BACTERIA SOURCE TRACKING IN COPANO BAY

PHASE II

FINAL REPORT

PRINCIPAL INVESTIGATORS:

JOANNA B. MOTT AND ROY L. LEHMAN
TEXAS A&M UNIVERSITY- CORPUS CHRISTI
(361) 825-6024 FAX: 825-2742

TGLO Project Coordinator*:* Blake Traudt

Date of submission: June 30, 2005

SUBMITTED TO:

Texas General Land Office
1700 N. Congress Avenue
Austin, TX 78701-1495

Executive Summary

Copano Bay is a secondary bay located on the northwestern shore of Aransas Bay in the Mission-Aransas Estuary on the lower Gulf coast of Texas, approximately 50 km north of the city of Corpus Christi, Texas. It is classified as an oyster-harvesting bay and water quality is monitored by the Texas Department of Health (TDH) [now the Texas Department of State Health Services (TDSHS)] Seafood Safety Division, according to the National Shellfish Sanitation Program, using fecal coliform bacteria as the indicator for fecal contamination.

The Texas Commission on Environmental Quality (TCEQ) identifies Copano Bay/Port Bay/Mission Bay as water body segment 2472. The TCEQ is required, under Section 303(d) of the federal Clean Water Act, to identify water bodies for which effluent limitations are not stringent enough to implement water quality standards and list these on the Texas 303(d) list. The TCEQ Draft 2004 303(d) list includes the Copano Bay water body segment 2472, specifically, the area along the southern shore including Port Bay, and the area near Bayside for bacteria (oyster waters).

While fecal coliform levels are used to indicate fecal contamination and thus impaired water quality, these numbers do not provide information on the sources of the contamination. There has been discussion over the past few years regarding the relative risk associated with human vs. nonhuman (bird, wildlife, etc.) fecal contamination and the closure of shellfish harvesting waters due to contamination that could be of non-human origin.

In this study, bacteria source tracking (BST) was used to evaluate sources of fecal contamination in Copano Bay to provide data for use in future evaluations of standards for oyster harvesting waters. The project objective was to identify and quantify the relative contributions of various sources of fecal contamination to Copano Bay for development of a Total Maximum Daily Load (TMDL) by TCEQ and to support a potential bacteriological adjustment by TDH (now TDSHS).

Two bacteria source tracking techniques were used: antibiotic resistance analysis and pulse field gel electrophoresis. Antibiotic resistance is a well-established approach that has been used in a number of previous studies (for example: Hagedorn et al., 1999; Harwood et al., 2000; Parveen et al., 2001; Webster et al., 2004; Whitlock et al., 2002; Wiggins et al., 1999) and included in comparison studies and reviews (Scott et al., 2002; Simpson et al., 2002; Stewart et al., 2003; Stoeckel et al., 2004). Pulse Field Gel Electrophoresis (PFGE), considered the gold standard for epidemiological tracking of organisms by the Centers for Disease Control and Prevention, was used to develop "genetic fingerprints" of a subset of the isolates (due to the high cost and labor involved with this technique) to provide confirmation of bacteria sources. As the Copano Bay water body classification is based on fecal coliforms, *Escherichia coli,* a member of this group, was used as the bacterium for the study.

The objective for the project was accomplished as several tasks:

1. Expansion of the existing *E. coli* antibiotic resistance profiles library at Texas A&M University-Corpus Christi (TAMU-CC) to include fecal samples from the Copano Bay area for comparison with water isolates of *E. coli.*
2. Isolation of *E. coli* from Copano Bay water samples and comparison of their antibiotic resistance profiles and pulse field gel electrophoresis "fingerprints" with those in the TAMU-CC database for source tracking purposes.

3. Submission of data report to the TCEQ, Coastal Bend Bays and Estuary Program (CBBEP) and TDH Project Managers and the Texas General Land Office (TGLO) Project Coordinator, for use in determining sources of fecal contamination in Copano Bay.

In order to determine animal sources of contamination, libraries of antibiotic resistance profiles and PFGE molecular fingerprints of *E. coli* isolates from known sources in the Copano Bay watershed were developed. Animal sources were based on TDH Division of Shellfish Sanitation comprehensive sanitary surveys. For antibiotic resistance, isolates were obtained from human/sewage, cow, horse, duck, wildlife (javelina, deer etc.) and gull. The library included isolates collected during the study (winter/spring of 2004, November 2004) and was supplemented with isolates, representative of the watershed, from existing TAMU-CC libraries. A subset of the isolates from the spring 2004 collections were used for the PFGE library.

Fourteen of the stations in Copano Bay/Mission Bay currently monitored for water quality by TDH were selected by TDH for this study. Water samples were collected by TDH field personnel between October 2003 and May 2004, during eight sampling events, dates dependent on factors such as weather (rainfall), and following standard TDH procedures. Field parameters (salinity, air temperature, water temperature, wind direction, wind velocity, specific conductance, rainfall) were measured/observed by TDH personnel for each sample collection. Two bottles of water from each station were provided to TAMU-CC, a third was used by TDH for fecal coliform analysis. Water samples were analyzed for *E. coli* using EPA Method 1103.1. *E. coli* was isolated from fecal swabs using mTEC agar plates. For both fecal and water *E. coli* isolates transfers were made onto Rainbow® Agar plates (Biolog 1994) and confirmed as *E. coli* using MicroLog™ Microbial Identification System (Biolog, Inc., 3938 Trust Way, Hayward, CA 94545) following the MicroLog™ System Release 4.0 User Guide (Biolog, 1999).

