Fishes as Sources of *E. coli* Bacteria in Warm Water Streams

Final Report
August 4, 2010

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Executive Summary

Bacteria and other pathogens are a growing concern in Texas waters because the majority of water quality impairments on the 303(d) list are for bacteria. Nearly 50% of the designated streams in the Houston and Galveston area are impaired due to elevated levels of indicator bacteria. In some urban watersheds, streams routinely exhibit indicator bacteria levels that are more than ten times the contact recreation standard. Potential sources of indicator bacteria include humans, domesticated animals and wildlife. Determining the relative contributions from each source is a first critical step in determining the most effective management strategy. One potential source that has not been evaluated in past studies is freshwater fishes. This data gap is due in part to the prevailing opinion by various water quality professionals that only mammals and birds can serve as vectors for these bacteria. However, due to the subtropical climate and high diversity and numbers of fish that potentially inhabit coastal streams and bayous in Texas, it is imperative that this source be investigated.

The primary objectives of our study were to 1) conduct a comprehensive literature review of the likelihood of production of *E. coli* bacteria by freshwater fishes found in warmwater streams, 2) determine whether wild caught fish representing various species and trophic groups from Harris County waterways produce feces with detectable levels of the indicator bacteria, *E. coli*, and 3) determine whether native fish retained in aquaria transmit *E. coli* bacteria to ambient water, 4) conduct a literature review to determine likely densities of native fish in urban bayous and feces production rates and 5) using these data, estimate potential loading rates of *E. coli* bacteria into urban bayous from freshwater fishes. We therefore conducted a study during 2008-2009 to evaluate the potential role of fish as sources of indicator bacteria. During our study we utilized a multiple prong approach including a comprehensive review of scientific literature, review of historical water quality, intensive field surveys and laboratory bioassays.

Based on multiple lines of evidence obtained form our literature review, field surveys, and aquarium exposure studies we were able to develop information that will be useful for water quality managers dealing with human health microbiological criteria and water quality management. Based on data collected during our study and a careful review of the literature we concluded that wild fish in southeast Texas and elsewhere can contain high levels of indicator bacteria including *E. coli*. Past studies documented in the scientific literature provide numerous examples of various species of fish containing detectable levels of indicator bacteria. Highest levels of indicator bacteria in fish species appears to be correlated with warm eutrophic conditions associated with subtropical environments and/or areas containing sources of indicator bacteria. The levels of *E. coli* in wild fish species appear to follow trends similar to ambient water and sediment concentrations. There does not however appear to be a strong consistent pattern in *E. coli* levels between trophic levels or species groups of

fishes. The laboratory aquarium study documented low levels of indicator bacteria in water, while relatively high levels of bacteria in feces were detected in some of the fish. Reasons for lack of a strong correlation include reduced feeding and defecation rates and potentially low stocking rates.

Limited genetic data shows that bacteria that are found in fish are genetically related to forms found in warm-blooded species including waterfowl. Few data exists that supports the origin of fish specific forms or strains of *E. coli* or other indicator organisms. However, there is substantial evidence that depending on temperature and ambient conditions that *E. coli* picked up by foraging fish can survive for long periods in the digestive system and possibly increase in numbers.

Literature and limited site data provide sufficient documentation that rivers and bayous in our area likely harbor 1000's of fish per hectare of water weighing up to 100's of kilograms. These high populations of fish can produce large amounts of fecal material. Production of feces by fish is dependent upon feeding rates which is influenced both by ambient temperature, prey/food availability, and trophic level. These feces and associated bacteria can be a highly significant source of indicator bacteria. Upon defecation much of this material would most likely be deposited in bottom sediments. Fecal associated microorganisms would most likely thive in these fecal enriched sediments.

Whether fish are actually producing a new source of indicator bacteria within the watershed or simply harboring microorganisms that are being biologically transported is still debatable. Many species of fish can swim long distances. Therefore, fish provide an extremely efficient mechanism to transport indicator bacteria within and between watersheds. This movement can also occur upstream in unpredictable complex patterns depending on flow regime. This mechanism of transport is not currently addressed in any water quality model dealing with microbiological loading, growth and transport.

Fish are not likely major new source of bacteria but more field and laboratory research and monitoring is needed to evaluate the relationship between fish and indicator bacteria at elevated summertime temperature regimes. We recommend that additional laboratory and field experiments and monitoring be conducted to further quantify and clarify these relationships in an effort to develop predictive models. The focus of these studies should be to determine whether fish are serving as 1) simply transporters of indicators bacteria, 2) transporters and incubators for further growth or 3) transporters, incubators and serving as a new unique source of indicator bacteria. . Examination of feces obtained from wild fish in area streams using molecular genetic methods are needed to help evaluate the role of fish as transport vectors of indicator bacteria or originators of unique indicator bacteria.

Fish provide a major mechanism for transporting bacteria in non-linear fashion. We recommend that further studies be conducted with members of each major trophic group using mark-recapture and/or telemetry methods to evaluate short-term and long-term movement within streams and rivers in the Harris County area and adjacent counties.   This data along with population and fecal production estimates could be incorporated into future simulation models to estimate potential movement and fate of indicator bacteria transported by fishes. In addition, estimates of biomass and density are sorely needed for our area streams and rivers. New studies are needed to obtain this information. Related bioenergetics studies of native fish are needed to also estimate fecal production rates which can be combined with fish abundance and movement estimates to obtain overall loading estimates of indicator bacteria.

The role of other cold-blood aquatic vertebrates as sources of *E. coli* and other indicator bacteria such as amphibians, alligators, water snakes, and turtles should be investigated. The limited data on amphibians and reptiles suggest that they may actually serve as original sources of indicator bacteria in addition to serving as transport vectors. Unfortunately data on the abundance and movement of amphibians and reptiles in local waterways is relatively rare.

Introduction

Bacteria and other pathogens are a growing concern in Texas waters because the majority of water quality impairments on the 303(d) list are for bacteria. Nearly 50% of the designated streams in the Houston and Galveston area are impaired due to elevated levels of indicator bacteria (TCEQ 2007a; TSL 2000). In some urban watersheds, streams routinely exhibit indicator bacteria levels that are more than ten times the contact recreation standard. Indicator bacteria include fecal coliform, *Escherichia coli (E. coli)* and Enterococci bacteria. Although not generally harmful themselves, they are often correlated with the presence of pathogenic (disease-causing) microorganisms that are present in human and animal digestive systems. Therefore, high levels of these indicator bacteria in streams would indicate an increased risk of exposure to pathogenic microorganisms in water. People swimming in these streams would be at a higher risk of contracting waterborne pathogens. One of the barriers to effective management of indicator bacteria levels and associated pathogens is successful source identification.   Potential sources of indicator bacteria include humans, domesticated animals and wildlife. Determining the relative contributions from each source is a first critical step in determining the most effective management strategy.

Several potential sources of indicator bacteria exist within urban streams and bayous in Harris County including contaminated runoff and storm water, malfunctioning wastewater collection systems, improperly functioning wastewater plants, wildlife and domesticated animals. The final report to the TCEQ of Total Maximum Daily Loads for fecal pathogens in Buffalo Bayou and White Oak Bayou indicates a predominance of human sources for *E. coli* in Buffalo Bayou after rain events and a higher proportion of non-human sources in dry weather (Rifai 2006; Petersen et al. 2006). During dry weather wastewater plants and storm sewers were also a major source (Petersen et al. 2005). Determining the relative contributions from each source is a first critical step in determining the most effective management strategy. One potential source that has not been evaluated in past studies is freshwater fishes. This data gap is due in part to the prevailing opinion by various water quality professionals that only mammals and birds can serve as vectors for these bacteria. However, due to the subtropical climate and high diversity and numbers of fish that potentially inhabit coastal streams and bayous in Texas, it is imperative that this source be investigated (Rakocinski et al. 1997).

The primary objectives of our study were to 1) conduct a comprehensive literature review of the likelihood of production of *E. coli* bacteria by freshwater fishes found in warmwater streams, 2) determine whether wild caught fish representing various species and trophic groups from Harris County waterways produce feces with detectable levels of the indicator bacteria, *E. coli*, and 3) determine whether wild caught fish retained in aquaria transmit *E. coli* bacteria to

ambient water, 4) conduct a literature review to determine likely densities of native fish in urban bayous and feces production rates and 5) using these data, estimate potential loading rates of *E. coli* bacteria into urban bayous from freshwater fishes.

Methods

In order to evaluate the potential role of fish as sources of indicator bacteria our study utilized a multiple prong approach including a comprehensive review of scientific literature, review of historical water quality, intensive field surveys and laboratory bioassays.

Literature Review

A literature review was conducted to determine whether past investigators have found thermophilic indicator bacteria in freshwater fish. Starting in the late 1990's the State of Texas promulgated new water quality standards that designate *E. coli* bacteria as the primary indicator bacteria for evaluation of human health risks associated with contact recreation in freshwater. Therefore we focused our review on published data sets and studies that include information *on E. coli* bacteria and freshwater fish as potential hosts. Other data that was evaluated included past studies on the relationship of the production and survival of other indicator bacteria in fish including fecal coliforms and Enterococci. We focused our review on published agency reports and peer reviewed literature produced by researchers engaged in bacteriological research. We used electronic literature search services at University of Houston Clear Lake library, and internet searches (e.g. Google Scholar).

Historical Water Quality

Historical microbiological water quality data for each site was reviewed and obtained from either the Texas Commission of Environmental Quality (TCEQ) and Houston Galveston Area Council Clean Rivers Program water quality databases (HGAC 2010; TCEQ 2010). This data will be used to evaluate the overall potential for uptake of indicator bacteria in each stream that was surveyed during the field study described below. Fish inhabiting a stream with high levels of indicator bacteria would be expected to have higher levels of bacteria. We selected several sites that were within at least 1 mile of the field survey sites (Table 1).

Table 1. Historical water quality monitoring sites in Harris County, Texas used to assess microbiological conditions near fish collection sites.

TCEQ Site ID	Location	TCEQ Segment
11409	Horsepen Bayou @ Bay Area	Tributary to 1113 Armand Bayou Tidal 1113
15831	White Oak Bayou @ Tidwell Street	White Oak Above Tidal 1017
13778	Greens Bayou @ I-45 N.	Greens Bayou Above Tidal 1016

The following descriptions provided for each watershed where fish were collected are largely quoted verbatim from the *Basin Summary Report 2006* produced by the HGAC (HGAC 2006). The White Oak Bayou (TCEQ segment 1017) is approximately 23 miles long and has a drainage area of 109 square miles (HGAC 2006). The watershed contains widespread development, with more dense population found in the lower reaches inside Loop 610 (HGAC 2006). Heavy commercial development occurs along the Highway 290 corridor with residential and less dense mixed developments adjacent to the corridor. The designated uses for this waterbody are limited aquatic life and contact recreation (HGAC 2006). The entire segment and all sub-segments of White Oak Bayou Above Tidal are on the 2004 303(d) list for bacteria. Indicator bacteria *Escherichia coli* (*E. coli*) and fecal coliform have been used to assess the suitability of a water body for contact recreation use. Past monitoring shows that natural streams tend to exhibit low bacterial levels during dry weather conditions and there is an additional complicating factor in that essentially all of the dry weather flow is from treated human sewage (HGAC 2006). White Oak Bayou is highly urbanized and sanitary sewer inputs may be one of the major sources of bacteria (HGAC 2006; Rifai 2006). White Oak Bayou possesses 57 permitted wastewater outfalls and 3 industrial stormwater outfalls (HGAC 2006).

Greens Bayou (segment 1016) is 24 miles in length and has a drainage area of 139 square miles (HGAC 2006). The designated uses include contact recreation and limited aquatic life use. The watershed is heavily developed in the central and western sections with residential and mixed commercial developments as the predominant land uses. The T.H. Wharton power plant is located at the headwaters of Greens Bayou and its cooling water discharge provides year-round flow to the bayou (HGAC 2006). Beltway 8 runs though the middle of the watershed with large, high intensity developments and business districts found adjacent to and at the intersections with Interstate-45, US Highway 59 and State Highway 249. Bush Intercontinental Airport is located in the north central section of the watershed. The eastern most potion of the watershed is mostly undeveloped with mixed residential and commercial developments scattered thoughout. Sanitary sewer services are not found in all areas, despite the numerous wastewater collection and treatment systems located throughout the watershed. Failing septic systems are scattered thoughout the watershed.

Greens Bayou is listed for a bacteria concern in the 2002 and 2004 305(b) Reports and 303(d) lists (HGAC 2006).

Horsepen Bayou is a major tributary of the Armand Bayou watershed and enters Armand Bayou at the lower end of the watershed. The watershed is predominantly suburban land use with the downstream portion being largely undeveloped including a forested riparian zone associated with portions of the University of Houston Clear Lake campus and Armand Bayou Nature Center. Armand Bayou tidal (segment 1113) is 8 miles long and has a watershed Area of 60 square miles. Its designated uses include contact recreation and high aquatic life (HGAC 2006). A majority of the watershed is densely developed with the City of Houston (Clear Lake City) in the south, the City of La Porte in the east, and parts of the Cities of Deer Park and Pasadena in the north. High and low intensity residential and mixed commercial developments are the dominant land uses but large industrial facilities are scattered throughout the northern portion of the watershed. Ellington Air Field is a prominent land mark located in the western part of the watershed adjacent to Hwy 3. Directly south of the air field, between Hwy 3 and IH-45, is a large open tract of land dotted with gas and oil wells and small gathering facilities with a few storage tanks. NASA's Johnson Space Center, the University of Houston – Clear Lake campus, and the Armand Bayou Nature Center and Preserve are other well known landmarks located in the southeastern end of the watershed. The center of the watershed is mostly grasslands, forestlands and wetlands. Municipal wastewater collection and treatment systems service majority of the watershed. Where developments are separated from municipal services by long distances, on-site wastewater disposal systems (septic systems) are used. No particular area is known to have a large number of failing septic systems. Rather, failing systems are scattered thoughout the watershed. Segment 1113 is on the 2002 and 2004 Texas 303(d) lists and the 2002 and 2004 305(b) reports for bacteria and depressed dissolved oxygen (HGAC 2006).

Fish and Indicator Bacteria Data Collection

During 2008 and 2009 we conducted an intensive survey of several Harris County waterways to determine whether wild caught fish from Harris County waterways representing various species and trophic groups produce feces with detectable levels of the indicator bacteria, *E. coli*. Our working hypothesis was that the indicator bacteria in fish guts are higher than surrounding waters and therefore a potential source contribution to the watershed. In order to test this hypothesis we collected representatives of multiple fish species representing various trophic groups from several urban bayous. Previous research suggests that there may be interspecific and intertrophic level differences in enteric bacteria levels (Trust et al. 1979). Therefore we attempted to collect bacteria from at least five different species from five related trophic levels (Linam et al. 2002)(Table 2).

Table 2. Candidate species for *E. coli* study.

Candidate Species	Trophic Groups[1]
Blue catfish	Benthic predator
Channel catfish	Benthic omnivore
Carp	Benthic omnivore
Smallmouth Buffalo	Benthic omnivore
Striped mullet	omnivore
Armored catfish	Herbivore
Grass carp	Herbivore
Gizzard shad or Theadfin Shad	omnivore
Gulf menhaden	omnivore
Bay anchovy	insectivore
Largemouth Bass or Spotted Bass	Predator
Green sunfish	Predator
Bluegill	Insectivore
Rio Grande Cichlid	Insectivore
Red Shiner	Insectivore
Mosquito fish	Insectivore
tilapia	omnivore

[1]. Trophic Groups based on classification scheme of Linam, et. al. (2002)

Sampling was conducted during dry weather base flow conditions during fall, spring and summer starting fall 2008 and continuing though summer 2009 at thee streams including White Oak Bayou, Greens Bayou and Horsepen Bayou (Table 3 and Fig. 1). These sampling events correspond to seasonal periods that could potentially affect levels of indicator bacteria in ambient water. For example, *E. coli* and other thermophilic indicator bacteria levels are often more elevated during warmer water temperatures present in summer months (Petersen et al. 2006).

Fish were collected during each sampling period using an electroshocking tote barge (Fig. 2). The barge uses pulsed DC electrical current to temporarily stun fish. Sampling consisted of 2 collectors with electrode poles and 2 collectors with dip nets attempting to collect the target fish that are stunned at each site. Effort was variable but usually lasted 1-3 hours depending on collecting efficiency. Once fish were collected they were placed in a holding tank temporarily. Prior to *E. coli* sampling each fish will be euthanized by dipping them in a net into a bath containing lethal levels of MS-222 according to UHCL approved institutional animal care (IACUC) protocol. Each specimen was identified to species and processed immediately (within 30 minutes). Total length and wet weight were obtained for each specimen using a measuring board and electronic scale. Fecal matter samples were then obtained using the following procedure. Each fish was dissected using previously sterilized scissors and tweezers. The intestine was then removed, and using gentle pressure the fecal material was squeezed out unto a previously tared bottle of sterile water. The estimated amount of fecal material, by weight, was then calculated from the increase in weight in the bottle. The bottle was then capped, shaken vigorously for 1-2 minutes, placed on ice, and returned to the lab for analysis within 6 hours.

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Table 3. Field monitoring sites and frequency.

Site	TCEQ Segment	Location	Dates
Horsepen Bayou	1113 Armand Bayou Tidal	Pineloch Street, Harris County	November 2008, April 2009 and July 2009
White Oak Bayou	White Oak Above Tidal 1017	Tidwell Street	November 2008, April 2009 and July 2009
Greens Bayou	Greens Bayou Above Tidal 1016	I-45 N., Harris County	November 2008, April 2009 and July 2009
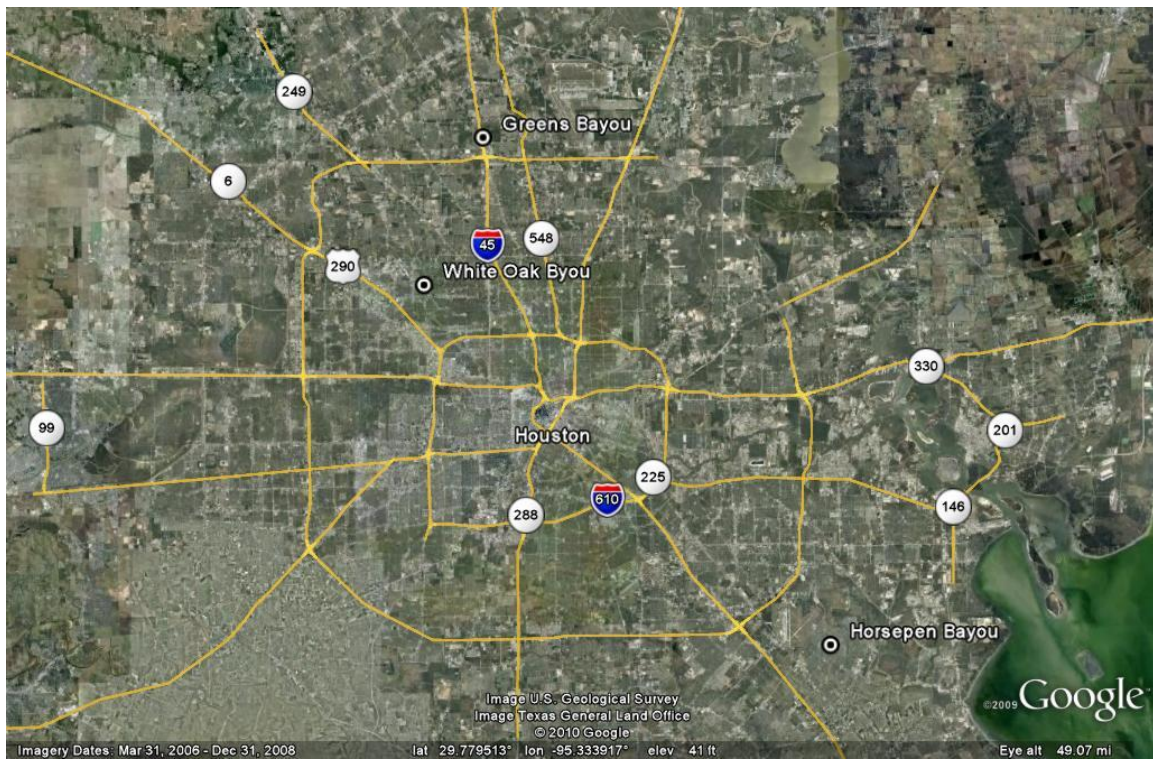
Figure 1. Sites were fish were collected during this study.

Figure 2. Electroshocking tote barge used to collect fish specimens.

During collection of fish, ambient measurements of water temperature, pH, specific conductance, dissolved oxygen and secchi disk turbidity were taken at each site. In addition, ambient water and shoreline sediment samples were collected for analysis of *E. coli* bacteria. Since streamflow can influence bacteria levels by either increasing concentrations due to contaminated runoff and by dilution of source water, it is important that sampling be conducted during similar flow regimes. Therefore, to increase the likelihood of detecting actual impacts associated with the fish fecal material loading and to reduce flow induced variation, we conducted our sampling during dry weather conditions.   Dry weather was defined as a period of at least 3 days of no significant precipitation within the watershed. Also sampling occurred when stream levels were low enough to safely wade the stream and collect fish. However to document stream conditions we obtained available precipitation and streamflow or stage from the nearest stream gage maintained by the USGS or the Harris County Office of Emergency Management (HCOEM)(Table 4). In the case of Horsepen Bayou due to tidal conditions flows were not measured.

Table 4. HCOEM and USGS stream stage and rainfall gages sites in Harris County, Texas that were queried to assess field conditions during fish collections.

HCOEM or USGS Site ID	Location	Data	Top of Bank
250-B104	Horsepen Bayou @ Bay Area	Stage	12.8
575-E100	White Oak Bayou @ Tidwell Street	Stage and Rainfall	66
USGS 08074020	White Oak Bayou at Alabonson Rd. (~ 2.5 miles upstream)	Stage and Flow	
1659-P100	Greens Bayou @ Knobcrest (near I-45 N).	Stage and Rainfall	84.8
USGS 08075900	Greens Bayou near US 75 (I-45)	Stage and Flow	

Water quality and bacteriological sampling was conducted using standard methods described in the TCEQ Surface Water Quality Monitoring and Clean Rivers Program guidance manuals and quality assurance project plans (TCEQ 2003a; 2008b). Ambient water bacteria samples were collected mid-stream using a previously sterilized sample bottle. Recently deposited shoreline sediment samples were only taken during spring and summer 2009. Water samples were be placed on ice and returned to the laboratory for analysis of *E. coli* bacteria. Sediment samples were placed into tared sterile water sample containers following protocol similar to the fish samples. During each sampling event a procedural microbiological blank was processed in the field.

Aquarium E. coli Production Study

Although results from the field portion of our study may demonstrate that various fish species create fecal material that contains *E. coli*, it does not answer the central question of whether these bacteria originate within the fish or are simply picked up from ambient water or while feeding on *E. coli* contaminated material and released back into the environment. Although complex molecular genetic approaches can assist in answering this question, we used a more simple approach to determine the potential for *E. coli* to originate within a fish. This was done by taking cultured fish which are subsequently fed prepared fish meal to determine whether *E. coli* levels increase in aquarium water after exposure to fish at various stocking levels. Afterward some of these fish are sacrificed to determine if *E. coli* are present in their intestinal material. If *E. coli* originated from the fish itself, we would expect levels of bacteria in water to increase with fish density and time irrespective of origin of the fish. However, if *E. coli* are primarily acquired from the environmental or ingestion of contaminated material, we would expect *E. coli* levels to decrease with time irrespective of stocking.

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We used 2 species and trophic levels of fish in this portion of the study. This included the channel catfish *Ictalurus punctatus*, a benthic omnivore, and primarily bluegill *Lepomis macrochirus* an invertivore. In a few cases longear sunfish *Lepomis megalotis,* another invertivore was substituted for bluegill. *In some cases we would* Channel catfish and bluegill were obtained from a local commercial fish farms, including Danbury Fish Farms and Fish Tales located in Angleton and Magnolia, Texas respectively, and delivered and stocked into our holding tanks within 4 hours. Fish generally ranged in size between 3-4 inches in total length. Fish were acclimated by floating them in bags to equilibrate the temperature in two separate 20-30 gallon holding tanks that contained outside filters with filter pads and activated carbon to maintain water quality. Also, the pH of the source transport water was tested and the aquarium water pH was adjusted if necessary to reduce stress. The pH was adjusted using a commercially available buffer to maintain levels between 7-8 standard units. We used commercially available conditioners AmQuel+® or Ammo-Lock®, to neutralize the chlorine in added tap water. Approximately 20 fish were kept in a 30 gallon holding tanks until testing began.

The aquarium *E. coli* production study was conducted during December 2-18, 2008 and January 20 to February 3, 2009 respectively. During the December experiment we used bluegill primarily, while channel catfish was used for the second. Water used in the experiment was previously sterilized (city chlorinated and/or UV sterilized) to reduce potential sources of indicator bacteria. This was further tested by bacteriological monitoring prior to addition of fish. Prior to stocking the tank was setup with individual aeration and in-tank biotreatment (sinking plastic media balls) and run for several days to establish bacterial colonies (Fig. 3). A very small portion of flake food was added to establish the bacterial colony.

A total of 15 tanks were used in our study. For each stocking level 5 replicate tanks were used. Stocking rates varied as follows; five tanks contained one fish, five tanks contained 3 fish, and five tanks served as controls. Once stocked, fish were fed twice daily a small portion of flake food sufficient for them to eat within 5 minutes. Aeration was provided by air stone. Ongoing filtration consisted only of in-tank plastic media balls to reduce the likelihood of removal of indicator bacteria by filtration Tanks were otherwise bare. Lighting consisted of fluorescent lights within the room and powered by a 24 hour timer to provide 12 hours of light and dark. We attempted to maintain water temperatures between 20 and 25 °C using a room heater. Water temperature was monitored daily. The initial fish were added to test tanks within 3 days of arrival and placement in the holding tanks. Fish were fed and monitored daily. Fish were fed once daily a small portion of flake food sufficient for them to eat within 5-10 minutes. In the control tanks we put food in a net & let it soak for 5-10 min, to simulate the amount of time the flakes were in the water. Water temperature, dissolved oxygen, conductivity, hardness and pH were initially measured 1-2 days prior to the bioassay, on day 6-7, and on day 14-15 at the end of the test.

