCGGCGGACGGGTGAGTAAAGCATAGGAATCTGCCTGTTAGAGG
GGGATAGCCCGGGGAAACTCGGATTAATACCGCATACGCCCTACGGGGGAAAGCAG
GGGATCTTCGGACCTTGCGCTAACAGATGAGCCWATGTCCGGATTAGCTAGTTGGT
AGGGTAAGAGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGAYCAGTC
ACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGG
GTTGTAAAGCACTTTCAGTGGGGAGGAAAGGGCAGTTGCTAATATCAGCTGCAGTT
GACGTTACCCACAGAAGAAGCACCGGCAAACTCCGTGYCAGCAGCCGCGGTAATAC
GGAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGTTGC
TTAAGCTAGATGTGAAATCCCCCGGGCWCAACCTGGGAACTGCATTTAGAACTGGG
CGACTAGAGTTTTGGAGAGGAGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAG
AGATCTGGAGGAATACCAGTGGCGAAGGCGGCCCTCTGGCCAAAAACTGACGCTGA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA
ACGATGAGAACTAGCCGTTGGGGTCCTTAGAGACTTTAGTGGCGCAGCTAACGCGA
TAAGTTCTCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTT
ACCTGGCCTTGACATCCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC
TGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTC
CCGTAACGAGCGCAACCCTTGTCCCTATTTGCCAGCACTTCGGGTGGGAACTTTATG
GGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGGC
CCTTACGGCCAGGGCTACACACGTGCTACAATGGGGCGTACAGAGGGTTGCGAAGC
CGCGAGGTGGAGCTAATCTCTTAAAGCGTCTCGTAGTCCGGATTGGAGTCTGCAACT
CGACTCCATGAAGTCGGAATCGCTATGTAATCGCGAATCAGAATGTCGCGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGA
ATGTA

Vita

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