For this project the analytical procedures for antibiotic resistance profiling followed the standardized Kirby Bauer Disk Diffusion method with a panel of 20 antibiotics. This provides an analysis based on standard clinical methodology and quality control (NCCLS 2000, 2002a, 2002b). The BIOMIC® image analysis system was used for an instantaneous reading of zones of inhibition and interpretation following NCCLS M100 (2002b). This system calculates antibiotic minimum inhibitory concentrations (MICs) and records zone diameters automatically from the standard disk diffusion method. The automated image analyzer ensured uniformity and included EXPERT© software which checks quality control, test results and unlikely results. This method has proven to improve reading consistency and speed thereby minimizing technologist variation. Zone diameters were analyzed using discriminant analysis.

The final antibiotic resistance profile known source library comprised 1058 isolates which were evaluated by two- (human/sewage vs. non-human), four- (human/sewage, cow, horse, wildlife) and six-way (human/sewage, horse, cow, duck, gull, wildlife) analyses. The library was tested for representativeness, cross-validated and challenged with known isolates not included in the library. The average rate of correct classification (ARCC) for two-way analysis was 72% with 80% of human source isolates correctly classifying. Six-way analysis ARCC was 59%, with a cross validated ARCC of 56%. Rates of correct classification for individual sources ranged from 82% (wildlife) to 44.5% (cows). Human (sewage) isolates were correctly classified for 63% of the isolates. Unknown source isolates were then

compared with the known source library to determine into which known source group each isolate could be classified.

PFGE analysis followed published standard Bio-Rad Methodology and Standards as described in Bio-Rad Laboratories (1995) (CHEF-DR III Pulsed Field Electrophoresis Systems: Instruction Manual and Applications Guide. Hercules, California). Digital images for analysis with Quantity One (Bio-Rad, Hercules, CA) were created. A Pulse Field Gel Electrophoresis database was constructed by the modeling of each isolate and each band of the entire set of both known and unknown samples. A Complete Linkage cladogram using Jaccard Coefficient Method was generated for each data set of knowns to determine the number of clusters of closely matching sample bands. Each of the unknown isolates was run against the entire database and the known source isolate with the most similarity was used to determine the identification of the source of the unknown. Isolates which did not show similarity with library isolates were classified as 'no match'. The results of a subset of unknown isolates analyzed using PFGE were compared with the results of the ARA analyses in order to confirm results.

For the water samples there was a lack of isolates from some stations/events so additional isolates were analyzed from events where high numbers of *E. coli* were isolated, to compensate for reduced numbers from other stations/events. More than 6,900 colonies were isolated on mTEC plates. Antibiotic Resistance Profiles (ARP) were developed for 2,811 of the verified *E. coli* isolates and were classified by source using the library of known source isolates. In two-way analysis, 42% of the isolates were classified as human (sewage) and 58% were classified as non-human. Six-way analysis, used for the majority of the discussion, showed 22% human (sewage), with 20-35% as each of cow, horse and duck. Few isolates were identified as wildlife or gulls. Percentage of isolates classified as each source is only based on comparison with the Copano Bay library developed for the study and a certain level of misclassification between groups must be assumed. PFGE confirmations, especially for human/sewage source isolates (63.9% of a subset analyzed by both ARP and PFGE), provided an additional level of confidence.

Isolates were separated by station and event, and percentages of each source described for each. Few isolates were found at the stations near the Copano Bay-Aransas Bay interface, whereas highest numbers were obtained from stations receiving inflow from Copano Creek, Mission River or Aransas Rivers, especially after rainfall. Both antibiotic resistance profiling and PFGE results suggest a human/sewage contribution to fecal contamination of Copano Bay, under dry weather as well as wet weather conditions. Livestock (cow, horse) appeared to contribute to fecal contamination at many stations under certain environmental conditions, such as rainfall and high river water flow. Wildlife and gulls, as assessed by antibiotic resistance profiling, were found to contribute relatively little contamination (in terms of *E. coli*) compared with human/sewage, cow and horse. Isolates identified as duck were found in areas known to be colonized by either migratory or resident ducks suggesting these birds contribute to fecal contamination of the Bay.

Additional studies are needed to examine loadings and sources in the contributing rivers: Mission and Aransas, and Copano Creek. Other questions such as potential contribution of fecal bacteria from sediments still need to be addressed.

The strategy of using a screening phenotypic technique in conjunction with a genotypic technique to analyze a subset of the data and provide a level of confirmation shows promise; however, library sizes were a constraint for both techniques. Comparisons between

the two techniques for non-human sources were particularly limited as the PFGE library was not only much smaller but contained a different, or restricted species group of isolates using isolates from the first group of collections only. The ARP library was expanded with isolates from a second period of collection and augmented with isolates from an existing TAMU-CC library. The PFGE library did not contain wildlife and gulls, thus these groups were not included in the isolate identification comparisons. The library issues were primarily related to funding constraints. Ideally the ARA and PFGE library would include the same isolates, with a subset of unknown isolates analyzed to support the ARA results. In order to maximize confidence in the results libraries should ideally be developed for the watershed being studied, over the same time period of the water (unknown source) sample collections.