Top-Side view   **Front View**

Figure 3. Aquaria used during our study to evaluate fish *E. coli* production rates.

Water temperature, pH and ammonia nitrogen was monitored each day and any diseased or dead fish were removed and replaced with healthy individuals from the holding tanks as necessary. Redear sunfish were used occasionally to replace dead bluegill. If total ammonia levels became elevated above 2 mg/l, we would also add AmQuel+® or Ammo-Lock®, commercially available ammonia, chloramines and chlorine removal agents. Ammonia, alkalinity and hardness were measured using commercially available aquarium test kits produced by Aquarium Pharmaceuticals, API®. However, if conditions became critical fresh sterilized water from the same source and having the same water quality characteristics was added to reduce ammonia levels.   Resulting dilution rates were noted to adjust for nominal bacteria concentration calculations if necessary. *E. coli* readings were taken before, and 1 day, 3 days, 7 days and 14 days after stocking from aquarium water. Laboratory procedures blanks were also taken at the same time. After the experiment was terminated, several specimens were sacrificed and fecal matter removed form the lower intestinal tract following protocol used in field collections. Remaining fish were acclimated to local water and released into ponds on the University property. As stated earlier, the experiment was run first with one species and then the other. Before and In between experiments the tanks were sterilized with chlorine bleach.

Bacteria Laboratory Analysis

Bacteriological analysis of ambient surface water and sediment, field collected fish feces, laboratory test fish feces, aquarium water, and blanks were conducted in the EIH laboratory according to the following procedures. Once field bacteria samples were collected and returned to the lab we vigorously shook the fish fecal, ambient water, and sediment samples. We then incubated and analyze water, sediment and fish fecal suspension samples using the IDEXX Colilert®

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defined substrate method which has been approved for use in Texas by both the TCEQ and EPA and is described in TCEQ (2003 and 2008b). *E. coli* will be reported as MPN/100 ml for water samples. Fish fecal and sediment suspensions were further converted to MPN/gram wet weight fecal material.

Statistical Analysis of Field and Laboratory Data

Bacteriological and water quality data were statistically analyzed and/or graphically displayed to evaluate differences in bacteria levels between fish species, seasons, aquarium stocking rates, water temperature and turbidity. These variables are known to potentially affect the survival and ambient levels of indicator bacteria both in water and/or the organisms. Preliminary tests of field bacteria data for normality indicated that both original and log transformed data did not fit the normal distribution. Therefore we utilized the non-parametric One-way Kruskal-Wallis Analysis of Variance by Ranks method to test the null hypothesis of no significant differences between fish species *E. coli* and aquarium water levels and to test whether these values are significantly different from zero (Daniel 1990; Dunn 1964; Orlich 2010). When statistically significant (p value < 0.05) differences were detected Dunn's multiple comparison test was used to conduct pair-wise comparisons to determine which groups were significantly different from each other. Supplementary graphical and correlation and regression analyses were conducted on water temperature, and turbidity to determine whether these factors may influence observed bacteria levels in water and sediment. When appropriate similar analyses were conducted on data from the aquarium study to determine whether there were significant differences between pre and post-stocking *E. coli* levels in aquarium water and whether these differences vary between species, and stocking rates. All statistical analyses were conducted using the Minitab® statistical software package.

Literature Review of Stream Fish Composition Densities and Composition and Fecal Production rates.

In order to estimate potential loading of *E. coli* associated with fish communities, it was necessary to develop an estimate of fish density within urban streams. This was an extremely difficult task to accomplish because there are few examples of complete censuses of fish communities in bayous provided in the literature. Most fish community censuses are based on rotenone studies of reservoirs and lakes, which contain species that are not typical of bayous and rivers. We conducted a literature review of both peer reviewed literature and agency reports. Agency reports used in our study were limited to studies conducted within southeast Texas bayous and rivers. In some cases it was necessary to convert values obtained by these studies into standardized densities in terms of number of fish per hectare. Since these studies for the most part used selective sampling gear it will be necessary in the future to conduct more comprehensive field studies using intensive collection techniques to refine these estimates. Finally, we reviewed the literature to obtain estimates of the

amount of fecal material produced by fishes. Estimates of fecal production in the literature were largely dominated by data obtained from aquaculture research and applications.

Estimation of Loading Rates of E. coli bacteria

Using our estimates of numbers of *E. coli* per gram of fecal material, and literature estimates of fish densities and feces production rates; we attempted to develop relative estimates of potential loading rates of *E. coli* bacteria into urban bayous and streams from fishes.

Results

Literature Review

E. coli are members of the Enterobacteriaceae and possess the traits of being gram negative straight rods. Sanders and Fryer (1988) indicated that most members of this group are common inhabitants of man and other animals, and can be considered as potential isolates of fishes but are usually opportunistic pathogens. Horan (2003) identifies both *E. coli* and Enterococci bacteria as specific indicators of fecal contamination from warm-blooded organisms only. Maier et al (2000) also indicate that fecal coliform bacteria and *E. coli* originate exclusively in the guts of warm-blooded organisms. They, however, also state that significant regrowth is possible on biological films located in pipes and culverts and in warm sub-tropical environments in eutrophic turbid water.

One of the earliest studies to evaluate the relationship of fish and bacteria was a study conducted by Potter and Baker (1961). They conducted a microbial analysis of 60 fish caught in thee areas of Flathead Lake, Montana. They collected numerous fungi and bacteria from their digestive tracts. They documented levels above 100,000 bacteria per square centimeter in the gut tissue of fish. The majority of these were gram negative chomogenic rod bacteria types. They concluded that fish operate as passive carriers of fungi and bacteria. No apparent pattern was observed between species.

Glantz and Krantz (1965) isolated and identified various serotypes of *E. coli* from fish and water. They conclude that it is likely that fish could pick up *E. coli* strains and carry them as transient or resident flora to other sources of water. They also summarized some of the earlier literature. Based on their review they provide evidence that fish harbor *E. coli* as part of their flora and the prevalence of coliform organisms increases when fish inhabit polluted waters (Evelyn and McDermott 1961; Griffiths 1937; Gibbons 1932; Snow and Beard 1938). Furthermore, fish are considered a conveyor of microorganisms and may therefore influence water quality indicators levels in the streams they inhabit (Potter and Baker 1961). This influence could be significant if multiplication of *E.*

coli occurs in fish intestines as was suggested by early investigators and if the bacteria are retained in the fish for any length of time (Johnson 1904).

An early study that investigated the occurrence, distribution, and persistence of coliforms, fecal coliforms, and fecal streptococci in the intestinal tract of freshwater fish was conducted by Geldreich and Clarke (1966). They examined a total of 132 fish representing 14 different species during their study. Examination of the intestinal contents of 78 fish from moderately polluted sections of the Little Miami River indicated that fecal coliform densities were lowest in bluegills (less than 20 per gram) and highest in catfish (1,090,000 per gram). Levels of fecal streptococci for these two species were 220 and 240,000 per gram, respectively. They concluded that the occurrence of fecal coliforms in fish caught in this stream actually reflected the warm-blooded animal pollution level of the water. All fish used in this phase of their study were caught during July, August, and September when the water temperatures were between 13 and 18 C. They further concluded that the fate of fecal coliforms and *Streptococcus faecalis* in the fish intestine indicated that these organisms can probably survive and multiply when fish and water temperatures are 20 °C or higher, but only when the organisms are retained in the gut for periods beyond 24 h. Based on the biochemical reactions for the 3,877 coliform strains isolated from 132 freshwater fish of 14 different species, 91.4% of all strains were composed of five IMViC types. They noted that in a similar study of the biochemical reactions of 850 streptococci isolated from the intestinal tract of 55 freshwater fish, the predominant strains included *S. faecalis* and various closely associated biotypes. No consistently recurring pattern for either coliforms or streptococci could be developed to identify species of fish investigated. They concluded that the composition of the intestinal flora is, however, related in varying degree to the level of contamination of water and food in the environment.

Buras et al. (1987) reared tilapia, common carp and silver carp in treated domestic wastewater. They found that the most sensitive to this environment was the silver carp, followed by common carp and tilapia. In healthy clean fish, bacteria were not found in the blood or the muscles. They were present in small numbers in various organs and in concentrations of 106–107 g−1 in the digestive tract content. In fish exposed to treated wastewater for the entire growing period, bacteria were found in the muscles. The number of bacteria recovered from various organs ranged between 104–106 g−1 and their concentration in the digestive tract content was 108–109 g−1. The number of bacteria in the pond water determined the presence and concentration of bacteria in the fish. The number of bacteria that caused their appearance in the muscles of fish has been named the "theshold concentration". Considering the public health aspects, fish can be reared in treated wastewater provided the bacteriological quality of the water is compatible with the "theshold concentration" levels of the fish grown in the ponds. The suitability of *E. coli* (fecal coliform bacteria) as indicators for the bacteriological quality of fish grown in wastewater-fed ponds was discussed.

Cahill (1990) reviewed the literature on the bacterial flora of fishes. She found that bacterial floras isolated from eggs, skin, gills, and intestines have been described for only a limited number of fish species. Generally, the range of bacterial genera isolated was related to the aquatic habitat of the fish and varied with factors such as the salinity of the habitat and the bacterial load in the water. The findings of Sugita et al. (1988) were reviewed and provide some evidence of continued proliferation of enteric bacteria within the digestive system of fishes. They found that the permanent intestinal microflora of goldfish *Carassius auratus* consisted of bacteria which were also present in the surroundings but which were able to persist and grow in the environment provided by the intestinal tract of the fish. Cahill (1990) pointed out that in many past investigations, identification of isolates to the genus level made it difficult to determine the precise relationships of aquatic and fish microflora. Microflora of fish intestines appear to vary with the complexity of the fish digestive system. The genera present in the gut generally seem to be those from the environment or diet which can survive and multiply in the intestinal tract, although they also state that there was evidence for a distinct intestinal microflora in some species. While obligate anaerobes have been recovered from carp and tilapia intestines, low ambient temperatures may prevent colonization by anaerobes in cold-water species such as rainbow trout.

Pullin et al. (1993) reported that there have been few studies of bacteria excretion although it is known that the guts of fish can act as proliferation sites for Enterobacteriaceae, *Aeromonas* and fecal streptococci which enter the culture systems though the influent water or feed. They reported that fecal coliform levels were 1,000 times higher in culture systems when compared to control ponds.

Public health studies have also been conducted that document the presence of *E. coli*, including pathogenic strains, in fish and shellfish offered for sale in fish markets or restaurants. Singh and Kulshestha (1994) screened 97 fresh water fish, 37 marine fish, 12 fresh water prawn, 13 marine shimp, 26 molluscs, 19 dried fish, 5 dried prawn, 20 fish pakoda (processed Indian seafood dish) and 6 fish egg samples for enterotoxigenic *E. coli* serotypes. They isolated a total of 17 *E. coli* strains. Out of these, 13 were typed as O: 87 (seven), O: 128 (thee) and one isolate each of O: 2, O: 20 and 0:3 serotypes (four isolates remained untyped). These were present in fresh water fishes, marine shimp, fresh water molluscs, fish eggs and marine fish samples. All the 17 *E. coli* isolates could be biotyped into 7 biogroups, 11 isolates belonging to biotype I and the other six isolates to 6 different biotypes. Out of 17 *E. coli* isolated, 7 were positive for enterotoxigenicity with the latex agglutination, coagglutination, mouse foot pad and asopermeability factor tests. They concluded that the antibiotic resistance and public health significance of these *E. coli* isolates deserve serious attention (Singh and Kulshestha 1994). The potential sources of these *E. coli* were not identified but could be introduced from improper handling of these food items.

Markošová and Jezek (1994) collected information on populations of indicator bacteria (mesophilic, coliform and fecal streptococci) together with relevant limnological parameters (temperature, oxygen, BOD and chlorophyll-α) during a 6 year study of thee eutrophic ponds. They manipulated the pond ecosystems with various levels of stocked carp (*Cyprinus carpio*) in 2-year management cycles. During the spring of the first year, the pond was stocked with young fish, and in the fall of the second year it was drained and mature fish were harvested. They found that fish management had a direct influence on bacterial numbers and on the basic parameters of the systems. A statistical analysis using t-tests found significant differences in oxygen concentration, chlorophyll-α and BOD during both years of fish management. During each season, populations of indicator bacteria increased with increasing water temperature, and maximal numbers of bacteria were recorded during the summer months. Correlation analysis confirmed that temperature had a positive significant effect on population dynamics. The results demonstrate that fish stocking can affect the bacterial population that is high fish biomass usually translated into higher numbers of indicator bacteria as well as BOD and phytoplankton. They concluded that if pond water quality must be maintained for the purposes of drinking or recreation, fish populations may have to be reduced.

Davis et al. (1995) documented high levels of fecal coliform bacteria from in the discharge of a channel catfish aquaculture facility. Biochemical differentiation of fecal coliforms showed most to have been *Klebsiella pneumoniae*, *K. oxytoca*, *E. coli*, and *Citrobacter frundii*. They also provided a review of the literature that documented high levels of fecal coliform bacteria levels in the effluent of many aquaculture operations despite the absence of any known source of warm-blooded animal waste loading (Wyatt et al. 1979; Shireman and Cicha 1994; Markošová and Jezek 1994).

Del Rio-Rodriguez et al. (1997) investigated the survival and persistence of *E. coli* bacteria in the intestine of cultured rainbow trout, *Oncorhynchus mykiss*. Infection was achieved by ingestion of contaminated feed but not by bath exposure. At 15°C *E. coli* was found to increase in number in the intestine of fish after an initial decline, and could still be detected after 4 days. At 6°C it was detected for 2 days but the numbers declined steadily. A similar trend was observed when extracted gut content was inoculated with *E. coli* in vitro; after an initial decline, bacterial growth recovered. These results showed that at temperatures around 15°C, the presence of *E. coli* in fish need not be an indicator of recent passage though polluted waters. It may be a consequence of infection established many days before, and perhaps some distance away.

Boyd and Tanner (1998) found that water from 48 channel catfish *Ictalurus punctatus* ponds at Auburn and Greensboro, Alabama, USA, usually contained less than 1,000 total coliform and 200 fecal coliform bacteria per 100 ml. There were no sources of human fecal matter to these ponds. Also, they reported that fecal coliform: fecal streptococci ratio was less than 1.0 and typical of fecal

contamination by warm-blooded animals other than humans. The abundance of coliforms was greater in spring and summer than in fall and winter in catfish and sportfish ponds at Auburn, Alabama. In spite of high organic matter inputs in feed, catfish ponds had no greater abundance of coliforms than sport fish ponds.

More recent studies of wild populations of animals have identified reptiles as potential sources of *E. coli* bacteria Gopee et al (2000). They found that the frequency of *E. coli* isolation was significantly higher in mammals when compared with birds and reptiles. Regardless of taxonomic grouping, the frequencies of isolation of *E. coli* from omnivores, herbivores, and carnivores were 87.2%, 70.0%, and 57.3%, respectively. These differences were statistically significantly.

Gordon and Cowling (2003) studied the prevalence of *E. coli* in a variety of Australian mammals, birds, reptiles, amphibians and fishes. *E. coli* were isolated from more than 2,300 non-domesticated vertebrate hosts living in Australia. *E. coli* was most prevalent in mammals, less prevalent in birds and uncommon in fish, frogs and reptiles. They found that the *E. coli* was isolated from 10 % of the fish examined (n=138). They also found that no differences in the prevalence of *E. coli* could be detected among the four species for which there were reasonable samples.

Al-Harbi (2003) and Al-Harbi and Uddin (2003) studied the causes of elevated *E. coli* and fecal coliform bacteria in intense tilapia aquaculture facilities. They found elevated levels in pond water, sediment, and tilapia intestinal samples. Levels were particularly high during warmer summer months. However, they identified pigeons that used the shoreline area around these ponds as an obvious source of indicator bacteria which contaminated the ponds and tilapia intestine. They reported that other studies had observed similar levels of bacteria in intensive aquaculture systems (Leung et al. 1992: Pullela et al. 1998).

Guzman et al. (2004) studied the uptake and levels of *E. coli* in laboratory tests of wild populations of a two freshwater fish species in Argentina, the orillero, *Jenynsia multidentata* and the mojarro, *Bryconamericus iheringi*. They also provided a brief review of the literature. They reported that penetration and establishment of bacteria in different tissues and organs of fish, such as digestive tract, gills, muscle, kidney, liver and gas bladder, have been reported in polluted environments (Gariboglio et al. 1976; Pal and Dasgupta 1991; and Pal and Dasgupta 1992). Pal and Dasgupta (1992) showed that bacterial concentration in different organs and tissues of fish increases with an increase in the bacterial load of the water and food. They conclude that because of their great capacity to move, fish are able to carry potentially pathogenic bacteria for humans to non-polluted waters causing infection when fish are consumed or handled (DePaola et al. 1994).

Based on their laboratory experiments they found positive linear relationships between bacteria levels in muscle and intestinal tissue of fish cultured in tanks with varying levels of introduced cultures of *E. coli* (Pal and Dasgupta 1992). In field experiments *E. coli* was recovered from the digestive tract and muscle of orillero. They concluded that the presence of *E. coli* in the fish suggests they can carry bacteria to non-polluted waters.

Recent studies have documented the presence of *E. coli* bacteria in some pelagic and demersal fishes (Clark et al (2007; Hansen et al. 2008). However, based on molecular genetic identification methods they found that the origin of some of these bacteria were Canadian geese and human sewage. The vast majority of these bacteria however could not be traced back to a specific warm-blooded animal. Although these studies demonstrated that benthic fish contain *E. coli*, it may be more appropriate to consider these fish as a vector of *E. coli* from other sources, rather than a new source of *E. coli* contamination in aquatic environments (Hansen et al. 2008).

Gaertner et al. (2008) found that fish from various trophic levels, including largemouth bass, *Micropterus salmoides*, channel catfish *Ictalurus punctatus*, common carp, *Cyprinus carpio*, and suckermouth catfish *Hypostomus plecostomus*, inhabiting the headwaters of the San Marcos River contained detectable levels of *Salmonella* species. This occurred both at upstream uncontaminated and downstream contaminated sites. Contamination was determined at each site by monitoring of water and sediment. They also found that the majority of the *Salmonella* was associated with particulate matter in the intestine. They conclude their data suggests that Salmonella are not components of the indigenous microbial community in fish intestines but rather are ingested with particulate matter. This study is also one of the few in the literature that illustrates potential upstream transportation of bacteria from contaminated to uncontaminated sites.

Although beyond the scope of this project as previously noted other species of cold-blooded vertebrates, including aquatic forms, have been shown to harbor and in some cases produce indicator bacteria (Mundt, 1963; Stevens and Hume 1998; Harwood et al. 1999; Gopee et al. 2000; Gordon and Cowling 2003).

Historical Data Review

Data compiled from the HGAC Clean Rivers Program indicated that the highest recent (2005-2008) levels of *E. coli* bacteria were found in White Oak Bayou, and the lowest levels were found at Horsepen Bayou (Table 5). Conductivity levels were highest at Horsepen Bayou which reflects the tidal influence on this stream. However, minimum conductivity levels were similar to the other two sites reflecting the more variable tidal conditions at Horsepen Bayou. These data suggest that there are probably greater and/or more numerous sources of *E. coli* in White Oak and Greens Bayou in contrast to Horsepen Bayou.

Table 5. Summary data from HGAC CRP online database collected during 2005-2008 (HGAC 2010).

Site	TCEQ Site ID	Parameter	Units	Min	Max	Count	Avg
White Oak Bayou	15831	Conductivity	uMHO	166	889	949	702.31
White Oak Bayou	15831	Dissolved oxygen	mg/l	5.1	12	949	7.83
White Oak Bayou	15831	PH	SU	7.6	8.5	949	7.96
White Oak Bayou	15831	NH3-N	mg/l	0.03	1.28	967	0.19
White Oak Bayou	15831	*E. coli*	MPN/100ml	70	19000	967	2658.21
Greens Bayou	13778	Conductivity	uMHO	197	881	1107	658.01
Greens Bayou	13778	Dissolved oxygen	mg/l	3.7	12.3	1107	8.55
Greens Bayou	13778	PH	SU	7.3	8.8	1107	8.01
Greens Bayou	13778	NH3-N	mg/l	0.03	2.42	1163	0.33
Greens Bayou	13778	*E. coli*	MPN/100ml	60	24192	1081	1883
Horsepen Bayou	11409	Conductivity	uMHO	180	12900	1297	1998.73
Horsepen Bayou	11409	Dissolved oxygen	mg/l	1.1	16.7	1319	6.04
Horsepen Bayou	11409	PH	SU	7.1	9.1	1319	7.71
Horsepen Bayou	11409	NH3-N	mg/l	0.03	2.72	1194	0.38
Horsepen Bayou	11409	*E. coli*	MPN/100ml	10	26	48	18

Field Collections: E. coli collection from Wild Fish

Hydrology and meteorology data compiled during our study indicated we collected our data during relatively low flow conditions (Table 6; Figs 4-15). Stream gage heights and flows were similar across each season. However in some cases we may have collected samples during the receding limb of the hydrograph which may have increased turbidity and *E. coli* levels slightly in comparison to base flow conditions.

Table 6. Conditions at fish collection sites and dates as recorded by HCOEM stream stage and rainfall gages sites in Harris County, Texas.

Date	Location	1 day rainfall (in)	3 day rainfall (in)	Stage (ft)	Top of Bank (ft)
11/10/08	Horsepen Bayou @ Bay Area	0	0	NA	12.8
11/20/08	Horsepen Bayou @ Bay Area	0	0	NA	12.8
4/8/09	Horsepen Bayou @ Bay Area	0	0	NA	12.8
6/22/09	Horsepen Bayou @ Bay Area	0	0	NA	12.8
11/19/08	White Oak Bayou @ Tidwell Street	0	0	64.66	66
4/13/09	White Oak Bayou @ Tidwell Street	0.90	0.94	52.54	66
6/24/09	White Oak Bayou @ Tidwell Street	0	0	52.72	66
11/25/08	Greens Bayou @ Knobcrest (near I-45 N).	0	0	NA	84.8
4/10/09	Greens Bayou @ Knobcrest (near I-45 N).	0.04	0.04	NA	84.8
6/29/09	Greens Bayou @ Knobcrest (near I-45 N).	0.00	0.00	NA	84.8

NA = not available

Figure 4. Stream gage height from USGS gage located on White Oak Bayou at Alabonson, Road. Fish collection occurred on November 19, 2008.

Figure 5. Stream discharge data from USGS gage site on White Oak Bayou at Alabonson Road. Fish collection occurred on November 19, 2008.

Fishes as Sources of *E. coli*   31
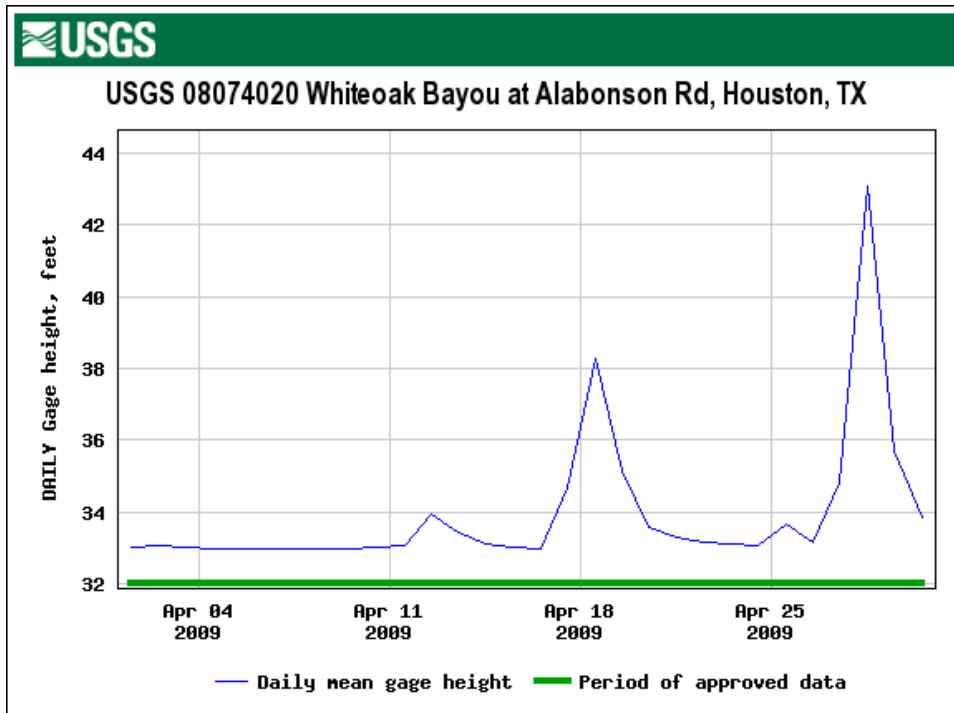
Figure 6. Stream gage height data from USGS gage site on White Oak Bayou at Alabonson Road. Fish collection occurred on April 13, 2009.
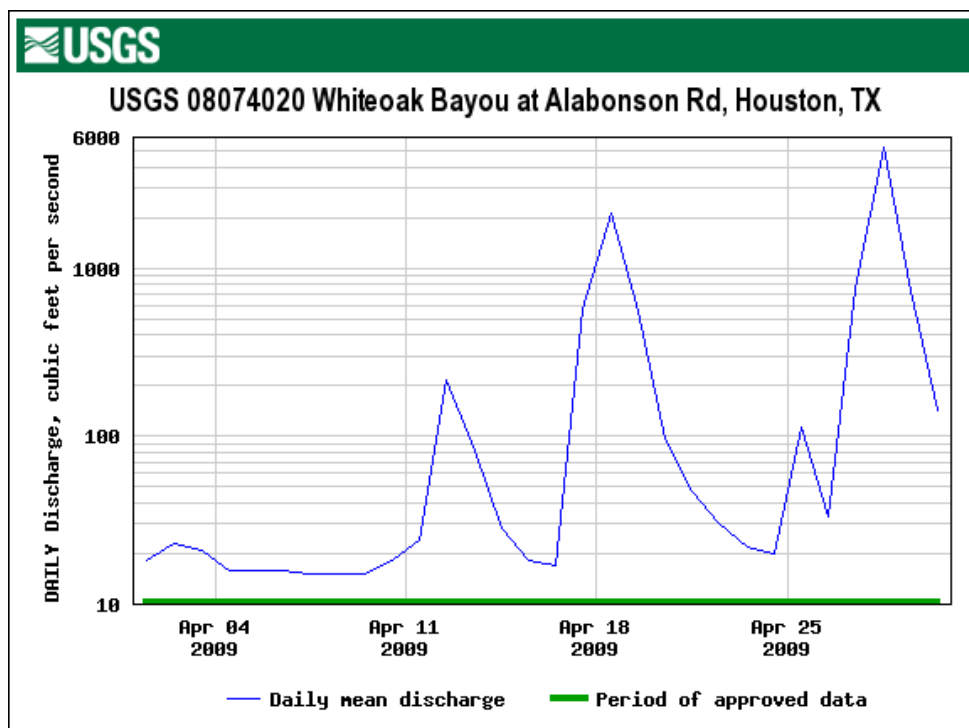
Figure 7. Stream discharge data from USGS gage site on White Oak Bayou at Alabonson Road. Fish collection occurred on April 13, 2009.
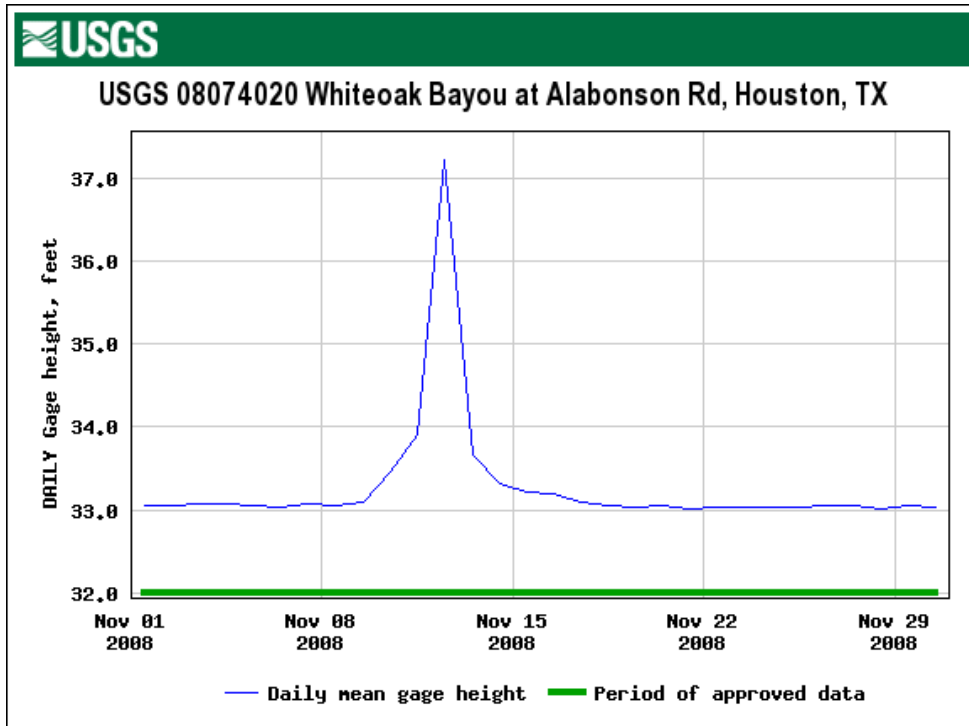
Fishes as Sources of *E. coli* 32
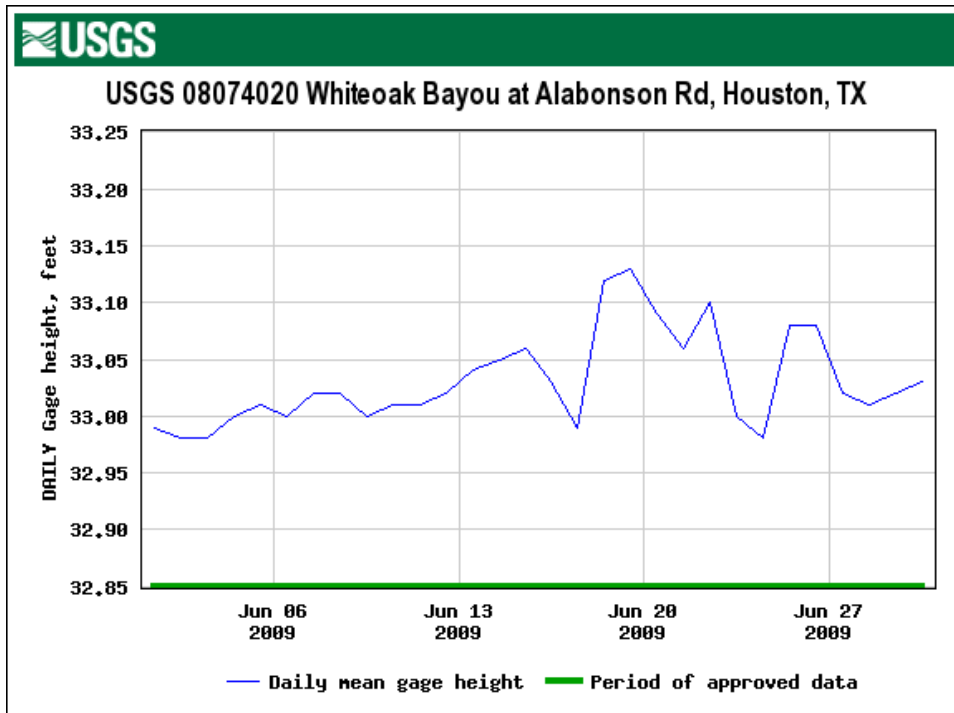
Figure 8. Stream gage height data from USGS gage site on White Oak Bayou at Alabonson Road. Fish collection occurred on June 24, 2009.
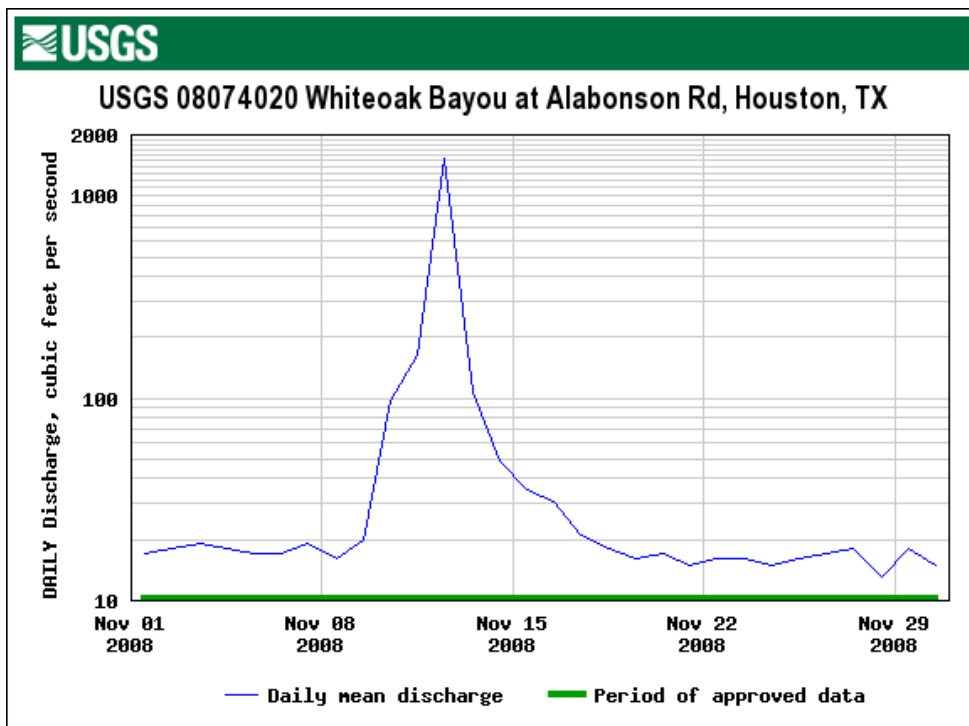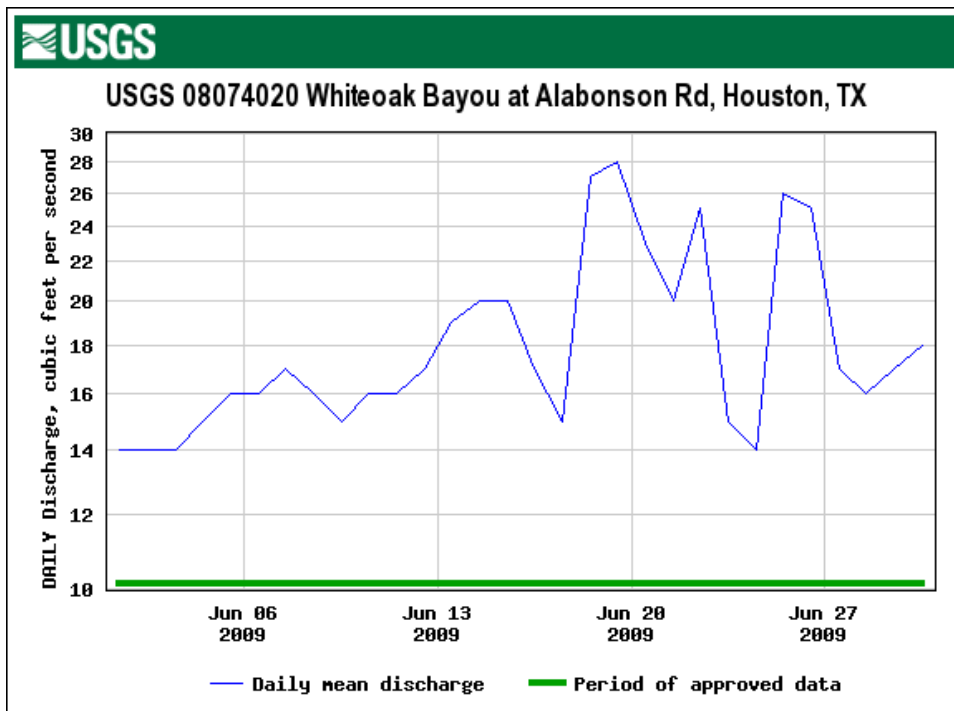

Figure 9. Stream discharge data from USGS gage site on White Oak Bayou at Alabonson Road. Fish collection occurred on June 24, 2009.

Fishes as Sources of *E. coli*   33
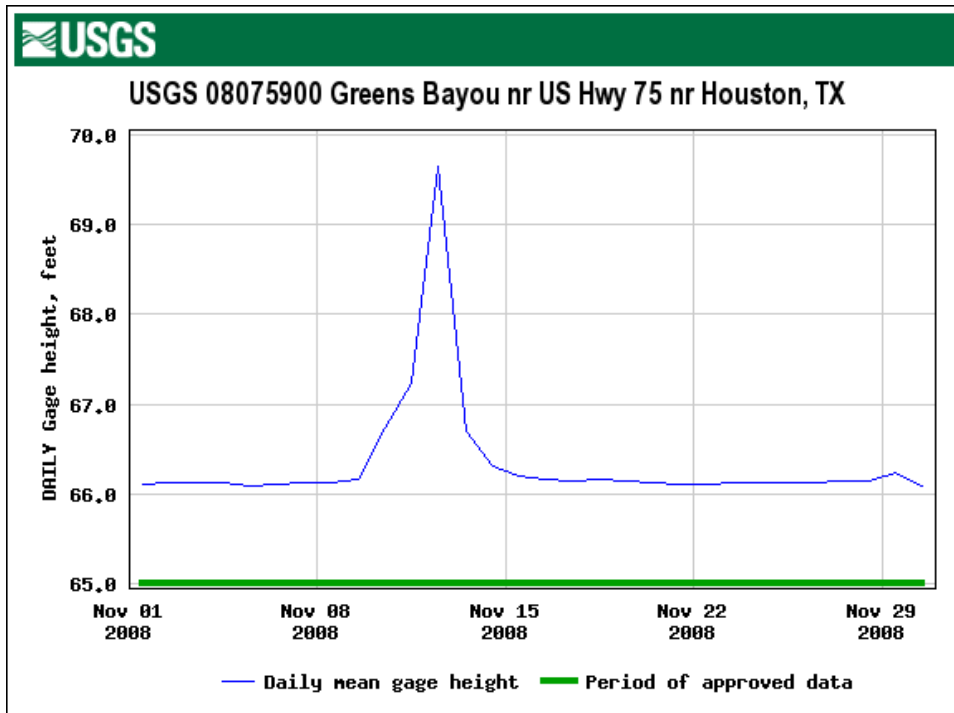
Figure 10. Stream gage height from USGS gage located on Greens Bayou near Hwy 75 (I-45). Fish collection occurred on November 25, 2008.
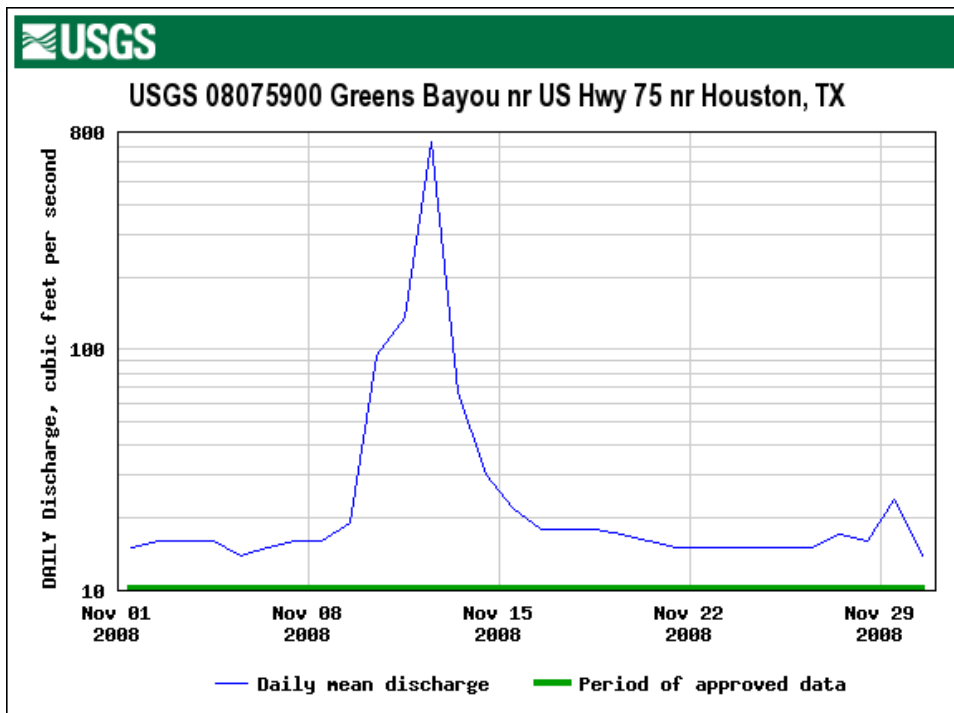
Figure 11. Stream discharge data from USGS gage site on Greens Bayou near Hwy 75 (I-45). Fish collection occurred on November 25, 2008.

Fishes as Sources of *E. coli*   34

Figure 12. Stream gage height from USGS gage located on Greens Bayou near Hwy 75 (I-45). Fish collection occurred on April 10, 2009.

Figure 13. Stream discharge data from USGS gage site on Greens Bayou near Hwy 75 (I-45). Fish collection occurred on April 10, 2009.

Fishes as Sources of *E. coli*   35
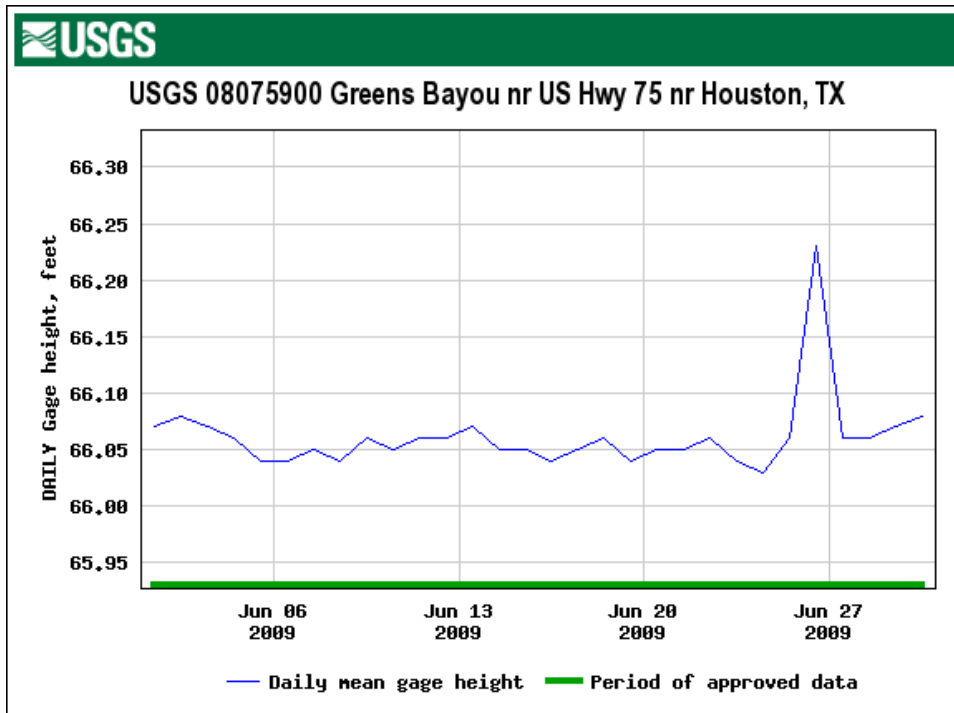
Figure 14. Stream gage height from USGS gage located on Greens Bayou near Hwy 75 (I-45). Fish collection occurred on June 29, 2009.
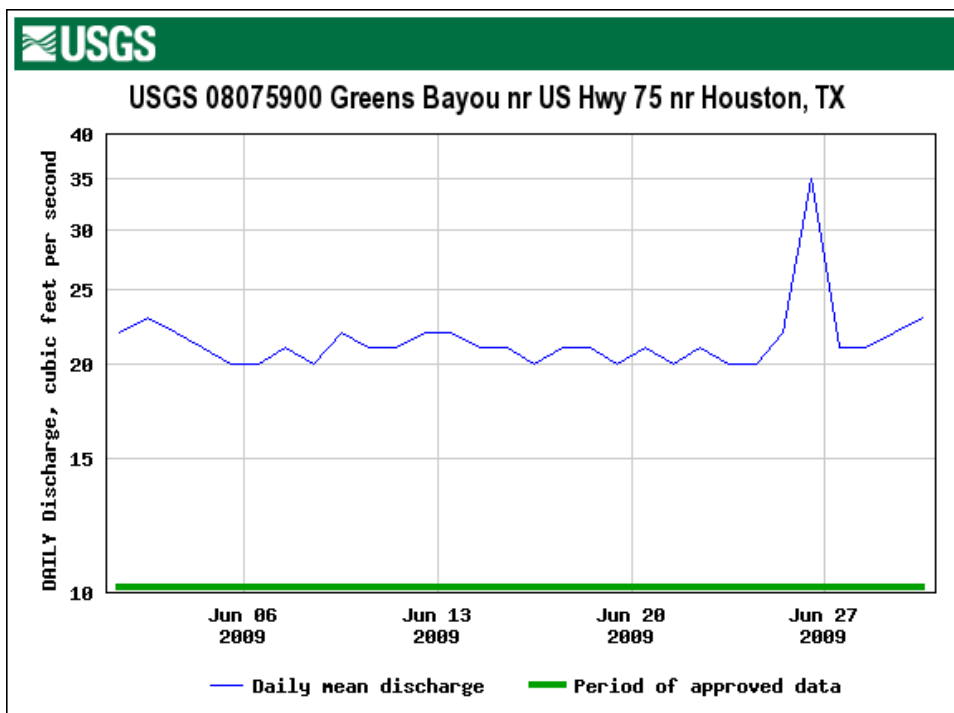
Figure 15. Stream discharge data from USGS gage site on Greens Bayou near Hwy 75 (I-45). Fish collection occurred on June 29, 2009.
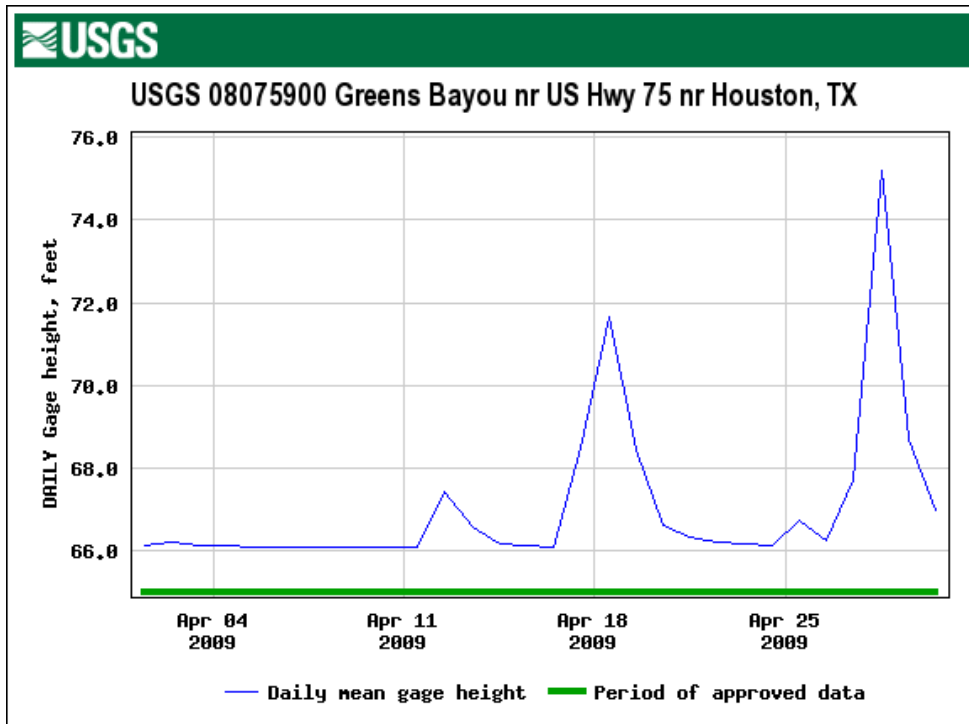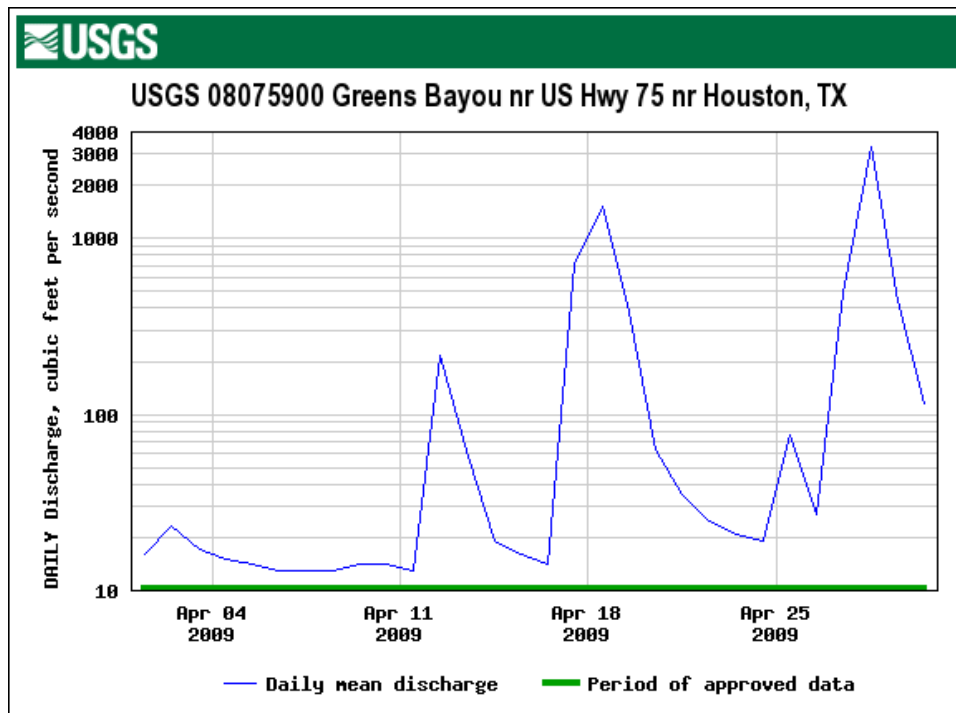
Fishes as Sources of *E. coli*   36

Water and Sediment Quality

Water quality conditions documented during our survey reveal spatial and temporal trends in several important variables (Figs. 16-26). Water temperatures generally followed seasonal trends (Fig. 16). However during April 2010, water temperature at Horsepen Bayou was noticeably lower that the other two sites. This may be due to the closer connectivity to coastal water bodies which delays spring time warming in comparison to shallower inland streams. Interestingly enough Horsepen Bayou, classified as a coastal tidal bayou, generally yielded lower specific conductance values (Fig. 17). Dissolved oxygen and pH levels measured at all sites were within levels that support all expected fish species (Figs. 18 and 19). Transparency and turbidity levels primarily reflect the amount of suspended sediments present in the water column (Figs. 20 and 21). With the exception of November 2008 collections, Horsepen Bayou usually displayed the lowest water and sediment *E. coli* levels (Figs. 22-26). Higher levels of *E. coli* were generally observed at intermediate temperatures but declined in summer months at highest temperatures. Based on examination of field data alone it appears that higher levels of *E. coli* occurred in the more urban bayous during warmer months of our study.
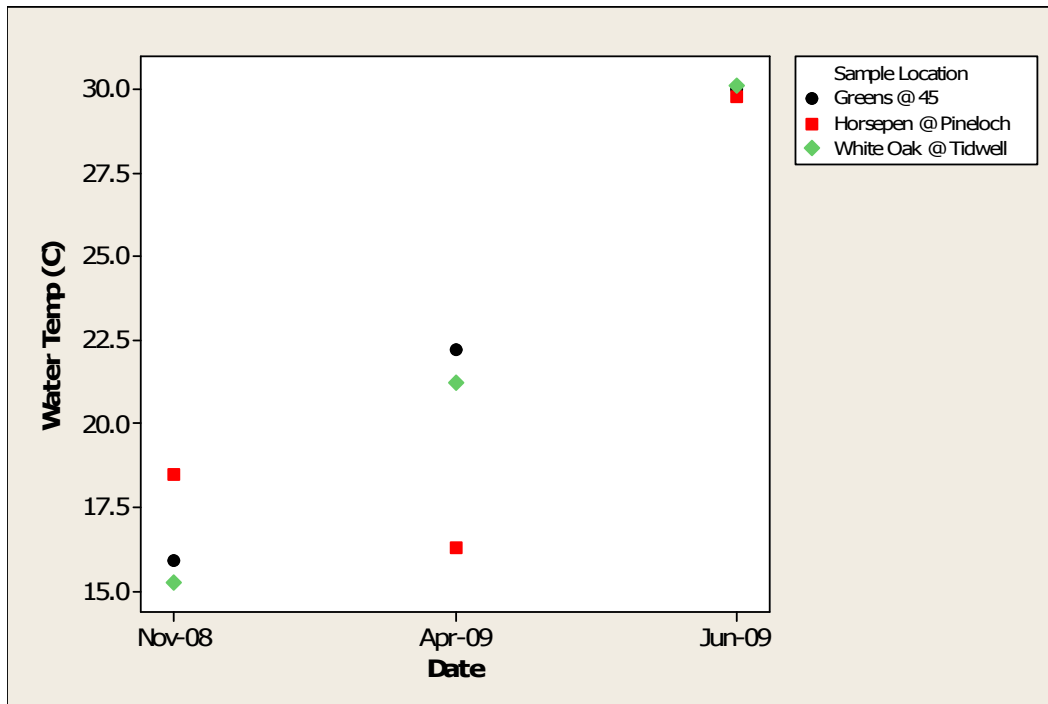
Figure 16. Water temperature measured during field collections for fish.