Since the inception of this project the science of bacteria source tracking and the techniques available have developed significantly. For future studies enterococci should be considered for study instead of *E. coli*. *E. coli* was used for this study as Copano Bay water quality is evaluated using fecal coliforms (due to its shellfish classification) a group of which *E. coli* is a member. An additional factor in the decision was the existence of libraries which could be expanded with Copano Bay watershed isolates, thus minimizing costs associated with library development. Carbon source utilization data, obtained when confirming *E. coli* colonies, is showing promise in another study (Mott and Lehman, unpublished).

TABLE OF CONTENTS

LIST OF FIGURES

Page

Page

Page

Page

LIST OF TABLES

Page

Page

Page

Page

Page

Page

Page

INTRODUCTION

The Draft 2002 Texas 303 (d) list includes Segment 2472 (Copano Bay/Port Bay/Mission Bay). Specifically, the area along the southern shore including Port Bay, and the area near Bayside is listed for bacteria (oyster waters) Category 5a with a rank of L (low priority), meaning a TMDL is underway, scheduled or will be scheduled. Rankings are based on the current understanding of the causes of the non-support of the water quality standards and the sources of pollution, the importance of the resource, the severity of the impact, and the likelihood of TMDL success. The Texas Administrative Code (2000) states that "The indicator bacteria for suitability for oyster waters is fecal coliform". The fecal coliform criteria for oyster waters is 14 colonies per 100 ml or no more than 10% of the samples are >43 MPN as specified in §307.7(b)(3)(B)".

Texas Department of Health (TDH), now the Texas Department of State Health Services (TDSHS), Seafood Safety Division monitors oyster waters according to the National Shellfish Sanitation Program, assessing water quality using the fecal coliform indicator group. There has been discussion over the past few years regarding the suitability of using fecal coliforms as the indicator of fecal contamination, and the relative risk associated with human vs. nonhuman (bird, wildlife, etc.) fecal contamination. In this study, bacteria source tracking (BST) was used to evaluate sources of fecal contamination in Copano Bay to provide data for use in future evaluations of standards for oyster harvesting waters.

Two bacteria source tracking techniques were used for the study. Antibiotic resistance is a well-established approach that has been used in a number of previous studies (for example: Hagedorn et al., 1999; Harwood et al., 2000; Parveen et al., 2001; Webster et al., 2004, Whitlock et al., 2002; Wiggins et al., 1999) and included in comparison studies and

reviews (Scott et al., 2002; Simpson et al., 2002; Stewart et al., 2003; Stoeckel et al., 2004). For this project antibiotic resistance was evaluated by the Kirby Bauer Disk Diffusion method with an image analysis system. This provides an analysis based on standard clinical methodology and quality control (NCCLS 2000, 2002a, 2002b), with an output that includes zone diameters and classification of each isolate as resistant, intermediate or susceptible to each antibiotic. Pulse Field Gel Electrophoresis (PFGE), a genotypic library-based technique is considered the gold standard for epidemiological tracking of organisms by the Centers for Disease Control and Prevention (Ferris et al. 2004, Lu et al. 2004, Singer et al. 2004). It is one of the best techniques to discriminate between strains of bacteria in complex bacterial matrices (Hanm et al. 2003, Meays et al. 2004, McLellan et al. 2003, Zhechko et al. 2005). It is standardized, reliable, and reproducible which makes it useful in comparative genetic analysis (Cameron et al. 1994, Lu et al. 2004, Okwumabuna et al. 2005). PFGE was used to develop "genetic fingerprints" of a subset of the isolates (due to the high cost and labor involved with this technique) to provide confirmation of bacteria sources. As the Copano Bay water body classification is based on fecal coliforms, *Escherichia coli,* a member of this group, was used as the bacterium for the study.

The project objective was to identify and quantify the relative contributions of various sources of fecal contamination to Copano Bay for development of a TMDL by TCEQ and to support a potential bacteriological adjustment by TDH (now TDSHS).

This was accomplished as several tasks:

1. Expansion of the existing *E. coli* antibiotic resistance profiles library at TAMU-CC to include fecal samples from the Copano Bay area for comparison with water isolates of *E. coli.*

2. Isolation of *E. coli* from Copano Bay water samples and comparison of their antibiotic resistance profiles and pulse field gel electrophoresis "fingerprints" with those in the TAMU-CC database for source tracking purposes.

3. Submission of data report to the TCEQ, CBBEP and TDH Project Managers and the GLO Project Coordinator, for use in determining sources of fecal contamination in Copano Bay.

STUDY SITE

General Description

Copano Bay (Texas Commission on Environmental Quality water body segment 2472) is a secondary bay located on the northwestern shore of Aransas Bay in the Mission-Aransas Estuary on the lower Gulf coast of Texas approximately 50 km north of the city of Corpus Christi, Texas (Fig. 1). Copano Bay measures approximately 112 km^2 in area and has an average water depth of approximately 1.5 m. Located in the northwest quadrant of Copano Bay is Mission Bay, a tertiary bay which measures approximately 14 km^2. A smaller tertiary bay, Port Bay, is located in the southwest quadrant. The northwestern portion of Copano Bay is located within Refugio County and the southeastern portion of the bay falls within Aransas County. Copano Bay is classified as a molluscan shellfish growing area by the Texas Department of Health (TDH) Seafood Safety Division due to the presence of the Eastern Oyster (*Crassostrea virginica*) (TDH, 2003a).