Fishes as Sources of *E. coli*   37
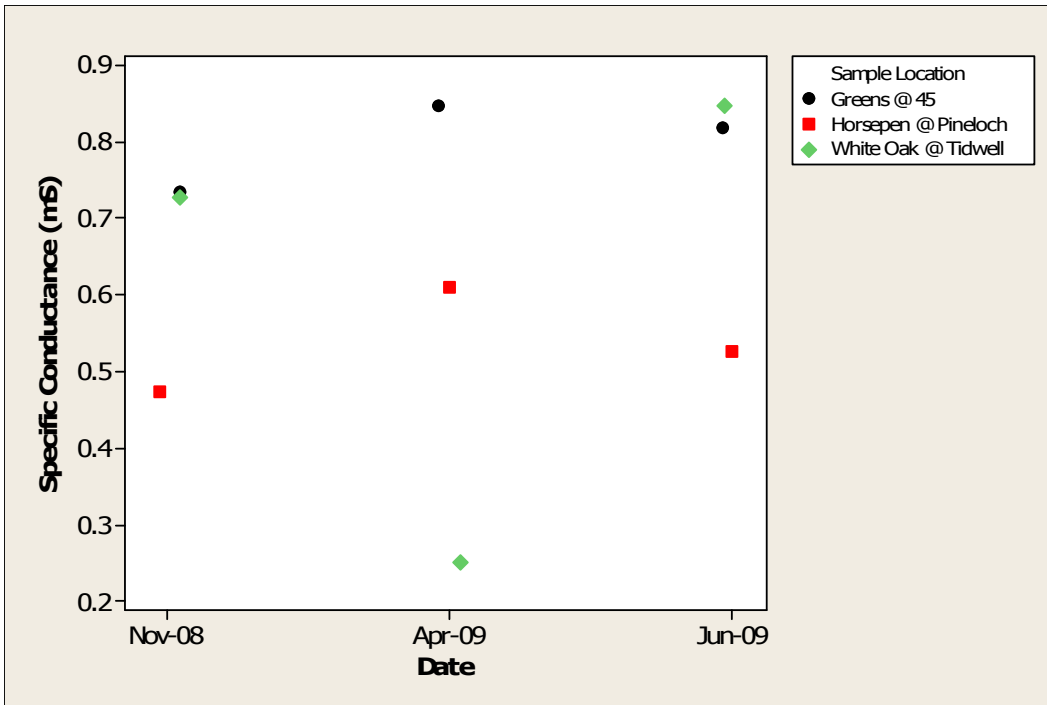
Figure 17. Specific conductance measured during field collections for fish.
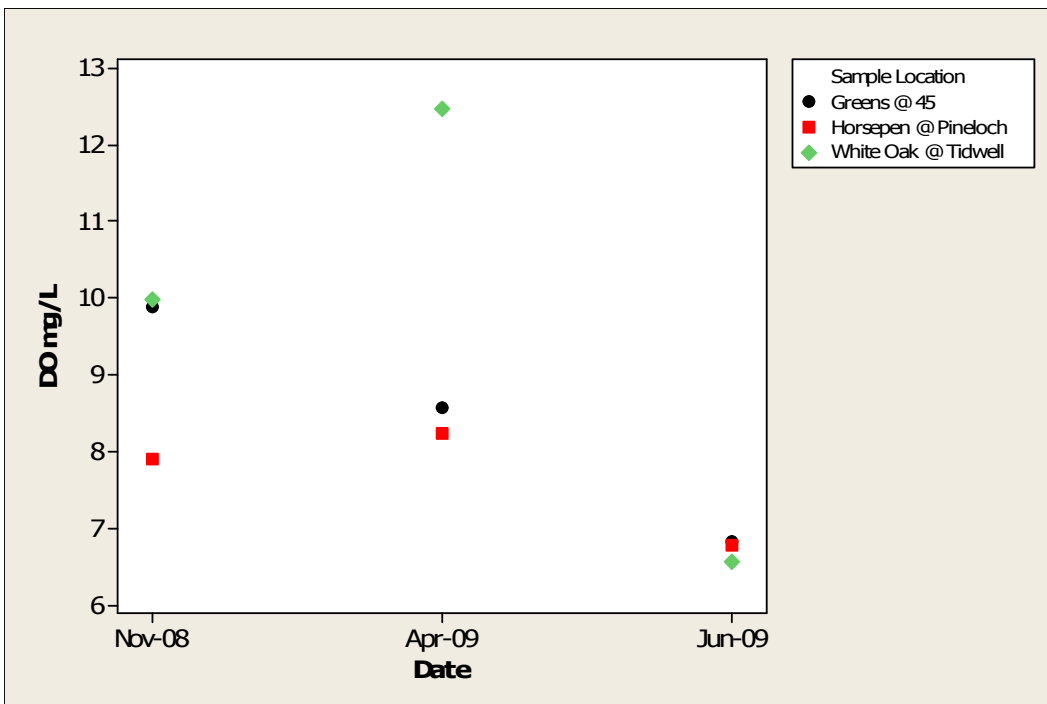
Figure 18. Dissolved oxygen measured during field collections for fish.

Fishes as Sources of *E. coli*   38
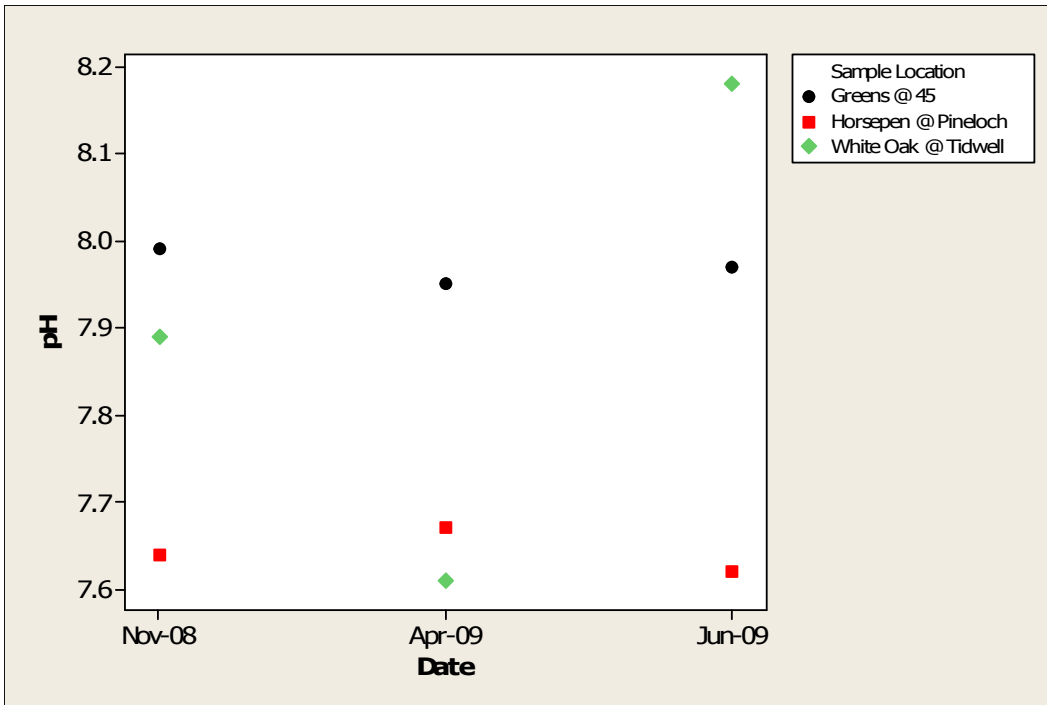
Figure 19. Surface water pH levels measured during field collections for fish.
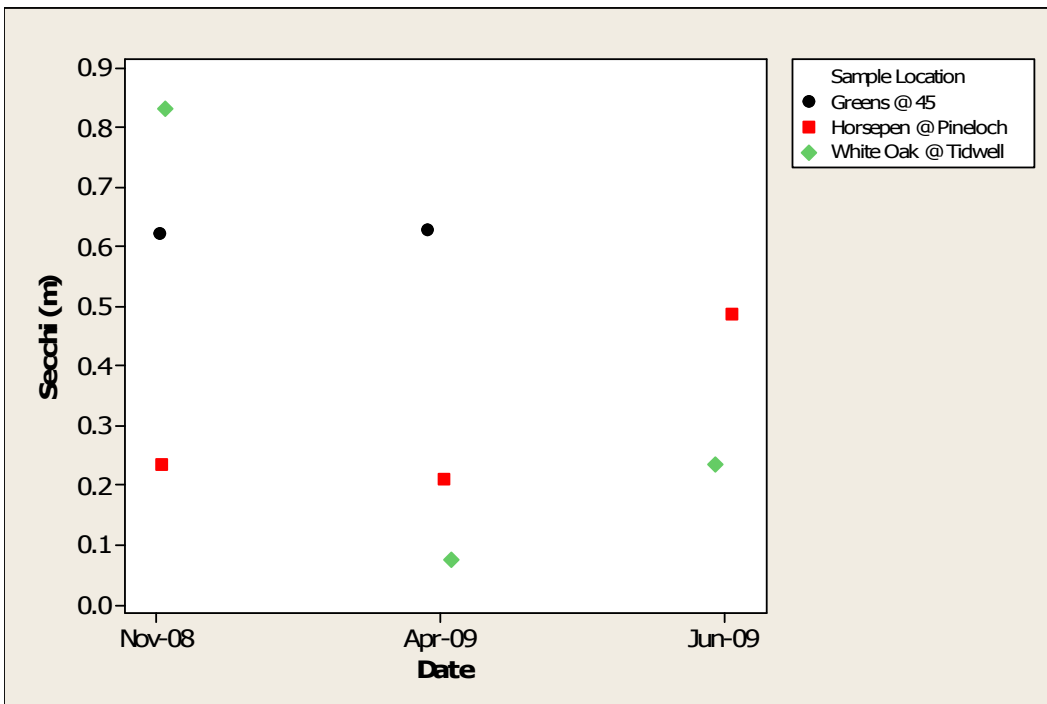
Figure 20. Secchi disk transparency measured during field collections for fish.

Fishes as Sources of *E. coli* 39
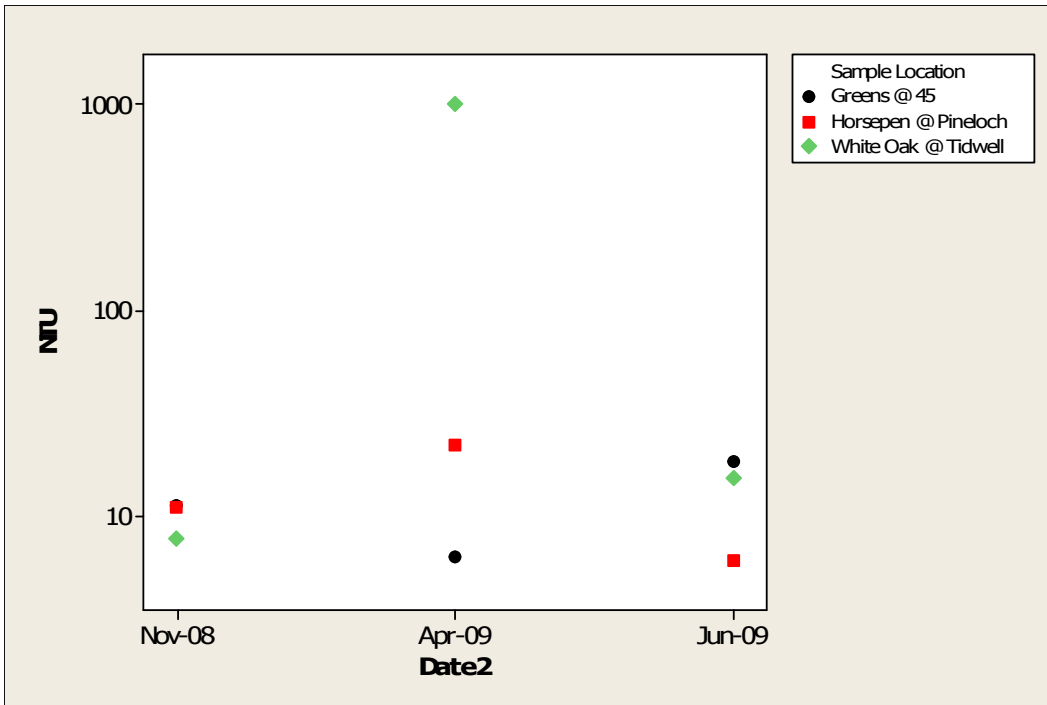
Figure 21. Turbidity (NTU) measured during field collections for fish. Note Y-axis is log scale.
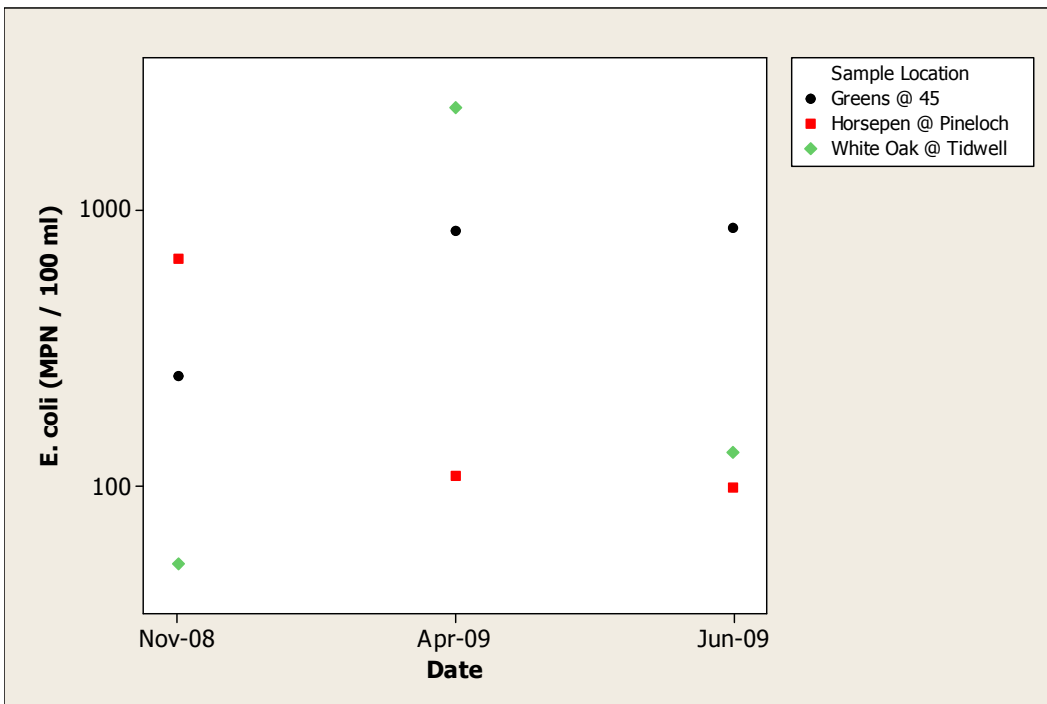
Figure 22. *E. coli* levels in water measured during field collections for fish. Note Y-axis is log scale.
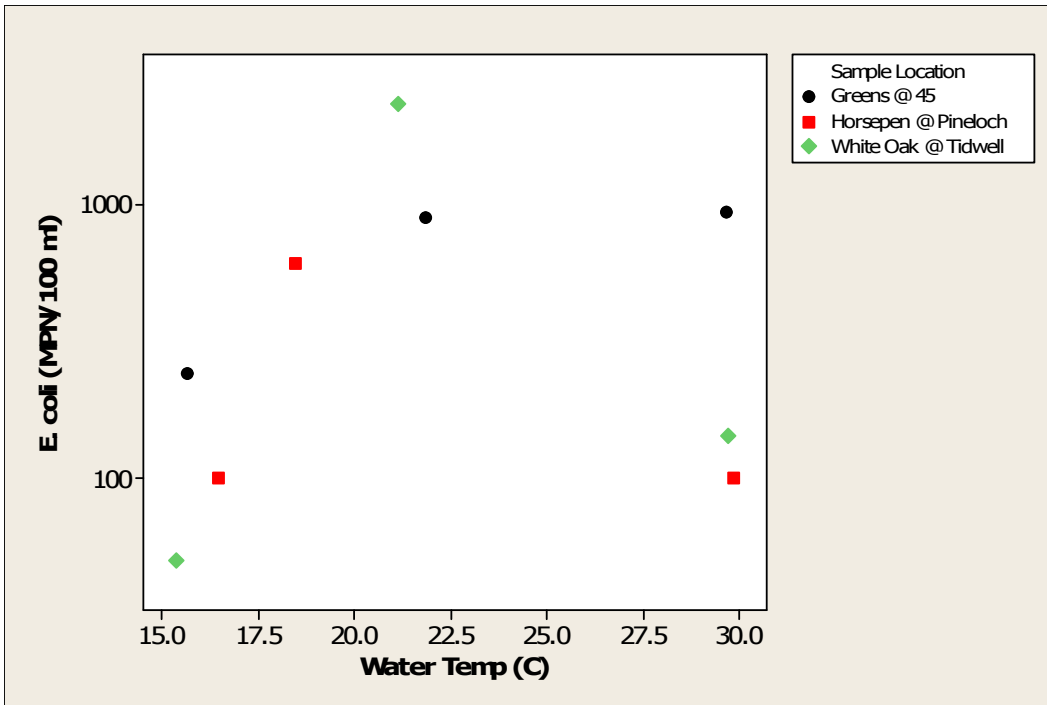
Figure 23. *E. coli* levels in water and water temperature measured during field collections for fish.
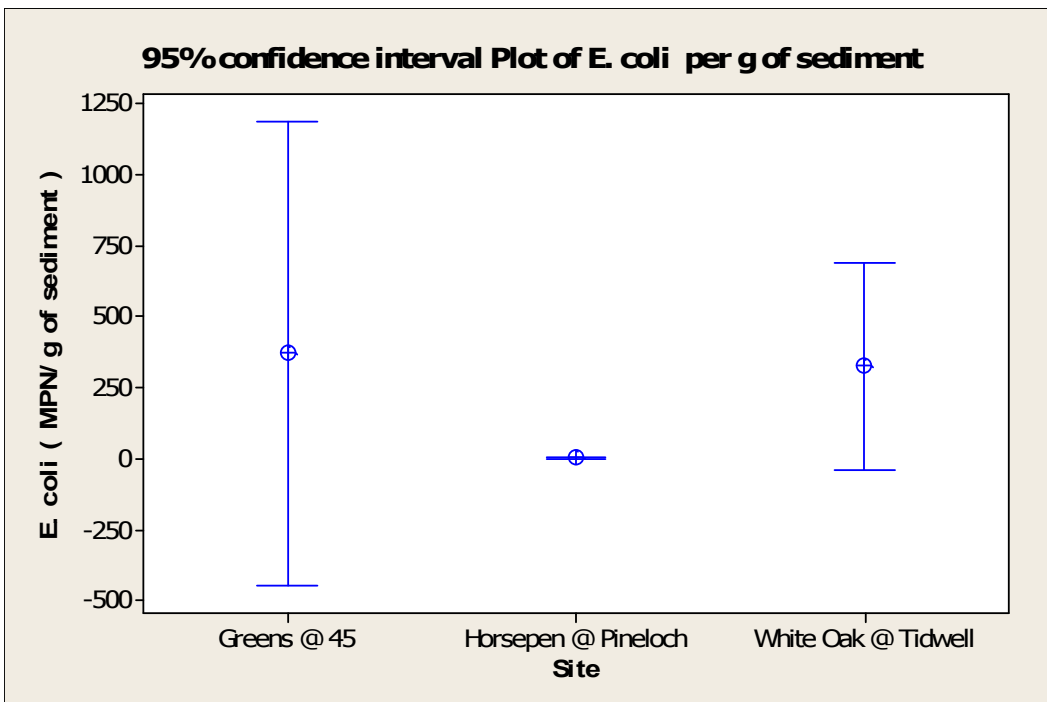
Figure 24. Confidence interval (95%) for mean *E. coli* levels in sediment measured during field collections for fish. Note Y-axis is log scale.

Fishes as Sources of *E. coli*   41
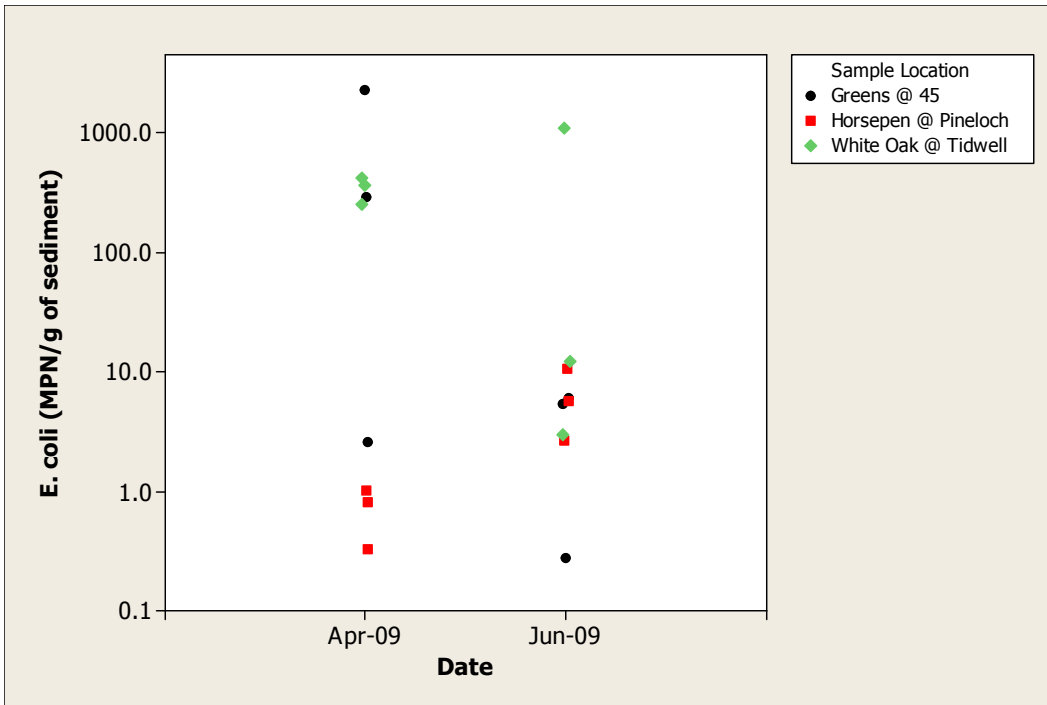
Figure 25. *E. coli* **levels in sediment measured during field collections for fish. Note Y-axis is log scale.**
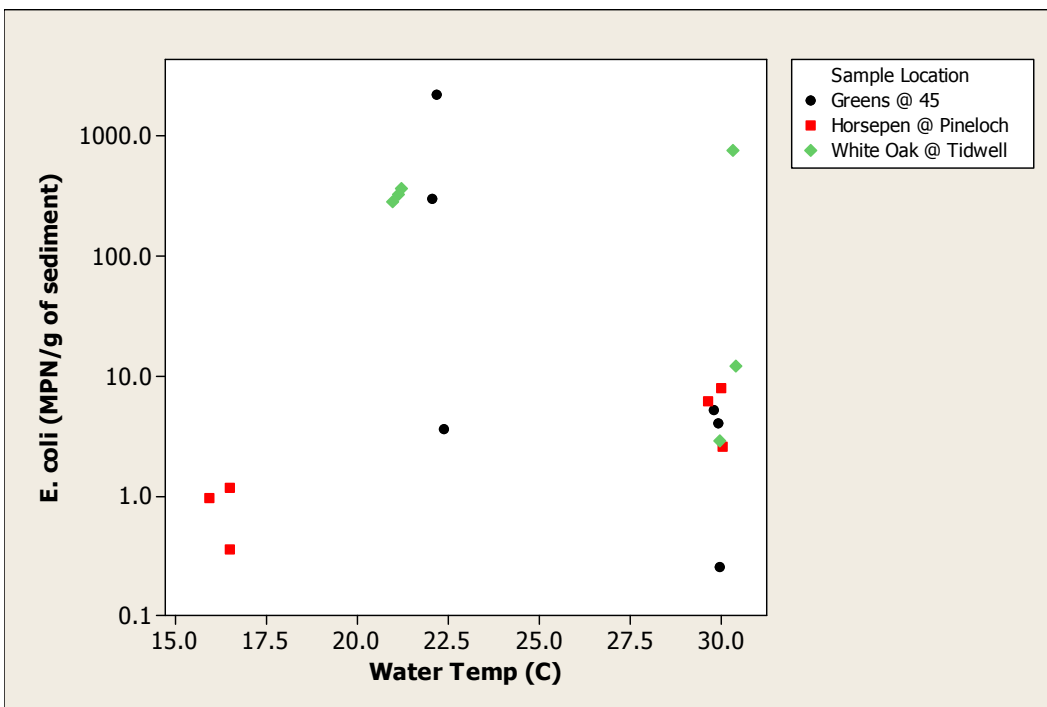
Figure 26. *E. coli* **levels in sediment and water temperature measured during field collections for fish. Note Y-axis is log scale.**

Fishes as Sources of *E. coli*   42

Wild Fish E. coli Levels

Overall production of *E. coli* in fish feces was generally highest at the Greens Bayou site (Fig. 27). However, there was a considerable amount of variability the estimate of mean concentrations of bacteria.   Based on the results of the Kruskal-Wallis non-parametric one-way ANOVA (KWANOVA) and Dunn's multiple pair wise comparison tests (DMC), median *E. coli* levels in fish fecal material were significantly higher at the Greens Bayou and White Oak sites in contrast to the Horsepen Bayou site (Fig. 28, Table 7). There was also an indication of seasonal fluctuations in *E. coli* levels in fish feces (Fig. 29). Highest average levels of *E. coli* in fish feces were generally encountered during November 2008 (fall) in comparison to the spring and summer months that were surveyed. This is in contrast to the water quality data which suggested higher levels of *E. coli* in the environment occurs in the spring months. The data were however, extremely variable. Based on the results of the KWANOVA and DMC) median *E. coli* levels in fish fecal material were significantly lower in April in comparison to November and June (Fig. 30, Table 8). There were however interactions between seasonal and site levels of *E. coli* in fish feces (Figs. 31 and 32). Average levels of *E. coli* in fish feces were generally highest at Greens Bayou. White Oak Bayou had the second highest mean levels during 2 of the 3 months surveyed, whereas Horsepen Bayou generally had the lowest average levels except for June 2009 (Fig. 32).