The area is frequently utilized both recreationally and commercially as a molluscan shellfish resource. Currently, the Eastern Oyster is harvested from TDSHS approved areas during oyster harvesting season, November 1 through April 30. Occasionally, parts of the area may be closed to harvest by TDSHS if one or more of the following factors occur:

- biotoxins are detected,
- unacceptably high levels of bacteria are detected,
- two or more confirmed illnesses are linked by TDSHS to the area (TDH, 2003a),
- over 3 inches of precipitation occurs in the Copano Bay area, or
- any precipitation occurs when the ground is already saturated (TDH, 1994).

Figure 1: Map showing Copano Bay study area, tertiary bays, and surrounding counties.

From November 1, 1994 through April 30, 2003 Copano Bay was closed to harvest a total of 156 days. Closures were due to heavy rainfall (13 days), bacterial contamination (29 days), and red tide (*Karenia brevis* formerly *Gymnodinium breve*) (114 days) (Table 1).

Description of Surrounding Area

Copano Bay is surrounded by marshes, tertiary bays, higher grasslands, and developing residential areas. The northern shoreline is mostly rural with mixed and low density residential development occurring along the southern and eastern shores. No industrial development exists in the area (TDH, 2003a). The incorporated city of Bayside (population 360) is located on the southwest shoreline of Copano Bay.

Table 1. The number of days by season (November 1 – April 30) that Copano Bay was closed to molluscan shellfish harvest and the reason for closure (TDH 1994, 2003, 2004).

Season	Number of days of closure	Reason for closure
1994-1995	0	-
1995-1996	4	Rainfall event
1996-1997	77	Red tide (*K. brevis*)
1997-1998	0	-
1998-1999	14	Bacteria
1999-2000	15	Bacteria
2000-2001	37	Red tide (*K. brevis*)
2001-2002	0	-
2002-2003	9	Rainfall event
2003-2004	7	Bacteria (fecal coliform)

Refugio County (The northern shore)

Refugio County encompasses approximately 770 square miles and was estimated by the U.S. Census Bureau to have 7,625 inhabitants in 2003 (U.S. Census Bureau, 2000), a slight decrease from the 2000 census. The upper northern shore of Copano Bay, from Copano Creek south past Plumbers Slough encompassing Mission Bay, is mostly undeveloped farm and ranch land (TDH, 2003a). The primary crops grown are corn, sorghum, and cotton. The chief use of the range/grassland is beef cattle production. Although no industrial facilities exist in the immediate study area, oil and gas production is a major industry, and production wells are numerous. Tourism is also a growing industry in the area and several hunting and fishing camps have been established and are operating (USDA, 1988).

The City of Bayside is located south of Mission Bay and north of the Aransas River inlet. In 2000, the population of Bayside was 360 individuals with 266 total housing units and an average household size of 2.35 individuals (U.S. Census Bureau, 2000). In 2003, a

comprehensive sanitary survey conducted by TDH determined that Bayside had 273 residences utilizing septic systems. Approximately 85-95% of these residences were occupied throughout the year (as opposed to seasonal vacation residences). Near Bayside, on Egery Road, but excluded from Bayside corporate limits, are 26 additional residences also on septic systems (TDH, 2003a).

Aransas County (the southern shores)

Aransas County encompasses approximately 252 square miles and was estimated by the U.S. Census Bureau to have 23,574 inhabitants in 2003 (U.S. Census Bureau, 2000), a nearly 5% increase from the 2000 census. The north and southeastern shore of Copano Bay, from the Copano Creek inlet south past the outlet to Aransas Bay continuing to Port Bay and west to the Aransas River is of mixed use. Much of the land is farm and ranch land, as is on the northwest shore. The primary use of farm and ranch land is production of cotton, sorghum, and beef cattle. As in Refugio County, oil and gas production is a major industry and many production wells exist in the area. Tourism is also an important industry and there are several tourism related areas including parks and bird sanctuaries (USDA, 1988).

The cities of Fulton (population 1,553) and Rockport (population 7,385) (U.S. Census Bureau, 2000) are located in Aransas County approximately 2-3 miles southeast of the bay. Though nearby, these two cities are serviced by municipal wastewater treatment facilities and are not within the watershed of Copano Bay.

San Patricio County (influence via Aransas River)

No part of Copano Bay actually falls within San Patricio County; however, the Aransas River, which is the boundary between Refugio and San Patricio Counties, is one of

the major sources of inflow to Copano Bay. Therefore, activities, which occur in San Patricio County, could potentially influence water quality of Copano Bay. As on the northwest shore, much of this land is farm and ranch land. The primary use of farm and ranch land is also production of cotton, sorghum, and beef cattle. As in Aransas County, oil and gas production is a major industry (USDA, 1988).

Septic Systems

There are a number of residences and businesses surrounding Copano Bay which were using septic systems as of 2003 (TDH, 2003a) (Table 2).

Soils

Aransas and Refugio Counties lie in the Western Gulf section of the Coastal Plain geomorphic region. The parent material of the soils is predominantly sedimentary in origin and the surface sediments are predominantly of Pliocene, Pleistocene, and Holocene (Recent) age (USDA, 1979, USDA, 1988). Soils surrounding Copano Bay are composed of four soil units: Aransas-Victine-Narta, Victoria-Edroy-Orelia, Narta-Aransas-Victine, and Galveston-Mustang-Dianola. All of these soil units are prone to flooding and wetness. With regards to sanitary facilities such as septic tank absorption fields and sewage lagoons, all of these soil units are also rated “severe” with soil properties or site features unfavorable for this use (USDA, 1988).

Soils of the upper north shore of Copano Bay (surrounding Mission Bay, in Refugio County) are predominantly Aransas-Victine-Narta soils, described as deep saline, moderately alkaline, clayey and loamy soils. These soils, formed in recent alluvium and marine

Table 2. Estimate of the number of residences and businesses surrounding Copano Bay (CB) using septic systems as of 2003 (from TDH, 2003) AB=Aransas Bay, AR=Aransas River.

Name	**Number of Units**	**Occupancy**	**Shoreline Location**
Bayside	273 houses	85-95% of the time	Southeast corner of CB north of the AR
Holiday Beach	560 houses	50% of the time	Northeast shoreline, north of CB/AB Interphase
Copano Cove	410 houses	year-round	Southern shoreline east of Port Bay
Copano Ridge	295 houses	year-round	South shoreline east of Port Bay
Copano Village	122 houses	year-round	Southeast shoreline south of redfish point
Cape Vallero	4 houses and 12 condo. units	year-round	Southern shoreline in Port Bay
Port Bay Club	8 houses	year-round	Southern shoreline in Port Bay
Copano Oaks	12 residences	year-round	East shoreline at the south side of the CB/AB Interphase
Heritage Oaks	13 residences	15% occupied currently	East shoreline at the south side of the CB/AB Interphase
Between Copano Ridge and Copano Village	30 houses	year-round	Southeast shoreline
FM 1781 between Hwy 35 and Copano Village	68 houses	year-round	Southeast shoreline
Ocean Hideaway RV Park	30 lots	40% occupied currently	Southeast shoreline
Evan Baitstand and RV Park	25 lots	32% occupied currently	Southeast corner just north of the AR
Marina Baitstand	3 boats	No data	No data
Keller Marina	4 boats	No data	No data
Redfish Bay Fishing Lodge	8 rental units,1 ramp	No data	South shoreline located on Rattle snake point
Bahia Vista RV Park	73 lots	30% occupied at the time of survey 80-90% occupied winter	East shoreline at the south side of the CB/AB Interphase
M.T.K. Boat Barns	30 boat storage units,1 ramp	No data	Southeast shoreline south of redfish point
Copano Bay State Fishing Pier	(2) 1000 gal septic tanks	No data	East shoreline south and north of CB/AB Interphase

sediment, are found on coastal flood plains and low terraces. Aransas-Victine-Narta soils are prone to flooding and ponding, and prevent rapid percolation (USDA, 1988).

Soils of the lower north shore of Copano Bay (surrounding Bayside, in Refugio County) are typically Victoria-Edroy-Orelia soils, described as deep, moderately alkaline to slightly acidic, clayey and loamy soils formed in clayey and loamy marine sediment, found on uplands. Victoria-Edroy-Orelia soils are also prone to wetness and ponding, and prevent rapid percolation (USDA, 1988).

Soils of the far southern shore of Copano Bay (surrounding Port Bay in Aransas and San Patricio Counties) and far northern shore of Copano Bay (east of Copano Creek, in Aransas County) are predominantly Narta-Aransas-Victine soils, described as nearly level, very slowly permeable, slightly to extremely saline, clayey and loamy soils found on flood plains and in low coastal areas. As with the Aransas-Victine-Narta soil unit, Narta-Aransas-Victine soils are also prone to flooding and wetness and prevent rapid percolation (USDA, 1988).

Soils of the mid eastern coast of Copano Bay (surrounding the inlet into Aransas Bay, in Aransas County) are classified as Galveston-Mustang-Dianola, described as nearly level to undulating, rapidly permeable, non-saline to extremely saline, sandy soils. These soils are typically found in low coastal areas. Galveston-Mustang-Dianola soils, although much more permeable than the previously mention soil units, are also prone to flooding and wetness as well as cutbacks and caves. Blowing soil, salinity, high water table, and the potential for flooding limit the use of this soil (USDA, 1988).

Copano Bay Tides and Current

Copano Bay is subject to tidal exchange occurring via the 2.7 km wide inlet connecting it with Aransas Bay. Tidal activity is diurnal to semi-diurnal with tides ranging from approximately 15cm to 61cm. Tidal amplitude in this shallow bay is heavily influenced by wind speed and direction (TDH, 2003a). Strong northwesterly winds associated with north fronts frequently reduce tide in Copano Bay by forcing water through the inlet into Aransas Bay. Conversely, strong southeasterly winds often push tides higher to the north side reducing tides in the southerly portions of the bay. Southeasterly winds prevail along the south Texas coast throughout the majority of the year, creating the dominate current pattern in Copano Bay with winds pushing water from the southern portion of the bay to the northern reaches (Fig. 2). There is little submerged vegetation on the bay bottom, which is generally silty clay. Numerous oyster reefs exist primarily in a southeasterly to northwesterly direction (Fig. 3), perpendicular to the dominant water flow pattern. They have the potential to reduce the movement of water in the bay (TDH, 2003a).