Almost all species of fish yielded detectable levels of *E. coli* levels in their feces (Fig. 33). Although no consistent relationship was observed between bacteria levels and fish species composition, certain species including common carp, redear sunfish, green sunfish, and largemouth bass often yielded high (>1,000 MPN / g feces) levels of *E. coli* . Intermediate (1,000 – 100 MPN / g feces) levels of *E. coli* in feces were observed in several species of fish including striped mullet, common carp, channel catfish and tilapia. Lowest bacteria levels (< 200 MPN / g feces) were usually observed in bluegill, largemouth bass and spotted gar. When grouped into trophic groups several patterns were observed. However, most trophic groups exhibited considerable variation in fecal *E. coli* levels. Species with highest (>1,000 MPN / g feces) *E. coli* levels were generally classified as piscivores and insectivores (Figs. 34 and 35). However, many members of these groups also exhibited low (<100 MPN) *E. coli* levels. In contrast, herbivores exhibited intermediate levels of *E. coli* in their feces. However, the KWANOVA and DMC failed to reject the null hypothesis of zero differences in median *E. coli* levels between trophic groups (Fig. 36, Table 9). Based on the results of the field surveys of fish fecal material there appears to be very high levels of *E. coli* in various species of fish that vary both seasonally and spatially. This may be due to exposure to additional site specific and seasonal external loads of *E. coli.*
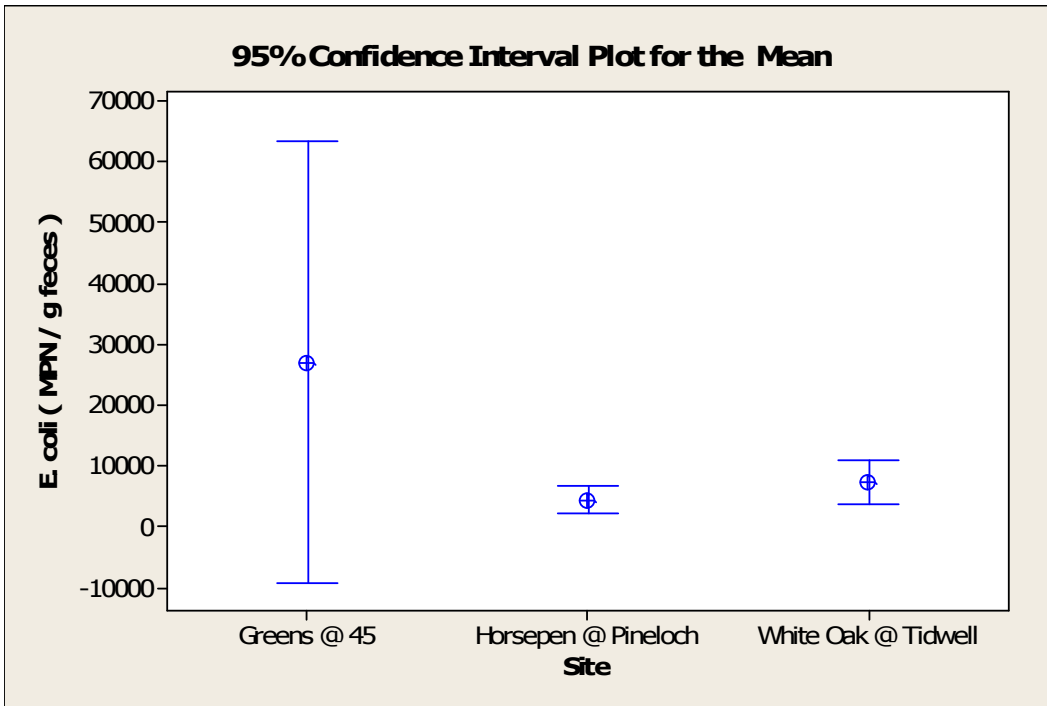
Figure 27. Average *E. coli* levels in fish feces at each site.

Figure 28. Kruskal-Wallis non-parametric one-way ANOVA and Dunn's multiple pair wise comparison test on median site *E. coli* levels in fecal material from all species of fish and sites.

Fishes as Sources of *E. coli*

Table 7. Results of Kruskal-Wallis one-way ANOVA and Dunn's multiple comparison tests on median fecal *E. coli* levels for all species of fish and seasons.

```
Kruskal-Wallis Test on the data

Site       N  Median  Ave Rank      Z
Horsepen   70   225.5      90.7  -2.50
Greens     69  1433.0     111.6   1.02
W. Oak     71  1823.3     114.1   1.47
Overall   210             105.5

H = 6.29  DF = 2  P = 0.043
```

* Sites	Z-value vs. Critical value	P-value
Horsepen vs. W. Oak	2.28887 >= 1.834	0.0221
Horsepen vs. Greens	2.03111 >= 1.834	0.0422

```
* The following groups showed significant differences
```
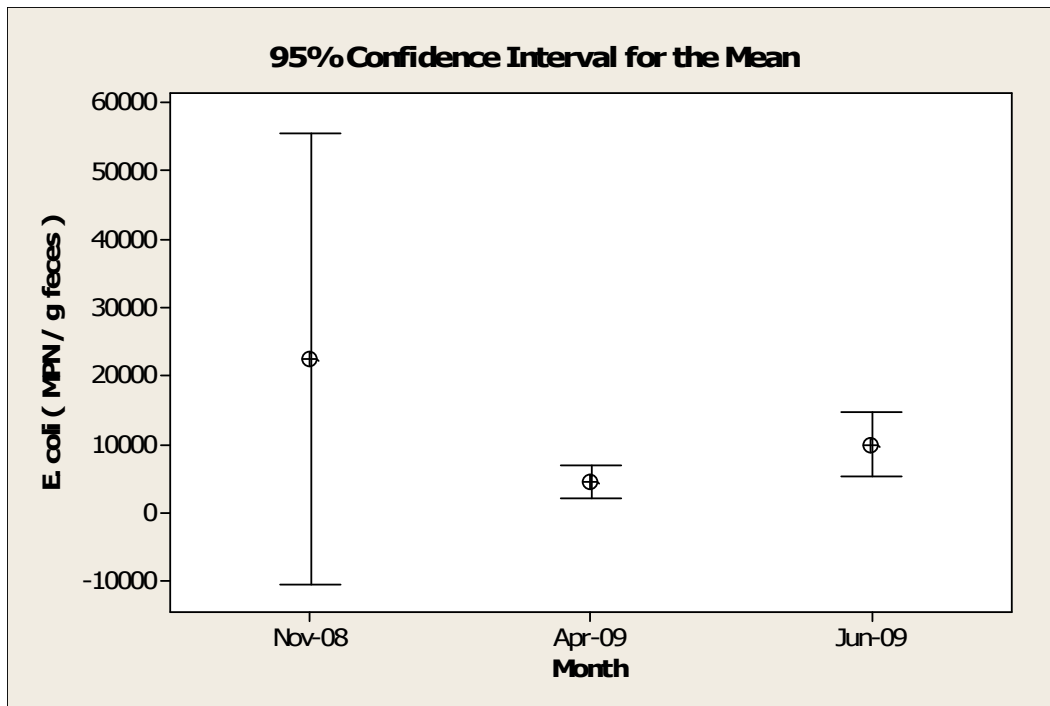

Figure 29. Average *E. coli* levels in fish feces during each month of collection.

Figure 30. Kruskal-Wallis non-parametric one-way ANOVA and Dunn's multiple pair wise comparison test on monthly median *E. coli* levels in fecal material from all species of fish and sites.

Table 8. Results of Kruskal-Wallis one-way ANOVA and Dunn's multiple comparison tests on median fecal E. coli levels for all species of fish and seasons.

```
Kruskal-Wallis Test on the data

Season    N  Median  Ave Rank      Z
Spring   64   196.6      89.1  -2.59
Fall     76  1485.1     110.4   0.88
Summer   70  2876.5     115.2   1.64
Overall 210             105.5

H = 6.95  DF = 2  P = 0.031
```

* Seasons	Z-value vs. Critical value	P-value
Apr-09 vs. Nov-08	2.35531 >= 1.834	0.0185
Apr-09 vs. Jun-09	1.96321 >= 1.834	0.0496

* The following seasons showed significant differences (adjusted for ties).

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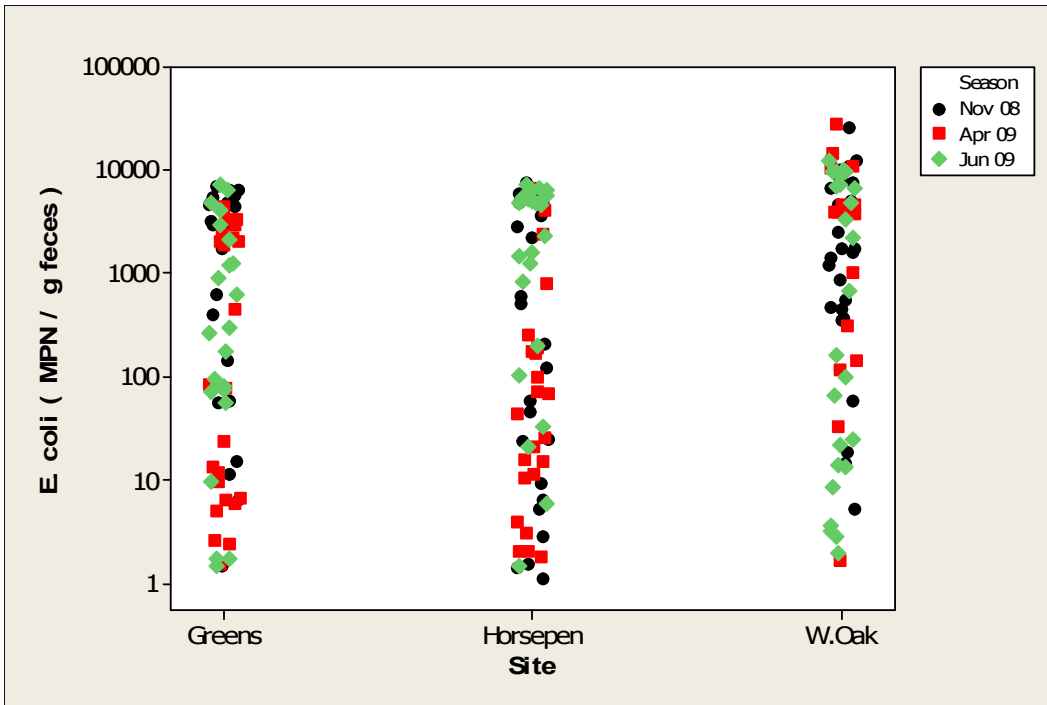
Figure 31. *E. coli* levels observed in fish feces at each site and season.
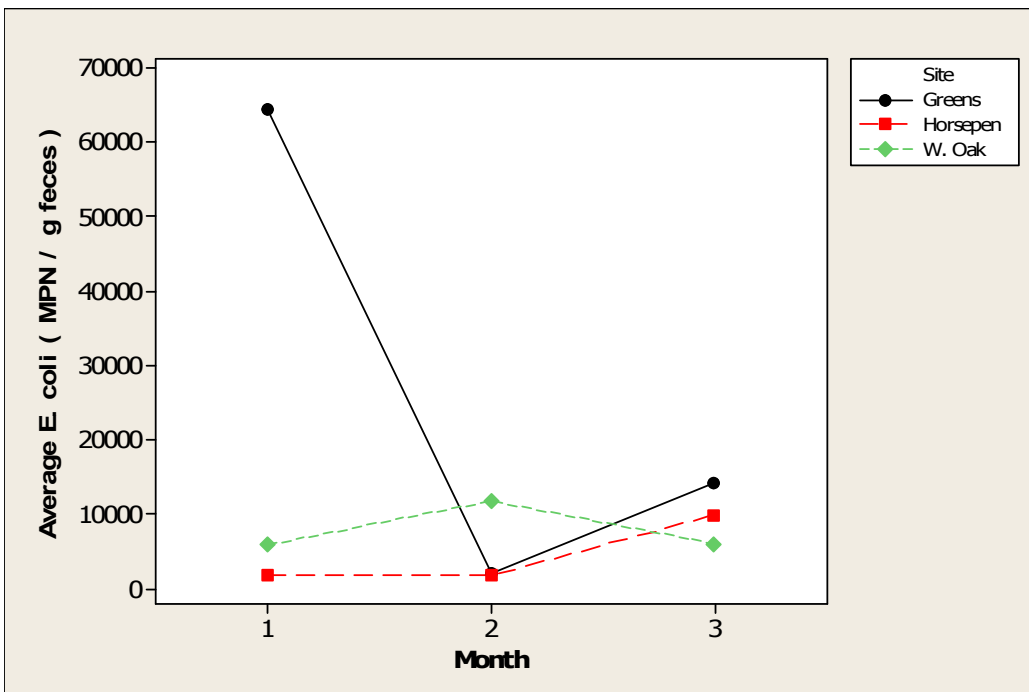
Figure 32. Average *E. coli* levels in fish feces at each site and month. Month 1 = Nov-08; 2 = Apr-09; 3 = Jun-09.
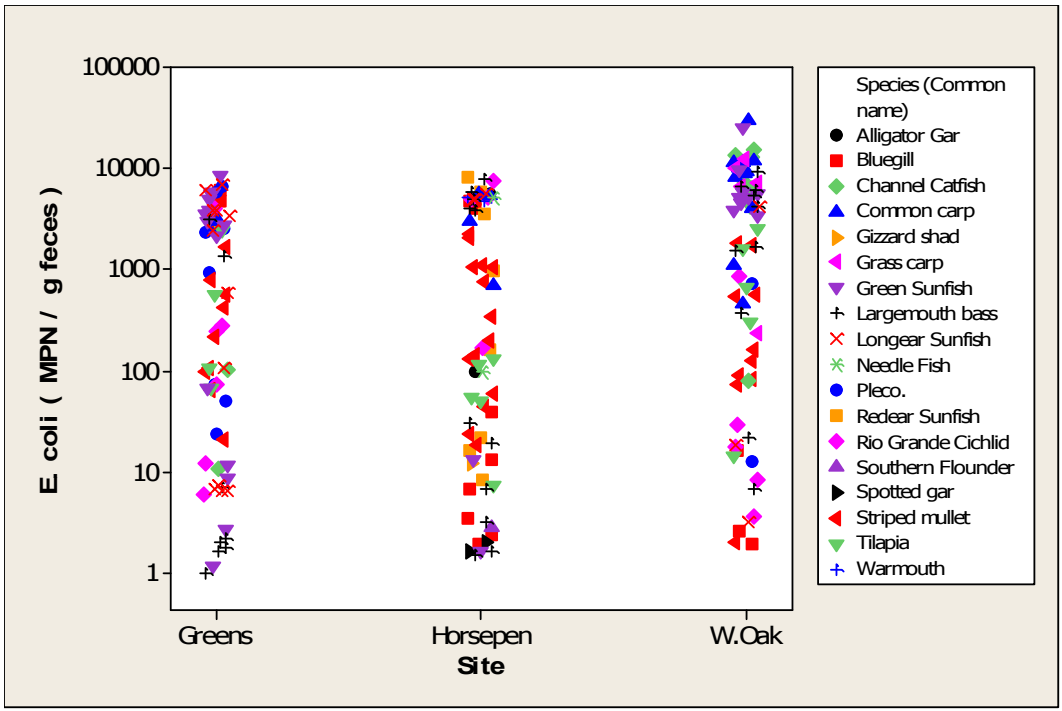
Figure 33. Levels of *E. coli* detected in feces of fish species collected at each site.
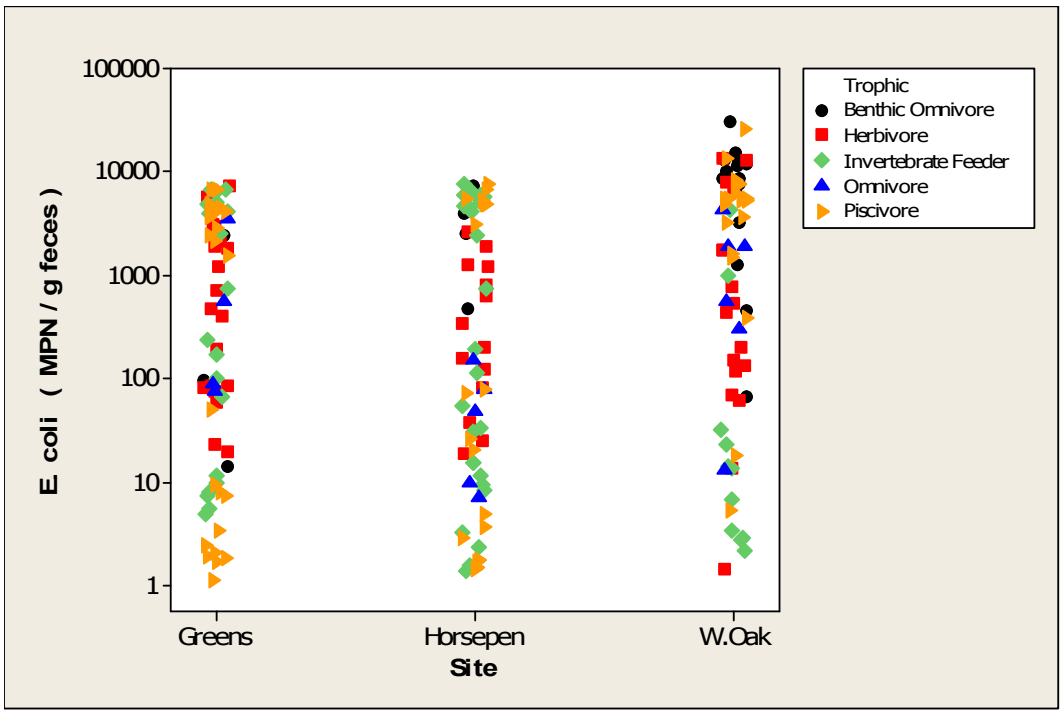
Figure 34. Levels of *E. coli* in detected in fishes from each trophic level at each site.

Fishes as Sources of *E. coli*   48

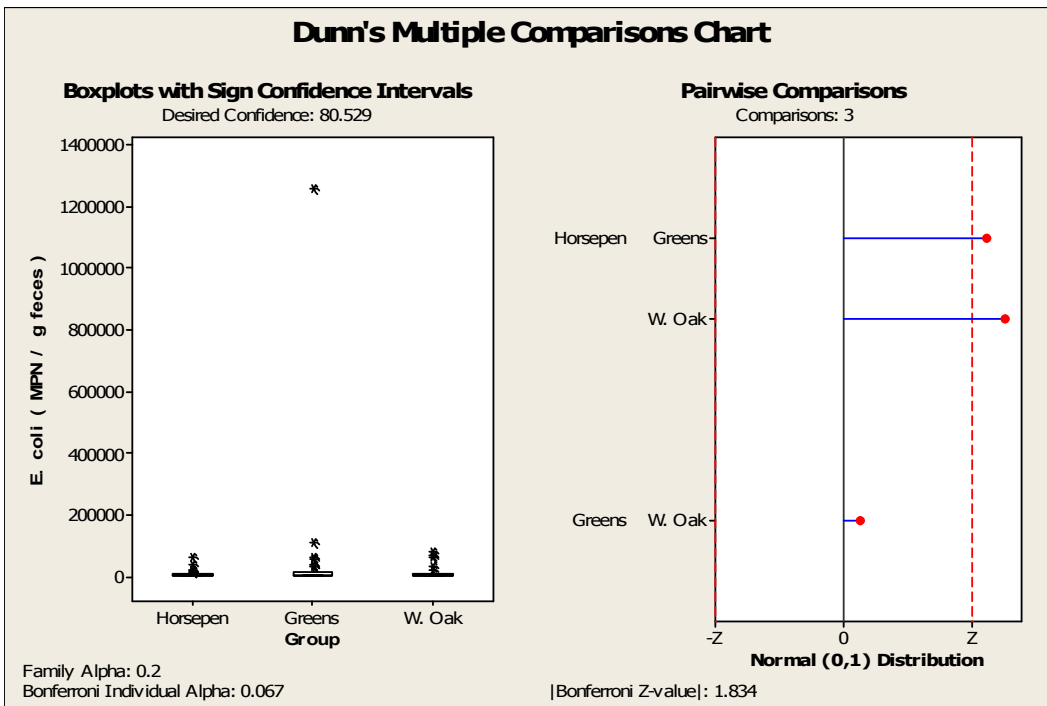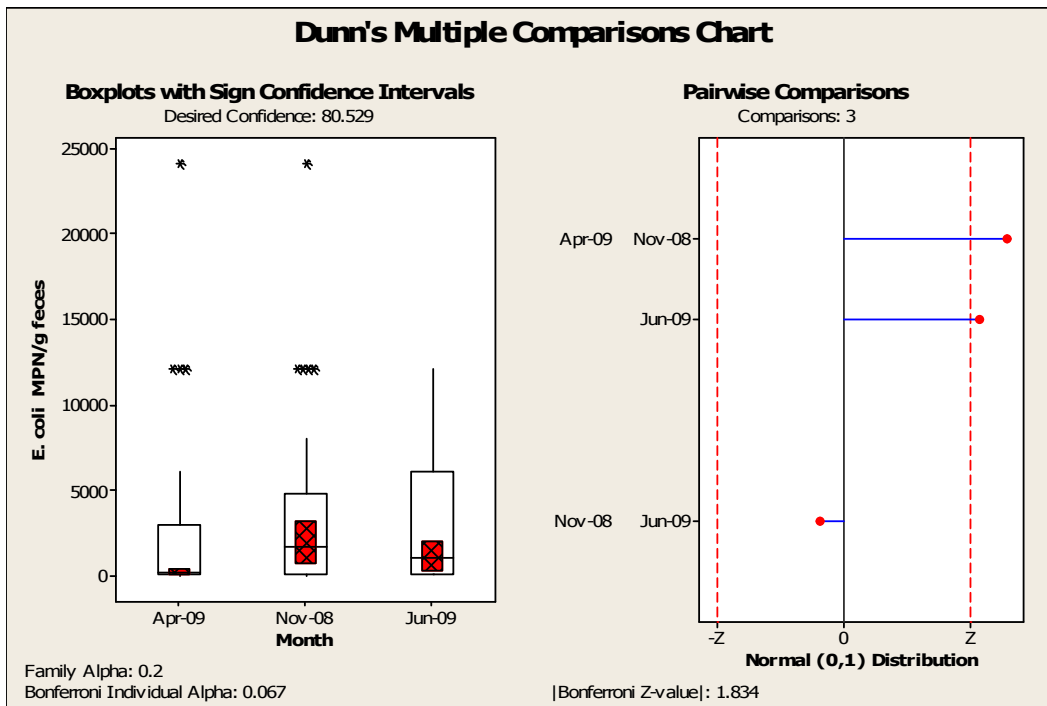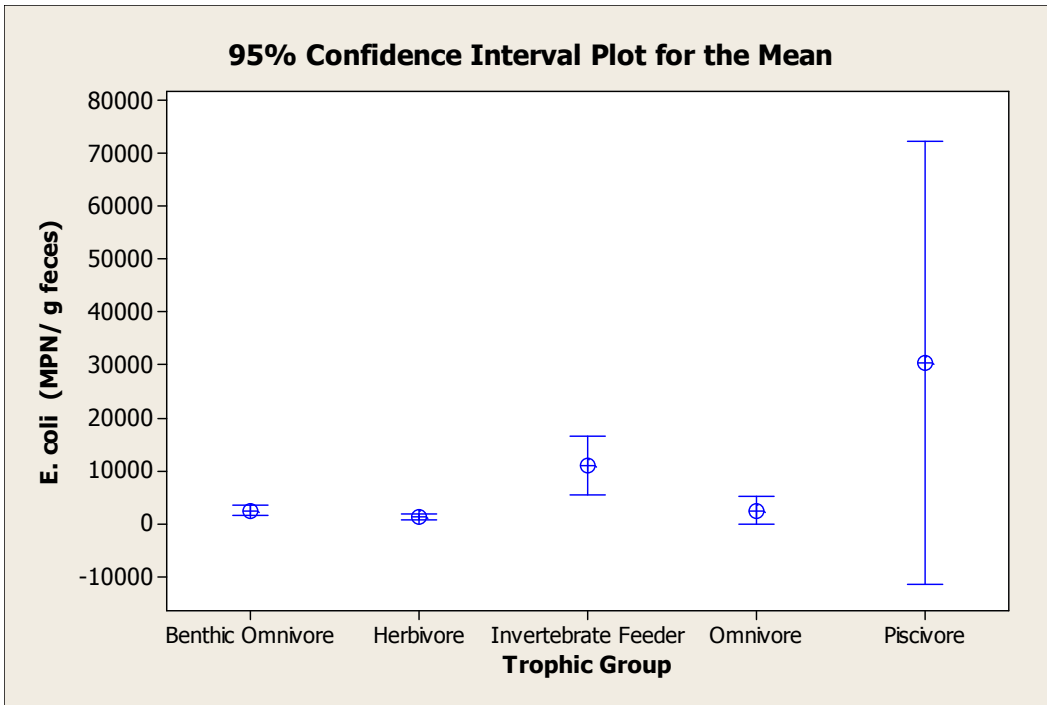**Figure 35. Average *E. coli* levels in feces of fish from each trophic group.**
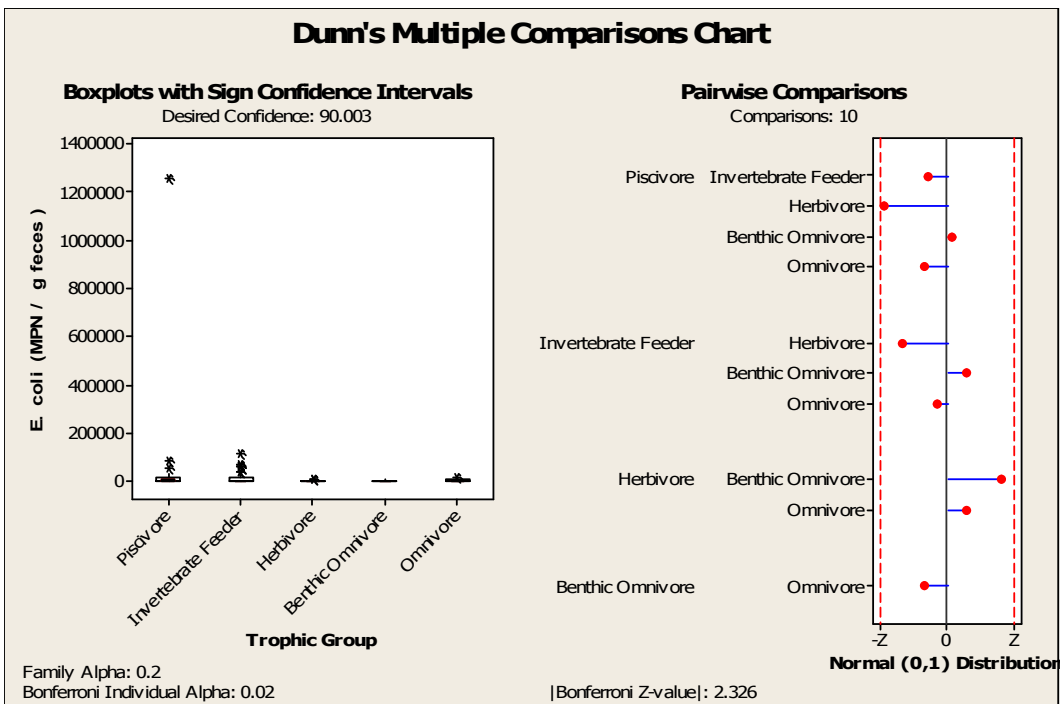
Figure 36. Kruskal-Wallis non-parametric one-way ANOVA and Dunn's multiple pair wise comparison test on median trophic group *E. coli* levels in feces.

Table 9. Results of Kruskal-Wallis one-way ANOVA and Dunn's multiple comparison tests on median trophic level fecal *E. coli* levels.

```
Kruskal-Wallis Test on the data

Trophic Group         N  Median  Ave Rank      Z
Piscivore            60  4306.7     114.6   1.38
Invertebrate Feeder  60   462.6     106.9   0.21
Herbivore            51   333.7      89.0  -2.23
Benthic Omnivore     24  2092.5     117.1   0.99
Omnivore             15  1270.1     100.9  -0.31
Overall             210              105.5

H = 6.10  DF = 4  P = 0.192
```

```
* There were no significant trophic group differences.
```

Aquarium E. coli Production Study

The sunfish (bluegill and redear) and channel catfish aquarium studies were completed during December 2008 and January/February 2009 respectively. The aquarium study produced supplementary information that's supports data collected in the field.   During both the sunfish (bluegill and redear) and channel catfish aquarium studies unexpected mortality occurred in some of the tanks (Table 10 and 11). A combination of elevated ammonia levels and disease pathogens was suspected in most cases; however no detailed examination for pathogens was conducted. During the sunfish experiment dead or stressed specimens were replaced with bluegill or redear sunfish that were maintained on site. During the channel catfish experiment we also took a similar approach but unfortunately we ran out of fish and had to reduce the higher density stocking rates to 2 fish and/or reduce the number of replicates for both stocking rates of channel catfish. This may have affected potential *E. coli* production. In addition, AmQuel+® or Ammo-Lock® and common salt were added occasionally during the experiment to control ammonia buildup and/or treat stressed fish.

Water temperature fluctuated during the sunfish aquarium study between 20 and 26 °C (Fig. 37). These temperature changes were caused by changing room temperatures. During the period of the study the facility experienced some problems with the rooml heating system which required the installation of portable heaters in the laboratory. Unfortunately this allowed temperatures to fluctuate more than preferred. However, the temperatures were well within the temperature tolerance limits of the species and should not have stressed this species.   Specific conductance levels ranged between 300 and 600 uS and median values were between 440 and 450 uS which is well within the range of suitable rearing conditions for freshwater sunfish (Fig. 38).

Table 10. Observations on mortality and other significant events that occurred during the bluegill/redear aquarium study from December 3 to 18, 2008.

Day	Exposure (# Fish)	Tank	Comment	Experiment Design 5 control; 5 (1 fish) low; 5 (3 fish) high
5	3	13	Dead fish replaced	No effect
6	1	8	Dead fish replaced	No effect
8	1	8	Dead fish replaced with redear sunfish	No effect
9			Left uneaten food in control tanks for 24 hours, may effect ammonia in control	No effect; control may exhibit elevated ammonia
11	3	15	2 dead fish replaced	No effect
11	3	13	1 dead fish replaced	No effect
11	1	8	Replace live redear with bluegill	No effect
12	3	15	2 dead fish, replace 1 stressed fish left with 3 new fish	No effect
13	1	8	Replaced 1 diseased fish	No effect
13	3	14	Replace 1 dead fish	No effect
14	1	11	1 fish died at end of study	No effect

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Table 11. Observations on mortality and other significant events that occurred during the channel catfish aquarium study from January 20 to February 3, 2009.

| Day	Exposure (# Fish)	Tank	Comment	Experiment Design 5 control; 5 (1 fish) low; 5 (3 fish) high
4	1	6	Replaced dead fish	No effect
6	1	8	Replaced dead fish	No effect
7	1	8	Replaced dead fish	No effect
7	3	13	2 dead fish replaced	No effect
8	3	15	Replaced dead fish	No effect
9	1	7	Replaced dead fish	No effect
9	3	13	Replaced dead fish	No effect
10	1	7	Dead fish NOT replaced	4 reps (1 fish)
10	3	15	Dead fish NOT replaced, reduced to 2	4 rep (3 fish) + 1 rep (2 fish) = high
11	1	7	Stocked with 1 fish from tank 15	5 reps (1 fish) restored
11	3	15	Dead fish, move remaining fish to tank 7	4 reps (3 fish) high remain
12	1	6	Dead fish, none left	3 reps (1 fish) low
12	1	7	Dead fish, non left	3 reps (1 fish) low
13	1	9	Dead fish, replace with 1 fish from tank 13	3 reps (1 fish) low; High = 4 reps; 3 with 3 fish; 1 with 2.
13	3	13	2 dead fish. Move remaining 1 fish to tank 9	3 reps (1 fish) low 3 reps (3 fish) high
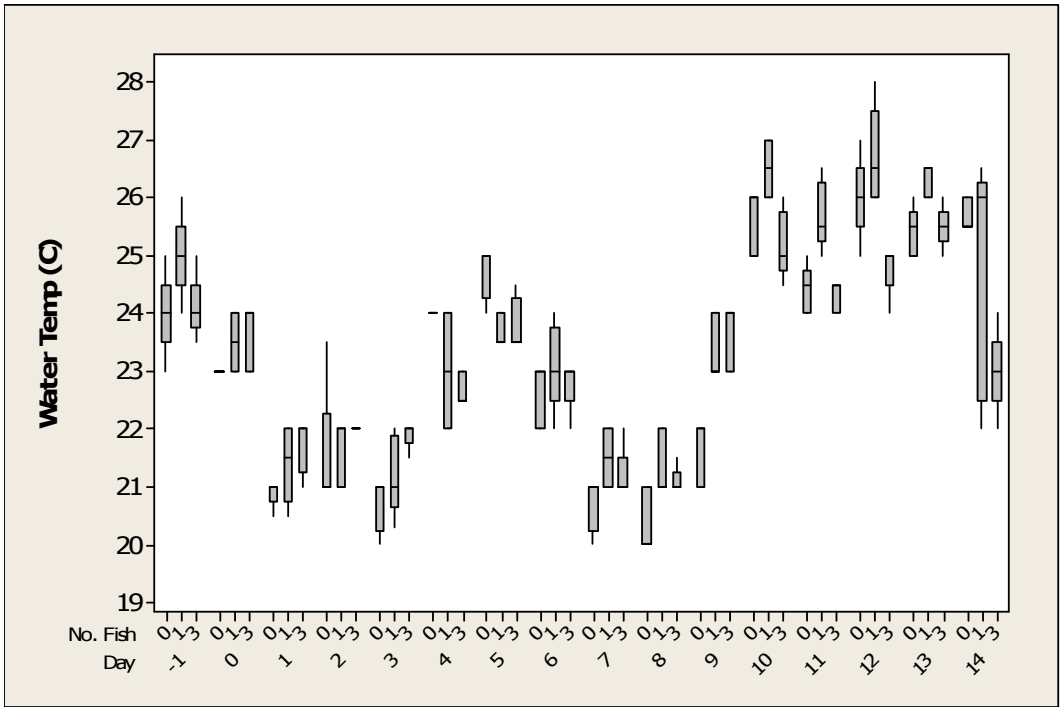
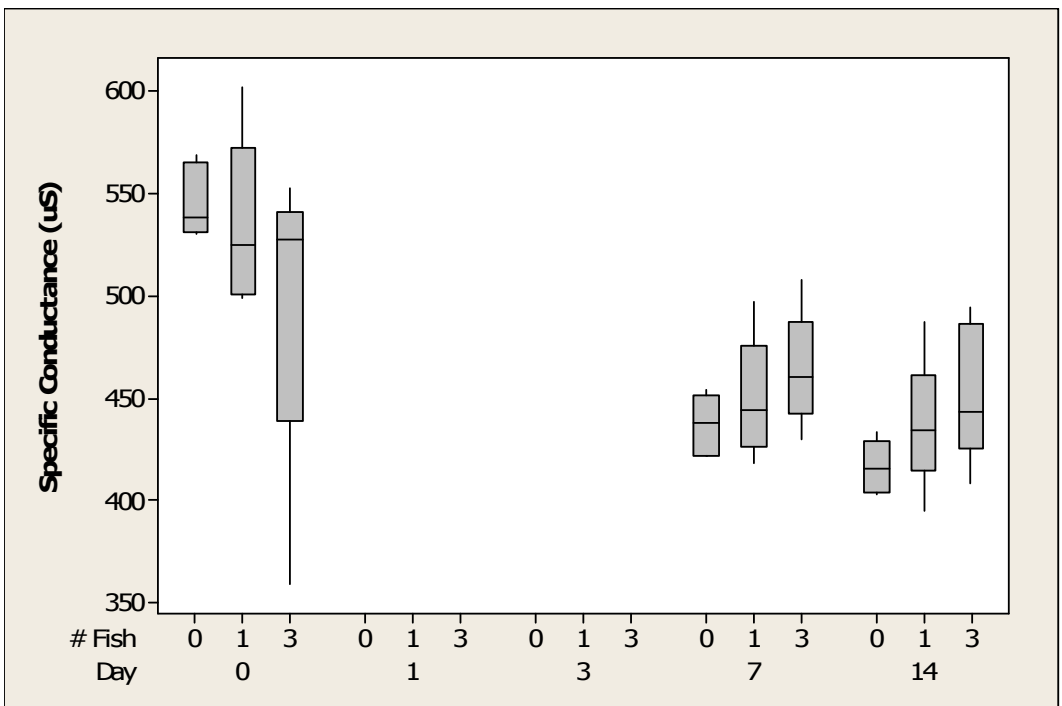**Figure 37. Water temperature recorded during bluegill aquarium study.**

Figure 38. Specific conductance recorded during the bluegill aquarium study.

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Dissolved oxygen levels were all above 4.0 mg/l and generally above 6.0 mg/l. We did observe a declining trend in dissolved oxygen over time and at higher stocking rates which reflects the influence of higher amounts of fish respiration and additional biochemical oxygen demand due to increased feeding rates and breakdown of uneaten food and feces (Fig. 39). The pH level of the test aquaria ranged between 7.4 and 8.3, which is within the range of tolerance for this species and a commonly encountered level in local streams (Fig. 40). The pH level did however decline over time suggesting the accumulation of waste products and increased concentrations of carbon dioxide. This patter of declining pH levels was most evident in aquaria with higher stocking rates (i.e. 3 fish). Total ammonia levels were generally low (median levels ≤ 1 mg/l) throughout the study, but did increase over time and with increasing stocking densities of fish (Fig. 41). This reflects the influence of increased feeding rates and waste production in these tanks. It is possible that these levels that occurred mostly after day 9, when combined with the presence of pathogens, may have stressed some fish and cause the observed mortality. However, the concurrent declining pH values would have decreased ammonia toxicity due to the decrease in the proportion of un-ionized ammonia. Un-ionized ammonia levels never exceeded 0.028 mg/l. The TCEQ requires treatment to 3 mg/l total ammonia in wastewater effluent to protect aquatic life (TCEQ 2003b). Previous studies have shown that levels of 0.82 mg/l un-ionized ammonia are acutely toxic to bass (Colt and Tommaso 2001). The highest un-ionized value we observed was usually far below this literature based criteria. However, total ammonia exceeded 3 mg/l occasionally. Median total hardness values ranged between 65 and 115 mg/l which are well within the tolerance levels of freshwater fish (Fig. 42).

Throughout the bluegill aquarium experiment *E. coli* levels in the water were extremely low (Fig. 43). The majority of measurements were below the detection limit of 1.0 MPN / 100 ml of water. The highest reading occurred in the days 1 and 3 in tanks containing one fish. This suggests that at the stocking levels employed and water quality conditions observed sunfish do not appear to be producing large amounts of *E. coli* bacteria that are detectable in the water column. It should be noted that relative feeding rates varied between aquaria and was a function of water quality conditions and health of individual organisms. In some cases we did not see fish feeding actively while others fed extensively (Fig. 44). On average about 3 out 5 (60%) of the tanks per treatment held actively feeding fish on any occasion as determined by visual examination of the fish after feed was administered.
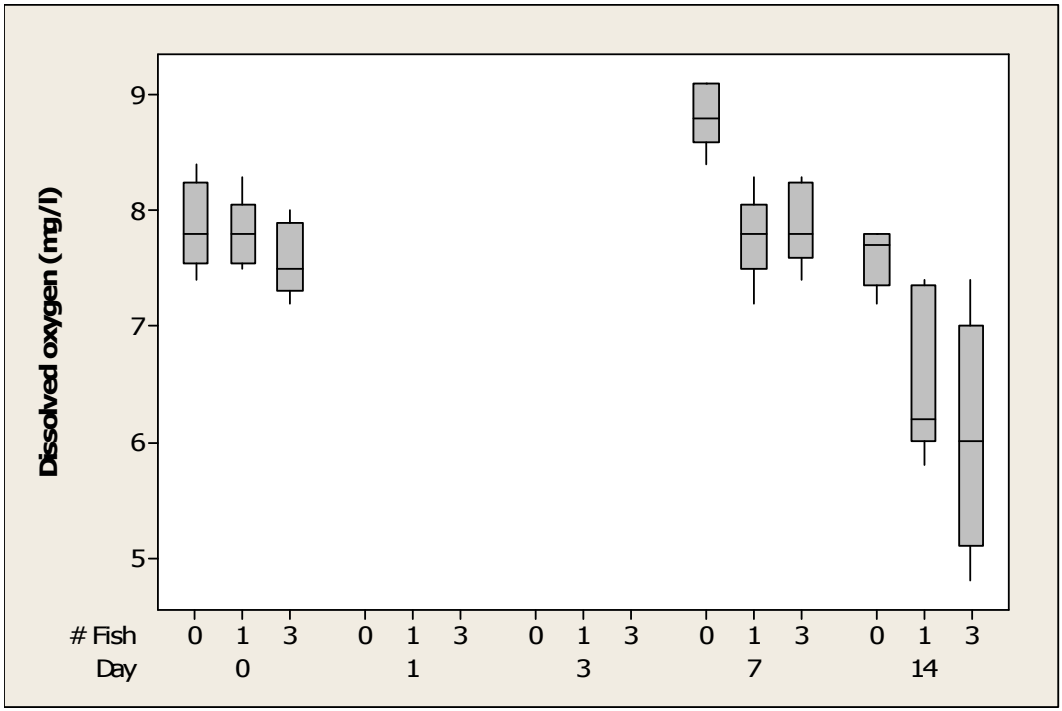
Figure 39. Dissolved oxygen recorded during the bluegill aquarium study.
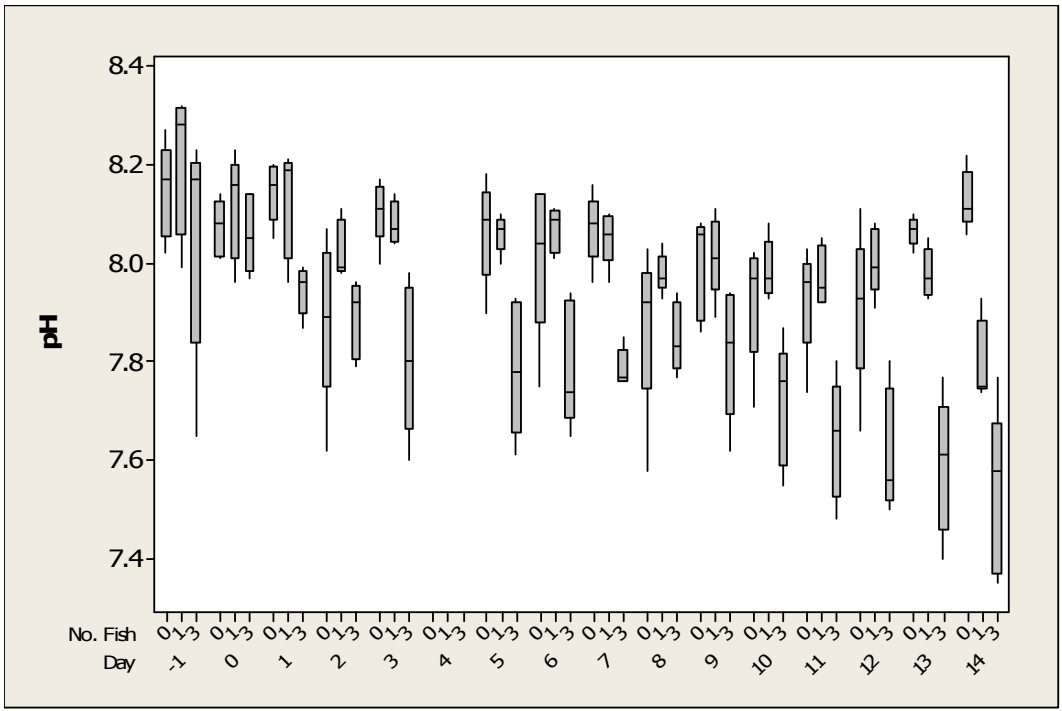
Figure 40. Recorded pH levels during the bluegill aquarium study.

Fishes as Sources of *E. coli*   55
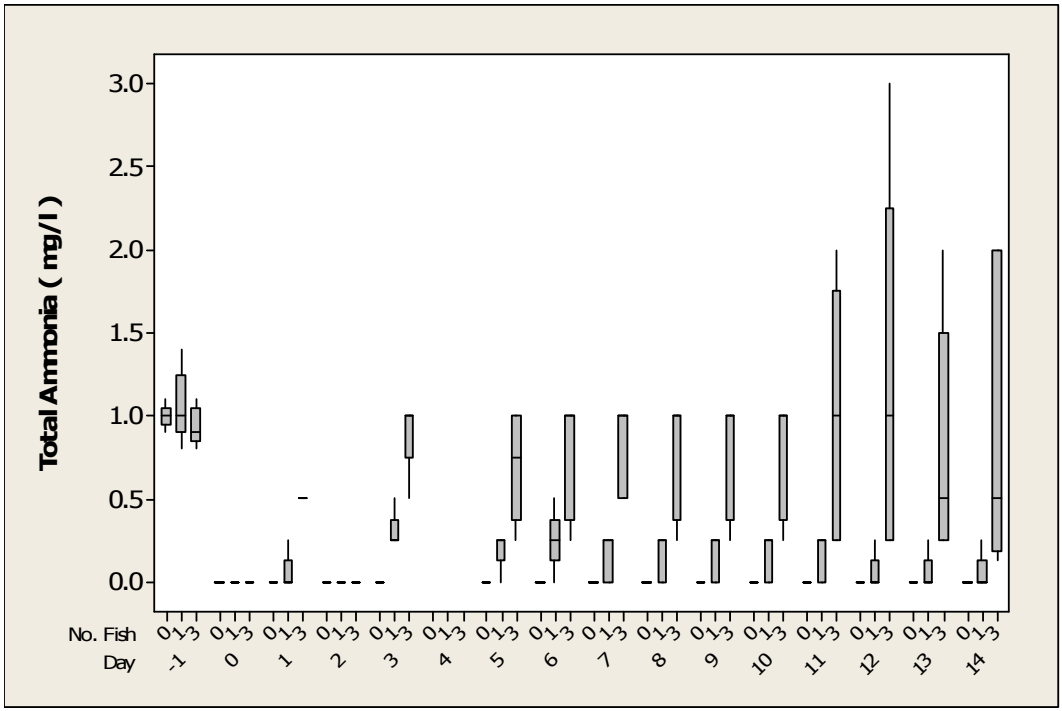
Figure 41. Total ammonia levels observed during the bluegill aquarium study.
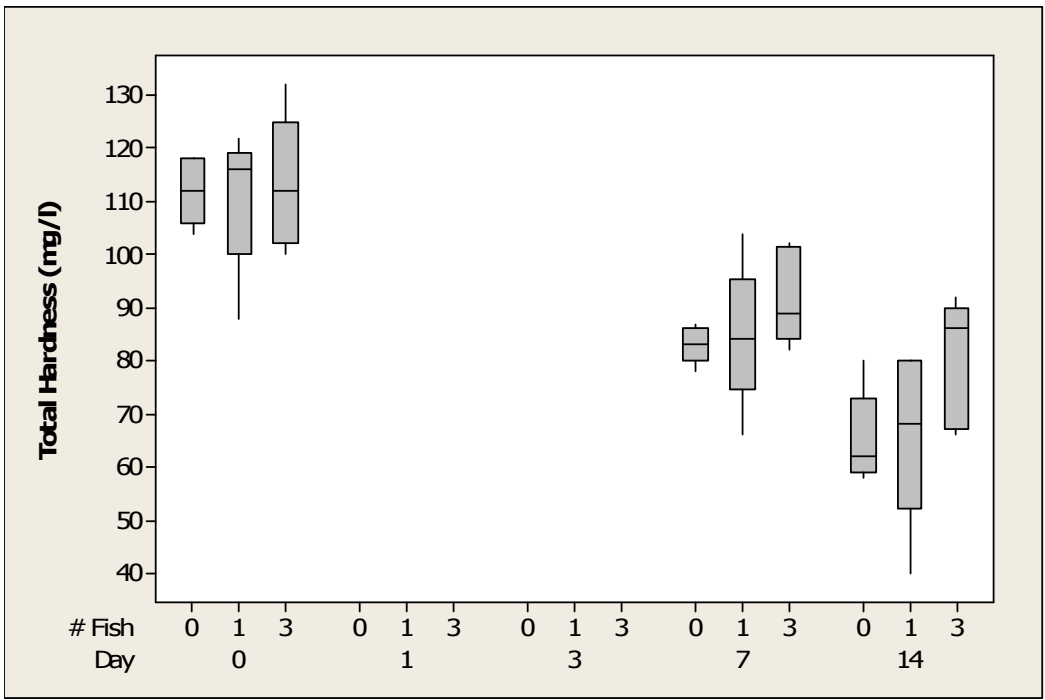
Figure 42. Total hardness levels observed during the bluegill aquarium study.