Climate

The climate of the Copano Bay area (Fig. 4 & 5) can be characterized as humid subtropical. Aransas County, being the more coastal of the two county counties included in the study area, has slightly less variation in climate. Data collected at the Aransas National Wildlife Refuge from 1942-1971 shows the highest monthly mean temperature of 33.3˚C occurring in both July and August (USDA, 1979). The lowest monthly mean temperature, 7.2˚C, occurs in January. For Refugio County, data collected from 1951-1980, shows the

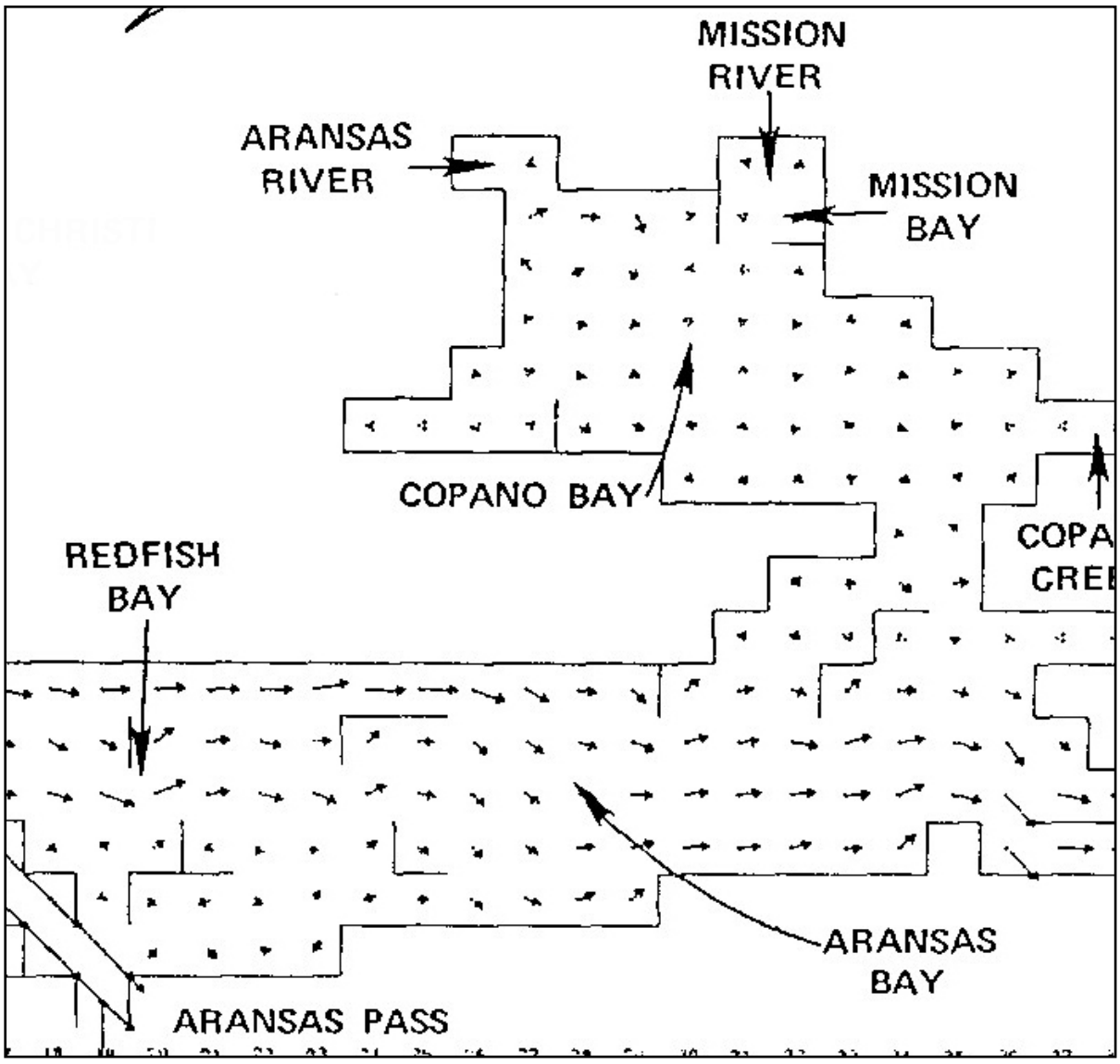


Figure 2. Chart showing water circulation in Copano Bay (after TDH 2003a).