Fishes as Sources of *E. coli*
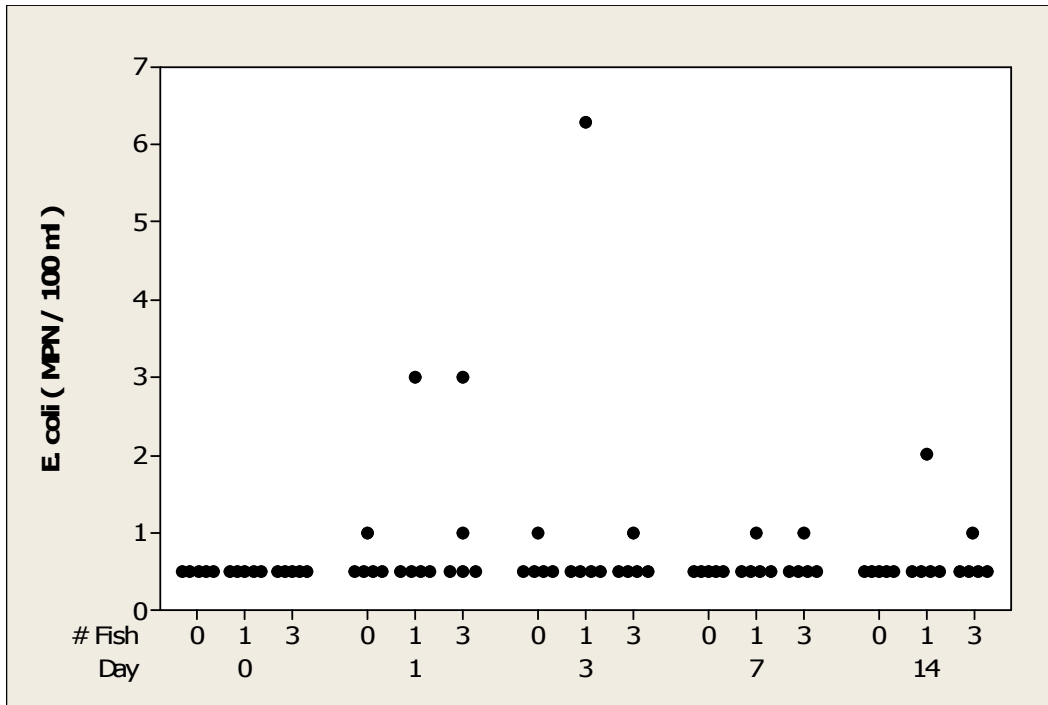
Figure 43. *E. coli* values observed in aquarium water exposed to captive bluegill and redear sunfish. Detection limit = 1 MPN. Values below 1 MPN reported as 0.5 MPN.
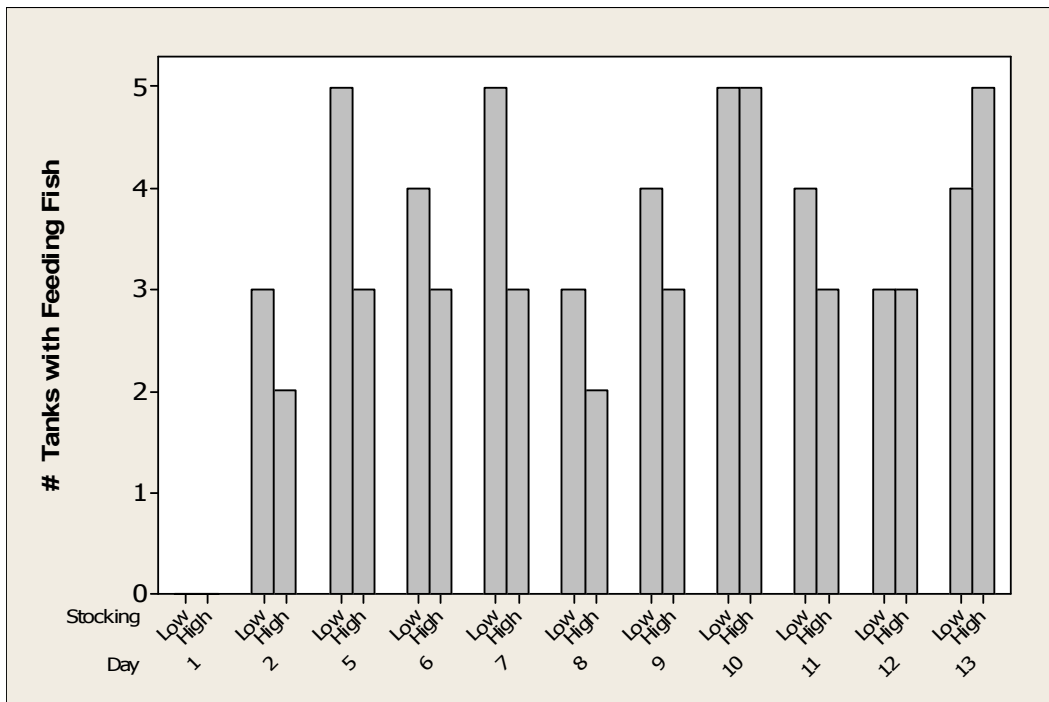
Figure 44. Number of bluegill aquaria out of a total of 5 with at least one actively feeding fish. Low = 1 fish per tank, High = 3 fish per tank maximum.

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Water temperature fluctuated during the catfish aquarium study between 20 and 29 °C (Fig. 45). As indicated earlier temperature changes were due in part to problems with the room heating system. This allowed temperatures to fluctuate more than preferred. However, the temperatures were well within the temperature tolerance limits of the species and should not have stressed this species.   Specific conductance levels ranged between 300 and 600 uS and median values were between 425 and 800 uS which is well within the range of suitable rearing conditions for freshwater sunfish (Fig. 46). There was an increase in conductivity levels near the end of the experiment. This may be due to the addition of AmQuel+® or Ammo-Lock® or salt during the experiment to control diseases and ammonia buildup.

Dissolved oxygen levels in channel catfish tanks were all above 4.0 mg/l and generally above 6.0 mg/l. We did observe a declining trend in dissolved oxygen over time and at higher stocking rates which may reflect the influence of higher levels fish respiration and/or additional biochemical oxygen demand due to increased feeding and deposition of uneaten food and feces (Fig. 47). The pH level of the test aquaria ranged between 7.2 and 8.7, which is within the range of tolerance for this species and is frequently encountered within local streams (Fig. 48). The pH level decline over time suggesting that the accumulation of waste products and increased concentrations of carbon dioxide due to fish and microorganism metabolism. This pattern of declining pH levels was generally higher in aquaria with higher stocking rates (i.e. 3 fish). Total ammonia levels were generally low (median levels $\leq$ 1 mg/l) up though day 4 of the study but then increased over time (Fig. 49). In general total ammonia levels were higher (median values 1- 2 mg/l) in the high density (3 fish) tanks (Fig. 49). This reflects the influence of increased feeding rates and waste production in these tanks. It is likely that these elevated levels along with the presence of pathogens, stressed some fish and cause the observed mortality (Table 11). The concurrent declining pH values would have a partially reduced the toxicity of ammonia due to the decrease in the proportion of un-ionized ammonia at lower pH values. The highest calculated un-ionized ammonia concentrations observed were 0.248 mg/l. The TCEQ requires treatment to 3 mg/l total ammonia in wastewater effluent to protect aquatic life (TCEQ 2003b). Previous studies have shown that levels of 2.90 mg/l un-ionized ammonia are acutely toxic to bass (Colt and Tommaso 2001). Based on these criteria, and unlike the bluegill aquarium study, un-ionized and total ammonia levels were both probably below critical lethal concentrations. Total hardness was only measured at the beginning of the channel catfish aquarium study. Median total hardness values ranged between 122 and 128 mg/l which are well within the tolerance levels of freshwater fish (Fig. 50).
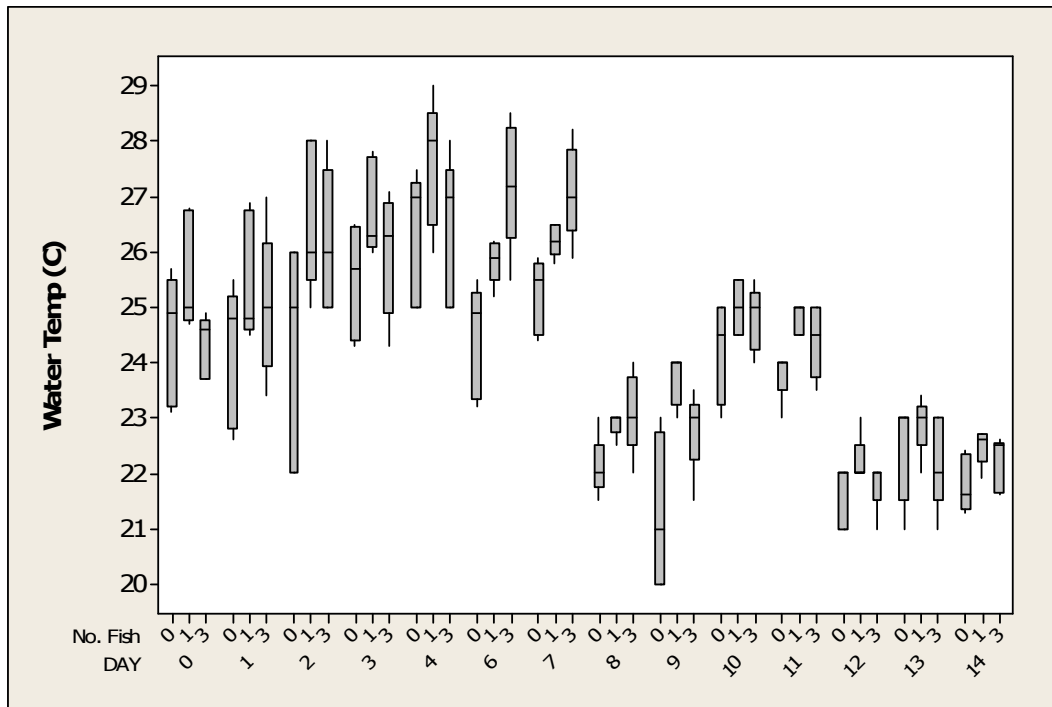
Figure 45. Water temperature observed during the channel catfish aquarium study.
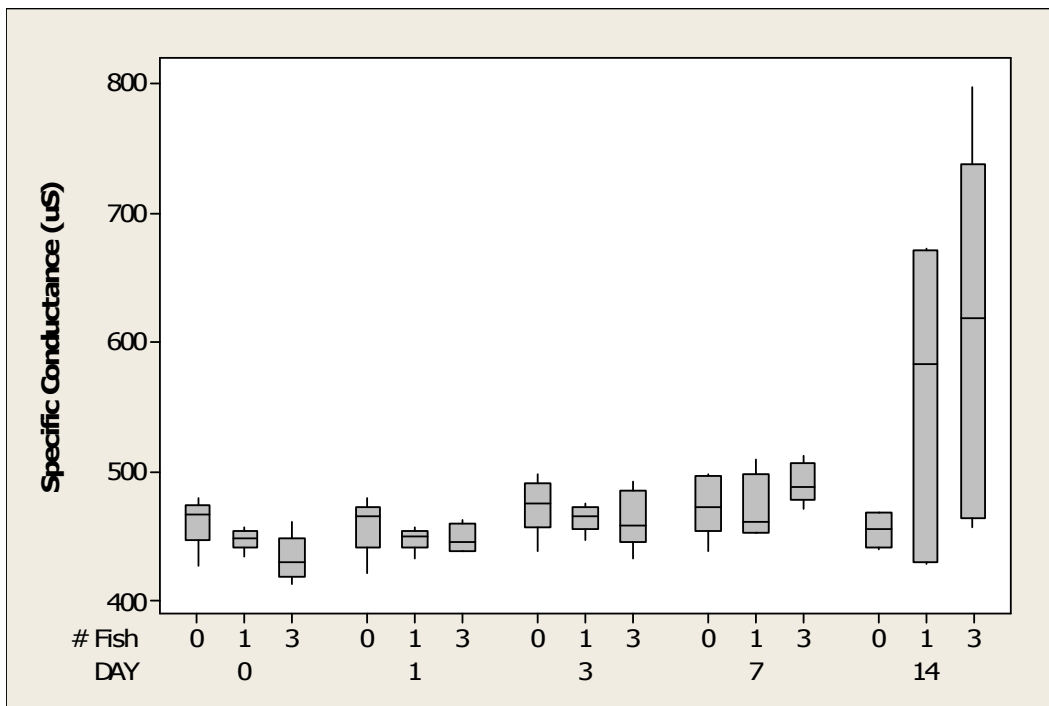
Figure 46. Specific conductance observed during the channel catfish aquarium study.
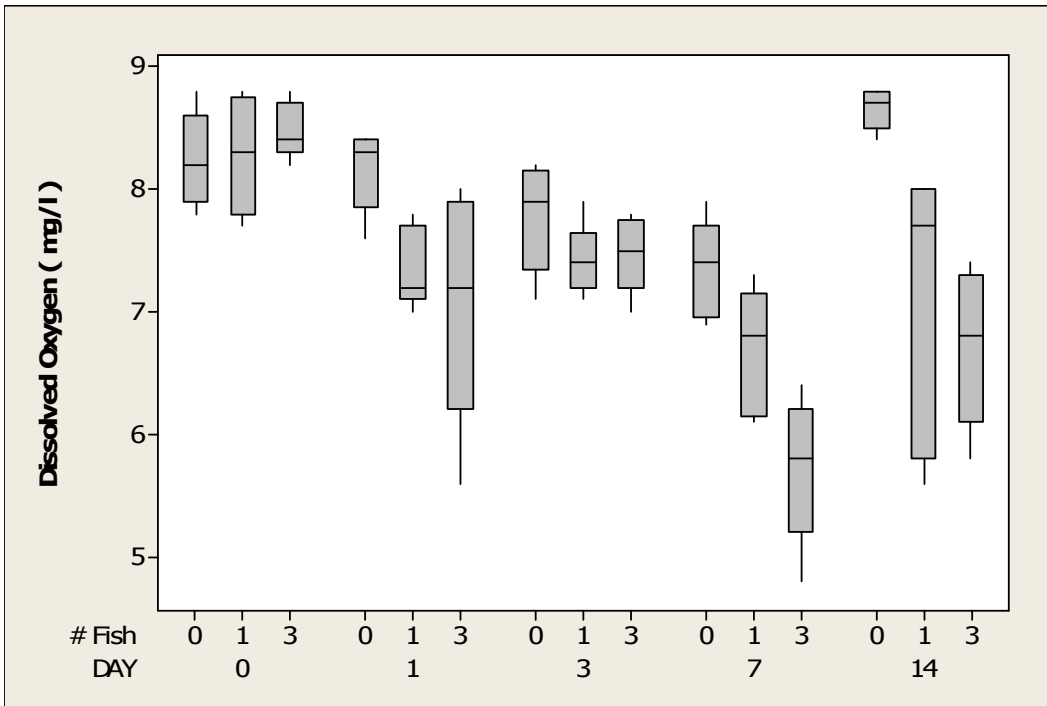
Figure 47. Dissolved oxygen levels observed during the channel catfish aquarium study.
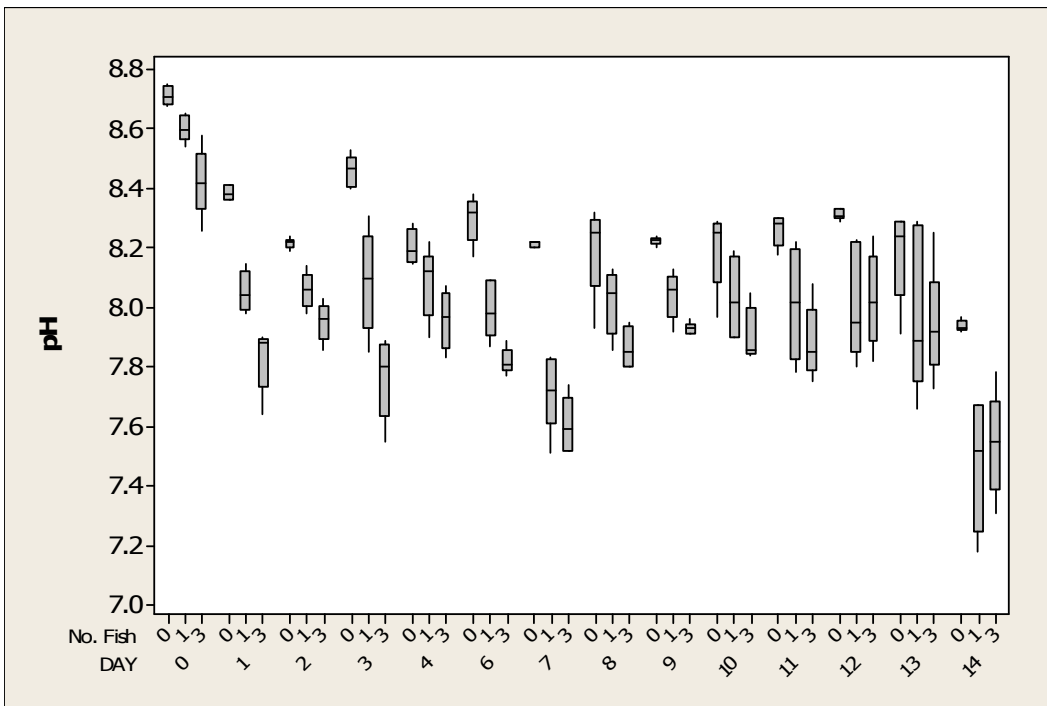
Figure 48. Recorded pH levels during the channel catfish aquarium study.
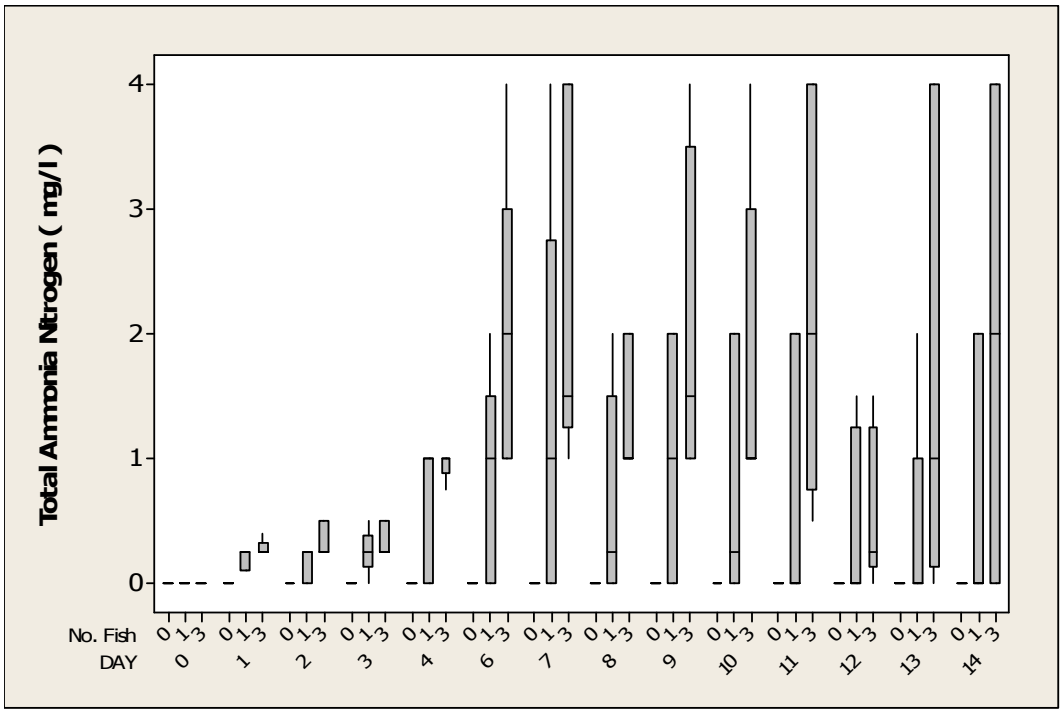
Figure 49. Total ammonia levels observed during the channel catfish study.
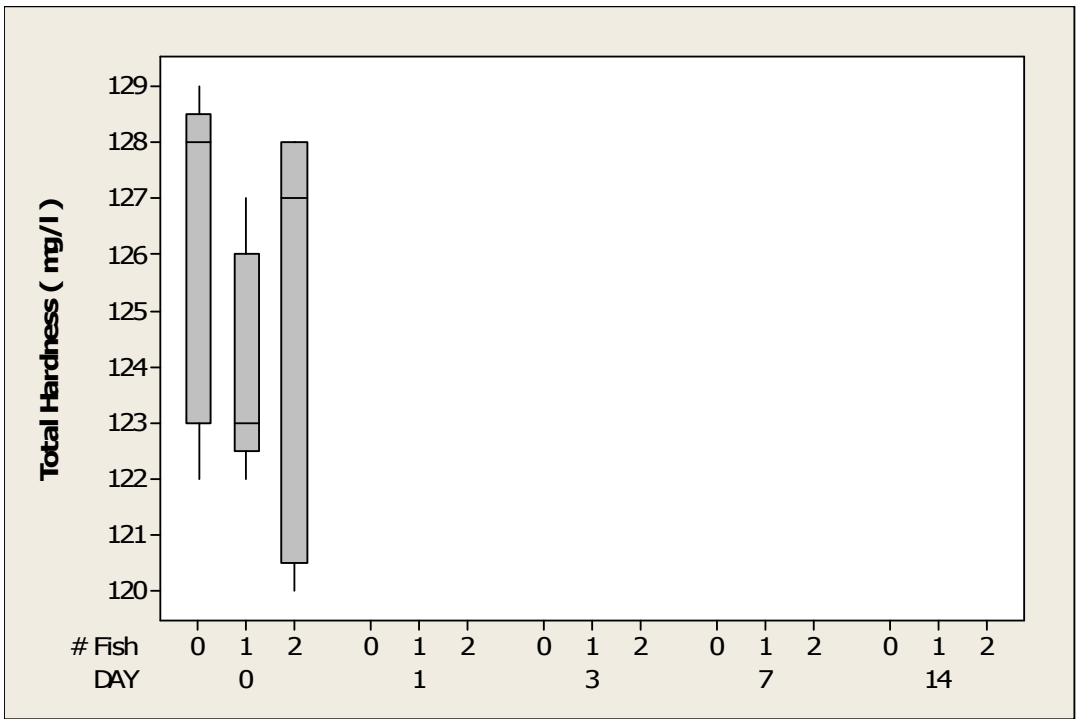
Figure 50. Total hardness levels observed during the channel catfish study. Note, no measurements taken after the initial readings.

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During the channel catfish aquarium experiment *E. coli* levels were below the detection limit of 1.0 MPN / 100 ml of water (Fig. 51). This suggests that at the stocking levels employed and water quality conditions observed channel catfish do not appear to be producing sufficient amounts of *E. coli* bacteria that can be detected in the water column. The relative feeding rates of channel catfish varied between aquaria, and were likely a function of water quality conditions and health of individual organisms. In some cases we did not see some fish feeding actively while others fed extensively (Fig. 52). On average about 3 out 5 (60%) of the tanks per treatment held actively feeding fish on any occasion as determined by visual examination of the fish after feed was administered. However, in some cases only 1 out 5 tanks (low stocking rate) exhibited active feeding. The rate of feeding generally decreased and mortality increased as the experiment progressed (Fig. 52 and Table 11).
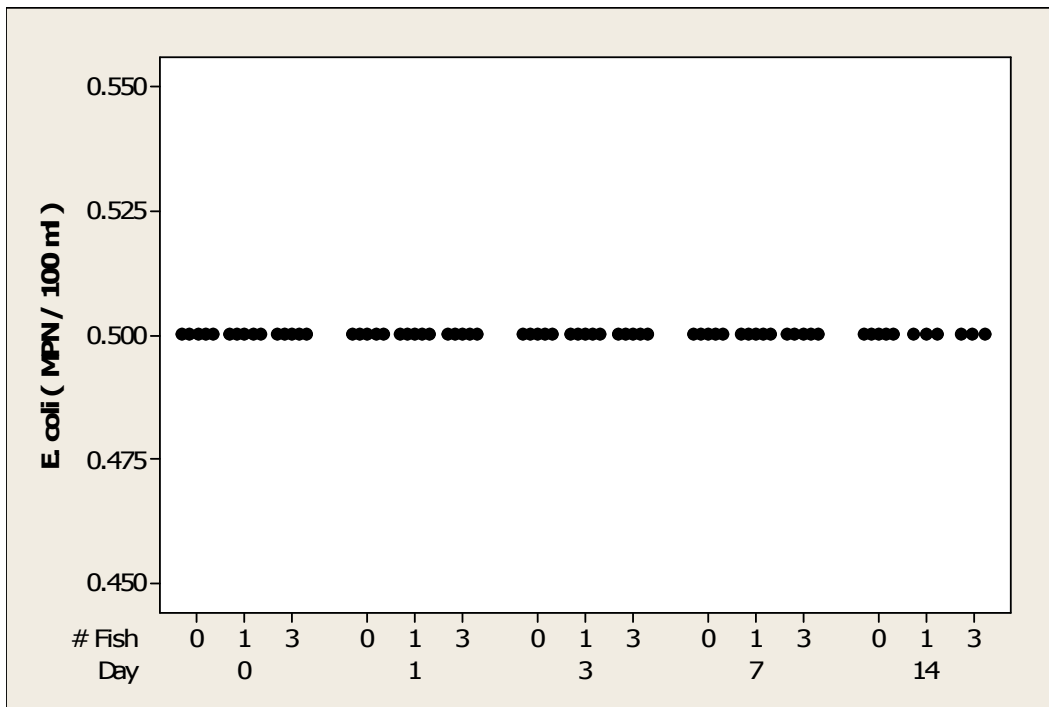
Figure 51. *E. coli* values observed in aquarium water exposed to captive channel catfish. Detection limit = 1 MPN. Values below 1 MPN reported as 0.5 MPN.
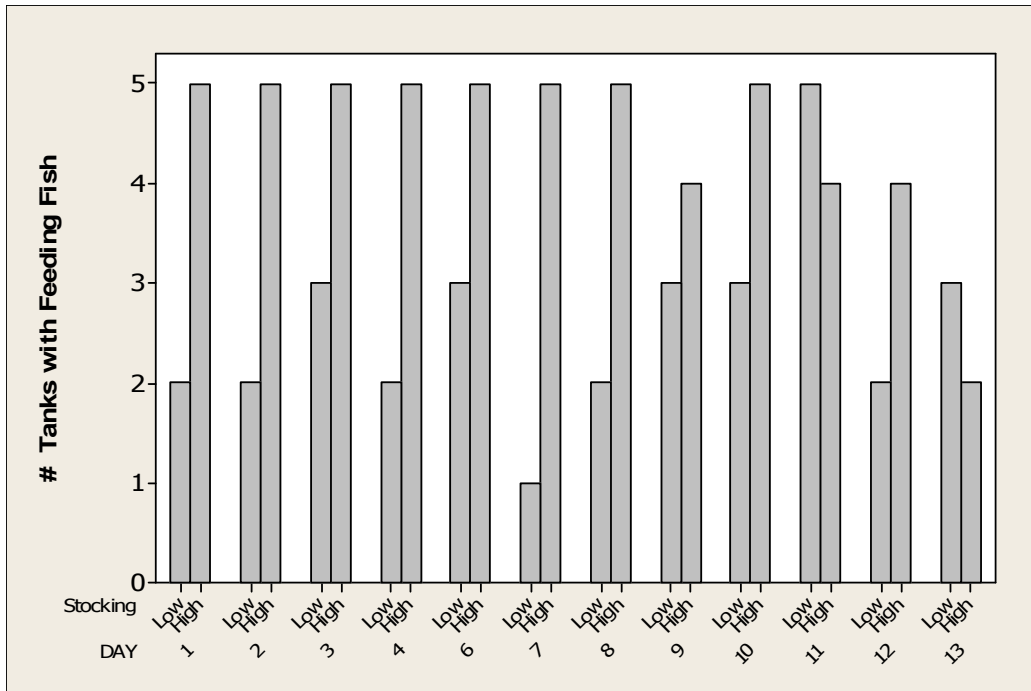
Figure 52. Number of channel catfish aquaria out of a total of 5 with at least one actively feeding fish. Low = 1 fish per tank, High = 3 fish per tank maximum.