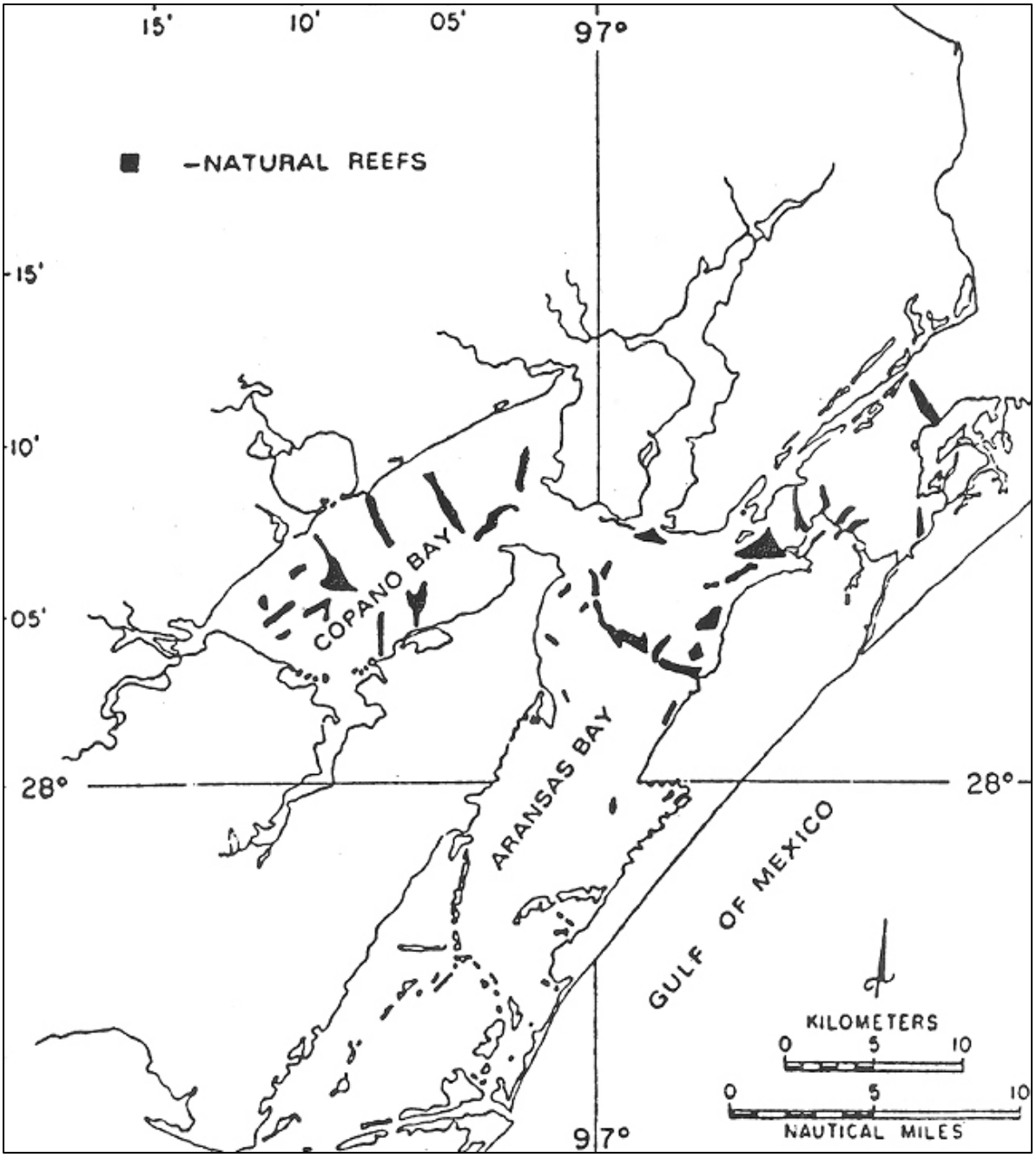


Figure 3. Map showing natural oyster reefs in Copano Bay (after TDH 2003a).

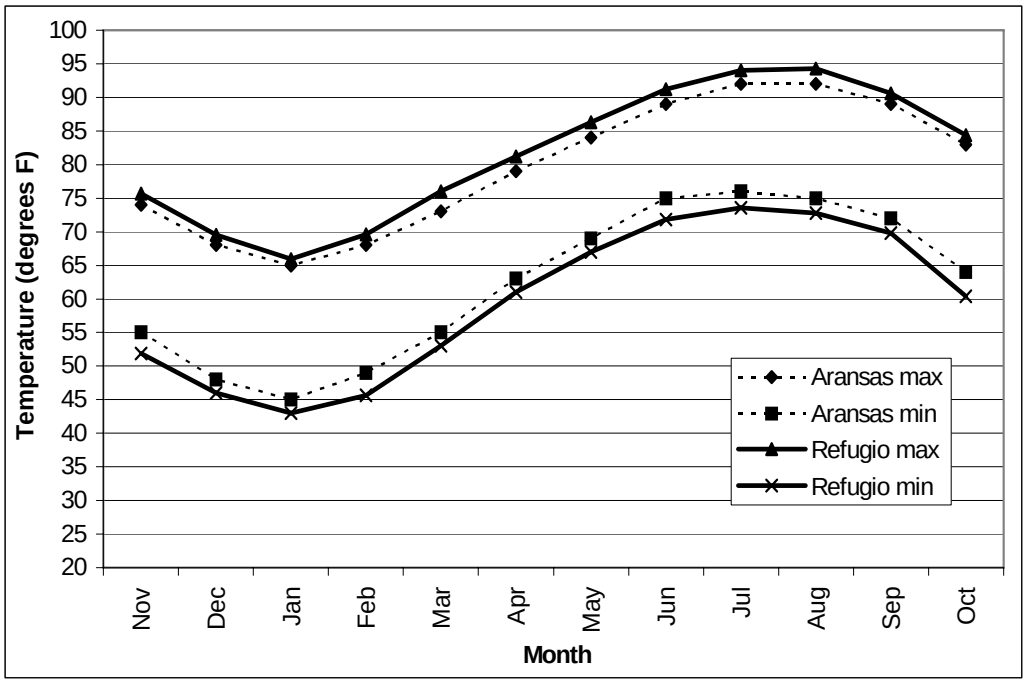


Figure 4. Mean monthly maximum and minimum temperature for Aransas County (1942-1971) and Refugio County (1951-1980).