These aquarium studies indicate that at the stocking rates used for both bluegill/ redear sunfish and channel catfish, *E. coli* that was present in their feces, were not detected in the overlying water column. However, variable feeding rates and the inability to stock fish at higher densities (> 1 fish per 5 gallons) due to water quality concerns probably reduced the probability of detecting indicator bacteria in water during our study. This issue coupled with the inability to filter the water sufficiently to reduce wastes without reducing bacteria levels, limits our ability to explore higher stocking densities and their influence on water quality, including *E. coli* production in a laboratory setting. Interestingly enough, *E. coli* were detected in the gut fecal matter of a sample of fish sacrificed at the end of the aquarium study (Fig. 53). In some cases these levels were extremely elevated (>10,000 MPN / g feces). This suggests that ambient water may be a poor medium to evaluate the entire impact of fish fecal production on indicator bacteria levels. This also suggests that some specimens of channel catfish and bluegill were able to maintain high levels of *E. coli* in their intestinal tract over a 2 week period without any obvious additional external source of *E. coli* bacteria.

All quality assurance blank *E. coli* samples yield values of less the detection limits (1 or 10 MPN / 100 ml) indicating sterile conditions were maintained during handling of fish in the field and laboratory.
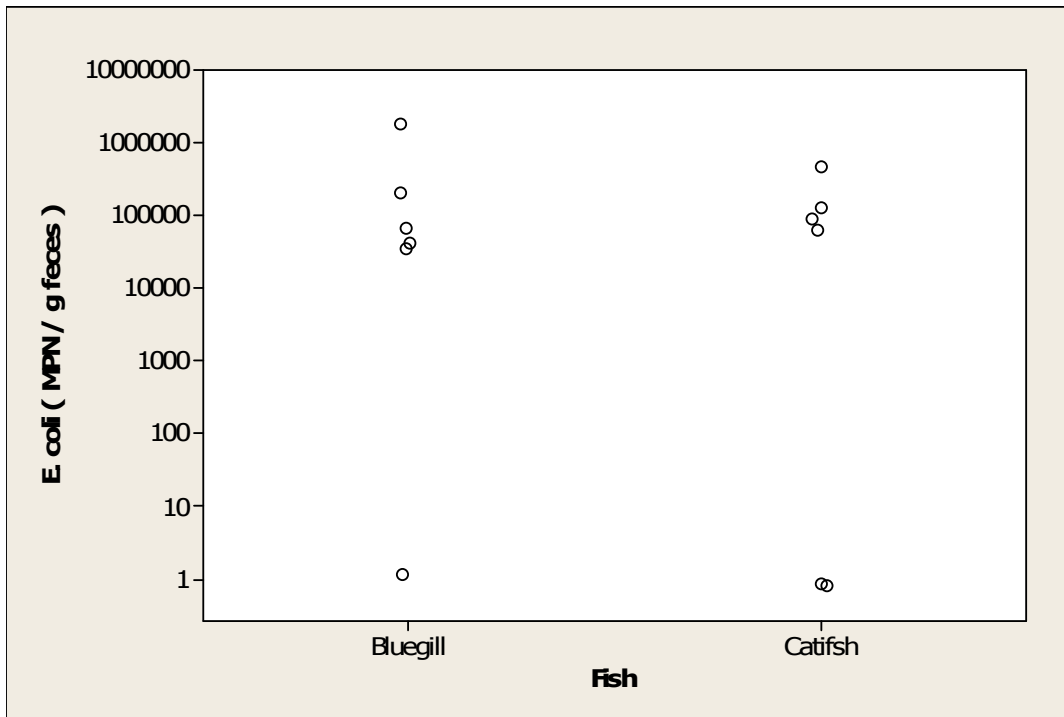
Figure 53. Estimated E. coli levels in feces of fish sacrificed at end of aquarium study.

.*Literature Review of Stream Fish Composition, Densities, and Fecal Production rates.*

It is difficult to estimate the absolute abundances and density of fishes in most rivers. This is due in part to the selective nature of all fishing gears and collection techniques (Bayley et al. 1989; Bonar 2009; Murphy and Willis 1996). For example seines and electrofishing capture on average only 70 to 75% of all species present respectively (Bayley et al. 1989). Furthermore in terms of abundance and species group characterization they found that seines and electrofishing gear efficiency ranged between 5 and 25% of the true abundance of these groups. In addition to gear specific efficiency, variable amounts of effort can result in different estimates of fish densities. Angermeier and Smogor (1995) found that in order to collect 90% of the species present in a stream it was necessary to sample 5 to 14 habitat units or a stream length equal to a distance of 22 to 67 stream widths. However, they concluded that in order to estimate the relative abundance of species less effort was necessary.

The majority of past fish studies in Harris County bayous and streams are based on biased sampling techniques that target selected species or size assemblages and have been used primarily to estimate catch per unit effort (CPUE) and develop community metrics, and not total abundance and biomass estimates (Guillen and Seiler 1991, Luedke 1994; Sneck-Faher and East 2007). In order to

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translate these catch per unit effort estimate of density into true estimates of abundance validation studies are usually needed where the bias collected method is compared to some true estimates of abundance. This includes the use of rotenone (a piscicide) or mark-recapture methods (Bonar et al. 2009). However, mark-recapture methods are usually confined to selected target species and not whole communities of fish. In contrast rotenone use for fish population estimation in public waters is labor intensive, heavily regulated by state and federal agencies, and may be unacceptable to local communities. However, even with these approaches other logistical issues remain including the dynamic seasonal and temporal interaction of fluctuating numbers of individual species which vary in size and resulting biomass.

Orth and Maughan (1984) provided estimates of annual fish biomass in Oklahoma warmwater streams. They found that the annual average total fish standing stocks were 60, 90, and 75 kg/ha, for riffle, pool and all sites, respectively. Total standing stocks differed among seasons with the annual average occurring in April-May and October-November, and the maximum occurring during July-August. Invertivore and invertivore-piscivore feeding guilds contained 79.9% of the total fish standing stock.

Randall et al. (1995) reviewed the world literature and found that the average fish community production for 55 rivers was 273 kg-ha^{-1}year^{-1}. Biomass estimates ranged between 7.4 to 1600 kg-ha^{-1} whereas estimated populations ranged between 3,714 to 778,000 fish-ha^{-1} for a variety of rivers from both temperate and tropical regions. A summary of the data used in their review is provided (Table 12). North American rivers yielded similar average statistics as data from the combined world literature database.

The Academy of Natural Sciences of Philadelphia conducted studies of fish communities in the lower Neches River in 1996 (ANSP 1998). During their study they estimated on average that there were 1.02 and 8.50 fishes per 100 m^2 present along shoreline areas of the Neches River. This translates into approximately 100 to 850 fish-ha^{-1}. These estimates were obtained using larger 50 ft (15 m) seines. However when smaller 20 ft (6.1 m) seines were used the estimates of average density of fish ranged between 572 to 399 fish per 100 m^2, which translates to 57,200 to 39,900 fish-ha^{-1} (ANSP 1998). These values are slightly lower than those reported by Randall et al. (1995) for North American rivers. However, estimates provided by ANSP (1998) are based solely on seine collections and therefore do not take into account fish in deeper portions of the river and/or more larger mobile adults. Additional fish community data was collected using trawls and dip nets, but no quantitative estimate of the area sampled was provided (ANSP 1998).

Table 12. Summary statistics on biomass, productivity and density of river fishes globally and in North America (NA). Data obtained from (Randall et al. 1995).

Parameter	Biomass kg/ha	Production kg/ha/yr	Density #/ha
N	58	55	42
Max	1,600	2,800	778,000
Mean	146	273	75,665
Min	7	26	3,714
N (NA)	25	18	11
Max (NA)	376	501	283,537
Mean (NA)	132	197	122,456
Min (NA)	7	29	13,300

Early fish surveys in Harris County waterways and adjacent areas utilized various methods to collect fish community data (Oborny 1997; Luedke 1994; Guillen and Seiler 1991; Seiler 1994; Chambers and Sparks 1959). This included the use of otter trawls, electrofishing (backpack and boat), seines and gillnets. However, most studies did not use standardized sampling distances or areas but rather used temporal effort such as electrofishing time (Luedke 1994). Therefore it is difficult to extract estimates of fish density per unit area based on many of these studies.

Seiler (1994) as part of a larger more comprehensive study collected shoreline fishes in the lower Houston Ship Channel using seines and on average swept an area equivalent to 208 m^2. His catches of fish ranged between 48 to 179,615 fish-ha^{-1} (0.1 to 303.2 kg- ha^{-1}) (Table 13). These values are generally lower than those reported by ANSP (1998) but are likely due to the fact these data were collected solely with shallow water seining gear. Seiler (1994) did collect other types of data on fish populations using gill nets but these data cannot be converted to spatial densities.

Table 13. Summary statistics of estimated biomass and density of fish collected in the lower Houston Ship Channel and San Jacinto River. Data obtained from Seiler (1994).

Parameter	Biomass (kg)/ha	Density #/ha
N	62	62
Max	303.2	179,615
Mean	39.9	11,438
Min	0.1	48

Luedke (1994) collected between 128 and 1,126 fish per site with an average catch of 400 fish. This was based on 20 minutes of effort/site. Stream widths were not reported but likely ranged between 3.3 to 15.4 meters wide based on past descriptions of the sites. Assuming operational speeds, the distances covered would likely not exceed 300 yards (274 meters). We can estimate that on average the area swept was equal 7.6 by 274 meters or 2,082 m^2. Therefore the densities of fish collected by electrofishing are probably on average 1,921

fish-ha^{-1}. As stated earlier though these estimates are probably biased low due to the selectivity of electrofishing towards larger surface fish.

Since the early 1990's there have been additional studies on fish communities by various investigators and biologists using standard protocol developed by TCEQ and predecessor agencies to evaluate fish community structure in relation to water quality (TCEQ 1999 and 2007; Parsons 2003). However, many of these studies are poorly documented in terms of spatial coverage or only provide data on time spent electrofishing and not area swept. It is difficult to estimate but based on standard 15 minutes of electroshocking effort this would likely translate to distances of approximately 100 to 300 yards in streams of varying width. Under these conditions total numbers of fish collected by recent investigators varied between 24 and 564 fish per site with and average of 335 specimens (Parsons 2003). Assuming the same physical dimensions used for estimation of total densities from data obtained from Luedke (1994), we estimated that fish densities for these more recent studies probably average 16,105 fish-ha^{-1}.

Sneck-Faher and East (2005) captured between 27 and 527 fish per collection per site during a study of Mustang Bayou during 2004 and 2005. Data were not available however in the report to generate estimates of fish density or biomass. Robinson and Culbertson (2005) surveyed several bayous using boat mounted electrofishing gear. They collected between 57 and 148 fish (average = 90) per 20 minutes of effort. They covered approximately 100 m of total shoreline distance. The average width of the streams surveyed was approximately 70 meters wide. Therefore using their average catch rates and the dimensions (70 X 100 m = 700 m^2) we estimate the average total density of fish to be 1,285 fish-ha^{-1}.

Ramirez (2008) documented large numbers of fish in local streams within southern Harris County using a combination of `seine and electrofishing gear. She found individual collections of fish using seines ranged between 0 and 1,750 (median = 500) fish per 9.1 m (30 ft) segment of stream. The majority of these streams were on average 15 ft (4.6 m) wide. Using the median densities we can convert this to estimated density of 11,933 fish-ha^{-1}.

Sandefur (2008) conducted a study of fish communities in various Harris County bayous and streams. She collected fish at 13 wadeable streams during thee sampling periods (early spring 2007, late spring 2007, and summer 2007). She collected up to 1,200 fish per 300 ft (91 m) segment of stream. Median values of 60 fish per collection (30 ft, 9.1 m) were common. Stream width varied between 5 to 55 ft (1.5 to 16.7 m) with a median value of approximately 30 ft (9.1 m). Using the median density we can convert this to estimated densities of 7,246 fish-ha^{-1}.

It is obvious that using past literature values and recent investigations of fish communities in Harris County that there are large numbers of fish and associated biomass in the local bayous and streams. Each of these organisms is capable of producing large amount of feces on a daily basis.

Fecal production in aquatic systems is a significant source of carbon transfer (Wotton and Malmqvist 2001). Horizontal transport of fecal pellets in rivers is an important flow of carbon. In a Swedish river approximately 100 meters wide, Malmqvist et al. (2001) documented mean transport rates of fecal pellets of 3.7, 1.0 and 2.7 tones C per day in each of thee consecutive summers. Much of this (20-30%) originates from seasonally abundant aquatic insects. Many pellets were deposited in dead water zones, backwaters, river margins, and lowland reaches, with the remainder being carried to the sea. They also noted that feces contain many groups of living organisms, including algae and bacteria, which pass though the gut little affected by digestion. The organisms in egested feces are thus likely to have the capacity for rapid growth as they are packed in close proximity to suitable substrates when deposited.

Fish digestive systems are highly variable in morphology which reflects their trophic level (Stevens and Hume 1995). The rate of food intake and digesta passage, and consequently fecal production in cold-blooded vertebrates including fish are highly dependent on ambient temperature (Stevens and Hume 1998; Clements and Raubenheimer 2006). Total retention times range from 10 to 158 hours for carnivores and are mostly less than 10 hours for herbivorous species (Clements and Raubenheimer 2006). This relationship along with concentrations of indicator bacteria present in fecal material would be the primary factors affecting potential loading rates into the environment. For example, the number of anaerobe bacteria in the intestine of grass carp (*Ctenopharyngodon idella*), a common invasive fish in Harris County, has been reported to be between 10^7 and 10^9/g digesta (Stevens and Hume 1998). However the amount of feces and associated bacteria produced will vary according to food availability, trophic level, and water temperature. Therefore it is very difficult to project fecal production rates for any poikilotherm including fish. Pullin et al. (1993) estimated fecal production rates based on studies of cultured fish that were given various feeds. They found that fecal production rates varied but were reported to be 2.5, 36, 27, and 36 g feces per 100 g of feed consumed for carnivorous fish, catfish, carp and tilapia respectively. Percentages of ingested food produced as waste for grass carp and yellow perch (*Perca fluviatilis*) range between 85 and 64.2% respectively as reported by Wetzel (1975) and cited in Jørgensen et al. (1991).

Another factor that influences the transfer and loading of indicator bacteria in streams is fish movement. Fish move within streams at varying distances. Small fairly sessile habitat specialists (e.g. madtoms, darters) seldom move long distances except during high flows. Highly migratory fishes include transients moving though the area in spawning migrations, such as white bass or migratory anadromous and catadromous species such as American eel. Resident species would tend to concentrate and recycle *E. coli* within the same stream reach they

inhabit whereas migratory species would disperse *E. coli* to other locations thereby contaminating ambient water and sediment.

Rodríguez, (2002) critically reviewed the "restricted-movement paradigm" (RMP) which states that adult fish in streams are usually sedentary and spend most of their lives in short (20–50 m) reaches of stream. He cites Hill and Grossman (1987) who found that several species of stream fish moved a maximum of 19.3 m over a 128 days. This paradigm has found wide acceptance by many biologists as a mechanism which explains the distribution of stream fishes. In many mark–recapture studies, however, many fish initially marked are often never recaptured. In addition, turnover rates of individuals in the home section (where fish were originally marked) can be high when marked fish moving out are rapidly replaced by unmarked ones. Recent challenges to the RMP have been based on the inference that high turnover indicates high mobility. However, when the home section is small many individuals may leave (high turnover) but not move far away (low displacement). Rodriquez (2002) used simulation models which showed that the empirical distribution of many cold-water stream fish can be explained by high turnover and low displacement rates. However, considerable intra- and interspecific heterogeneity exists in the extent of movement by various fish species.

Some species such as the blue catfish, *Ictalurus furcatus* a common species of catfish in southeast Texas bayous, can undergo considerable long-range movements up to 5-12 km from their release site within a period of 363-635 days (Graham, 1999). During longer time periods movement of up to 23.6 km over a 8 year period was documented for this species. In contrast, small catfish called madtoms show very little movement and high site fidelity (Burr and Stoeckel 1999).

The movement of common carp was recently studied by Butler and Wahl (2010). The common carp *Cyprinus carpio* is an introduced species that is abundant in many rivers including southeast Texas bayous. Radiotelemetry was used to determine long-term movement and habitat use patterns of common carp among flowing and impounded areas. They found that radio-tagged common carp displayed a variety of movement patterns but used impounded areas most frequently during all seasons. They documented many common carp were always located in impounded areas, but some individuals moved upstream into flowing areas in spring and summer and returned to impounded areas by fall. Common carp is a common bottom feeder and could easily transport *E. coli* laden fecal material between streams and rivers in local streams if long range movements occur as documented in the literature.

Detenbeck et al. (1992) reviewed the recovery of temperate-stream fish communities subjected to various disturbances. They reviewed case histories for 49 sites and recorded data on 411 recovery end points. Most data were derived from studies of low-gradient third- or fourth-order temperate streams located in forested or agricultural watersheds. They found that species composition,

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species richness, and total density all recovered within one year for over 70% of systems studied. Lotic fish communities were not resilient to "press" disturbances (e.g., mining, logging, and channelization). In the absence of mitigation efforts recovery time could range between 5 to greater than 52 years, and in these cases recovery was limited by habitat quality. However, following "pulse" disturbances, autecological factors, site-specific factors, and disturbance-specific factors all affected rates of recovery. Centrarchids (sunfish) and minnows were the most resilient group of fish to disturbance. Species within rock-substrate/nest-spawning guilds required significantly longer time periods to either recolonize or reestablish predisturbance population densities than did species within other reproductive guilds. They found that recovery was enhanced by the presence of refugia but was delayed by barriers to migration, especially when source populations for recolonization were relatively distant. Median population recovery times for systems in which disturbances occurred during or immediately prior to spawning were significantly less than median recovery times for systems in which disturbances occurred immediately after spawning. There was little evidence for the influence of biotic interactions on recovery rates. These data imply rapid movement and recolonization of warmwater streams subjected to periodic disturbances. This may also reflect extensive movement of fishes between various reaches, which would increase biologically mediated transport of *E. coli*.

Suski and Ridgway (2009) describe regular seasonal movements of various species of sunfish (largemouth bass, smallmouth bass, bluegill, and black crappie) associated with overwintering habitat in larger rivers. Some of these movements extend from < 1km to 69 km downstream depending on the severity (low temperatures) of winter conditions in shallow water areas. This movement was primarily from upstream shallower low order streams to downstream higher order deeper streams and rivers.

Peterson et al. (1993) conducted a fish colonization study of Illinois streams during late spring and early summer in 18 experimentally depopulated reaches that ranged from 46 to 113 m in length. They documented rates of colonization in terms of total fish numbers, number of species, individual species, and community structures were measured over time intervals ranging from 0.5 to 140 h. They found that both the proportion of original fish abundance (all species) and the proportion of original community structure (measured by a proportional similarity index) increased with time. They developed linear colonization models that indicated that 0.70 of the maximum proportional similarity index would be reached in 60–140 h (90% confidence limits of the model) and that 0.90 of the original fish abundance would be reached in 100–270 h. The concluded that the results of their study and previous studies indicate that in drainages dominated by surface runoff, disturbed fish communities in short stream reaches can quickly return to their original community structure and abundance without any aid, provided that fish have unrestricted access to the reaches and that the environment returns to its original state. This study supports the theory that there is extensive movement of fish within warm-water streams and rivers.

Conclusions and Recommendations

Based on various data sources including a literature review, field surveys, and aquarium exposure studies we were able to develop multiple conclusions that will be useful for water quality managers responsible for future TMDL's, and development of management practices to control indicator bacteria levels and associated pathogens.

Based on data collected during our study and a careful review of the literature we can make the following conclusions.

1. Wild fish in southeast Texas and in similar habitats contain high levels of indicator bacteria including *E. coli*.

2. Past studies documented in the literature provide numerous examples of various species of fish containing detectable levels of indicator bacteria. Highest levels of indicator bacteria in fish species appears to be correlated with warm eutrophic conditions associated with intensive aquaculture and/or tropical environments, although examples exist elsewhere including cooler temperate regions.

3. Levels of *E. coli* in wild fish species appear to follow trends similar to ambient water and sediment concentrations. That is in higher fish fecal *E. coli* levels are usually found at sites with high ambient concentrations.

4. There does not appear to be a strong consistent pattern in *E. coli* levels between trophic levels or species. Both herbivores, omnivores and carnivores appear to harbor indicator bacteria. However, trophic status can influence passage rates of undigested material and quantities of feces production.

5. Our aquarium study showed low levels of bacteria in water, while relatively high levels of bacteria in feces. Reasons for lack of a strong correlation include reduced feeding rates and potentially low stocking rates of fish.

6. Limited genetic data obtained from the literature shows that indicator bacteria that are found in fish are genetically related to forms found in warm-blooded species including waterfowl. Few data exists that supports the origin of fish specific forms or strains of E. *coli* or other indicator organisms. However, there is substantial evidence that depending on temperature and ambient conditions *E. coli* picked up by foraging fish can survive for long times in the digestive system and possibly multiply.

7. Fish can produce high amounts of fecal material depending on feeding rates which is influenced both by ambient temperature, prey/food availability, and trophic level. These feces and associated bacteria can be a highly significant source of indicator bacteria. Whether this represents a new source within the watershed or simply microorganisms that have been biologically transported to new sites is unclear.

8. Literature and limited site data provide sufficient documentation that rivers and bayous in our area can harbor 1000's of fish and 100's of kilograms of fish per hectare of water. During spawning aggregations and schooling this can increase dramatically and potentially increase exposure of rivers to large amounts of fish fecal material. During critical summer low flow conditions this may not be diluted immediately but instead become incorporated into the river sediment. Past studies have documented the significance of fecal material generated in aquatic systems and their role in carbon transport and sediment enrichment.   Associated microorganisms would most likely thrive in these environments.

9. Many species of fish can swim long distances. This may due to spawning migrations, immigration and emigration of young or adults, and flooding events. Fish can therefore provide an extremely efficient mechanism to transport indicator bacteria within and between watersheds. This movement can also be upstream in unpredictable complex patterns depending on flow regime. This mechanism of transport is not currently addressed in any water quality model dealing with microbiological loading, growth and transport.

Recommendations:

1. Fish are not likely major new source of bacteria but more field and laboratory research and monitoring is needed to evaluate the relationship between fish and indicator bacteria at elevated summertime temperature regimes. We recommend that additional laboratory and field experiments and monitoring be conducted to further quantify and clarify these relationships in an effort to develop predictive models.

2. Fish provide a major mechanism for transporting bacteria in non-linear fashion. We recommend that further studies be conducted with members of each major trophic group using mark-recapture and/or telemetry methods to evaluate short-term and long-term movement within streams and rivers in the Harris County area and adjacent counties.   This data could be incorporated into future simulation models to estimate potential movement and fate of indicator bacteria transported by fishes.

3. Estimates of biomass and density are sorely needed for our area streams and rivers. We recommend a combination of approaches to estimate these parameters including mark-recapture studies, multi-pass electrofishing methods, and acoustic methods and in limited areas use of rotenone.

4. Bioenergetic studies of native fish are needed to estimate fecal production rates.   A combination of approaches including laboratory and field studies is needed to better estimate this variable.

5. Examination of feces collected from fish captured in area streams using molecular genetic methods are needed to further evaluate the role of fish as transport vectors and/or sources of indicator bacteria.

6. The role of other cold-blood aquatic vertebrates as sources of *E. coli* and other indicator bacteria such as amphibians, alligators, water snakes, and turtles should be investigated. The limited data on amphibians and reptiles suggest that they may actually serve as original sources of indicator bacteria in addition to serving as transport vectors. Unfortunately data on the abundance and movement of amphibians and reptiles in local waterways is relatively rare.

Acknowledgements

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