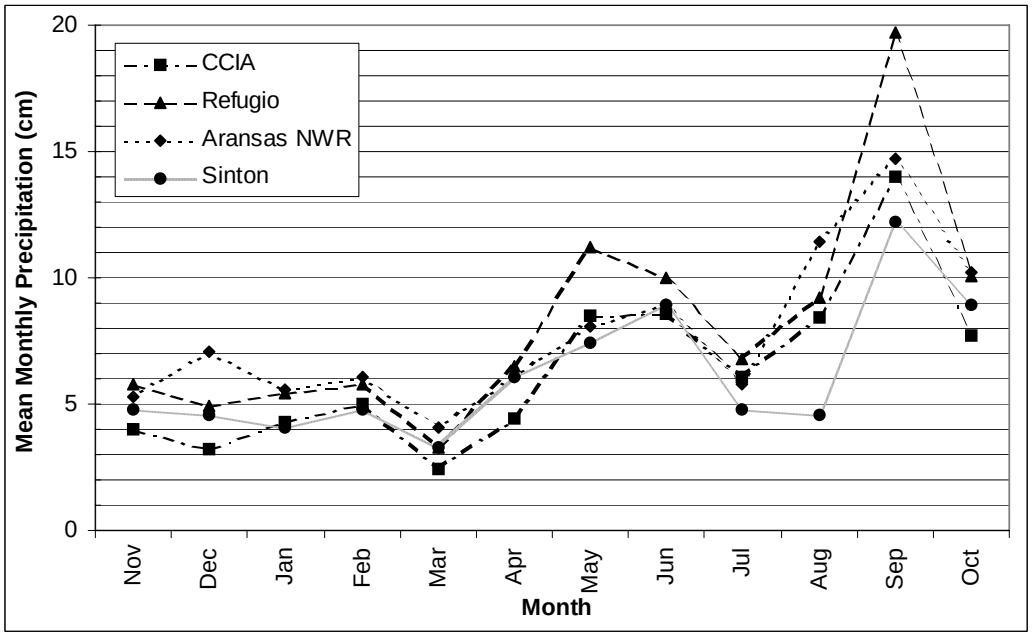


Figure 5. Mean monthly precipitation from Corpus Christi International Airport (1961-1990), the City of Refugio (1951-1980), Aransas National Wildlife Refuge (1942-1971), and the City of Sinton (1921 to 1973).

highest monthly mean temperature, 34.4˚C, occurs in July and August, and the lowest monthly mean temperature, 6.1˚C, occurs in January (USDA, 1988).

Precipitation

Precipitation in the study area often occurs in association with major weather events such as tropical storms which occasionally strike the study area during summer and early fall (USDA, 1979; USDA, 1988). Precipitation data exists from several nearby areas; the City of Refugio, the City of Sinton, Aransas National Wildlife Refuge, and Corpus Christi International Airport. The cities of Refugio and Sinton lie within the Copano Bay watershed. A general precipitation gradient exists along the Texas coast with precipitation amounts typically decreasing from east to west (South Texas Regional Water Planning Group. 2001).

Corpus Christi International Airport 30-year data (1961-1990) shows the mean annual precipitation was 76.5cm (NOAA NWS data). At the City of Refugio from 1951-1980 mean annual precipitation was 98.5cm (USDA, 1988). At the Aransas National Wildlife Refuge from 1942-1971 mean annual precipitation was 93.47cm (USDA, 1979), and at the City of Sinton from 1921 to 1973 mean annual precipitation was 74.4cm (USDA, 1979).

Typically, in the study area, less precipitation occurs during the traditional oyster season months, November through April. Thirty-year data (1961-1990) from Corpus Christi International Airport indicates that the mean monthly precipitation during the months of November to April was 3.9cm compared to 8.9cm for the months of May through October (NOAA NWS data) (Fig. 5). Data from Refugio shows mean monthly precipitation during the months of November to April was 5.3cm compared to 11.2cm for the months of May through October (USDA, 1988). Aransas National Wildlife Refuge data indicates

meanmonthly precipitation during the months of November to April was 5.7cm compared to 9.9cm for the months of May through October (USDA, 1979), and data from the City of Sinton shows mean monthly precipitation during the months of November to April was 4.6cm compared to 7.8cm for the months of May through October (USDA, 1979).

Watershed Description

Copano Bay lies almost entirely within the Aransas Bay watershed (USGS cataloging unit: 12100405) (Fig. 6), which also encompasses Copano Creek. Copano Bay receives most of its freshwater inflow from the Mission River via Mission Bay, the Aransas River, and Copano Creek. The Mission River lies within the Mission watershed (USGS cataloging unit: 2100406) to the northwest of Copano Bay. The river is approximately 97 km (60 mi) in length and drains approximately 187,774 hectares (725 square miles) of land in Goliad, Bee, and Refugio counties. Surface water flows into Mission Bay thence into Copano Bay (TDH, 2003). The Mission watershed encompasses the town of Refugio (population 2,941) (U.S. Census Bureau, 2000).

The Aransas River, which lies within the Aransas watershed (USGS cataloging unit: 12100407), is approximately 64 km (40 mi) in length and drains approximately 155,400 hectares (600 square miles) of land in San Patricio, Bee, and Refugio Counties. Surface water in the watershed flows into the Aransas River and then directly in to Copano Bay (TDH, 2003a). Copano Creek falls within the Aransas Bay watershed (USGS cataloging unit: 12100405) and drains approximately 22,792 hectares (88 square miles) in Aransas and Refugio counties.

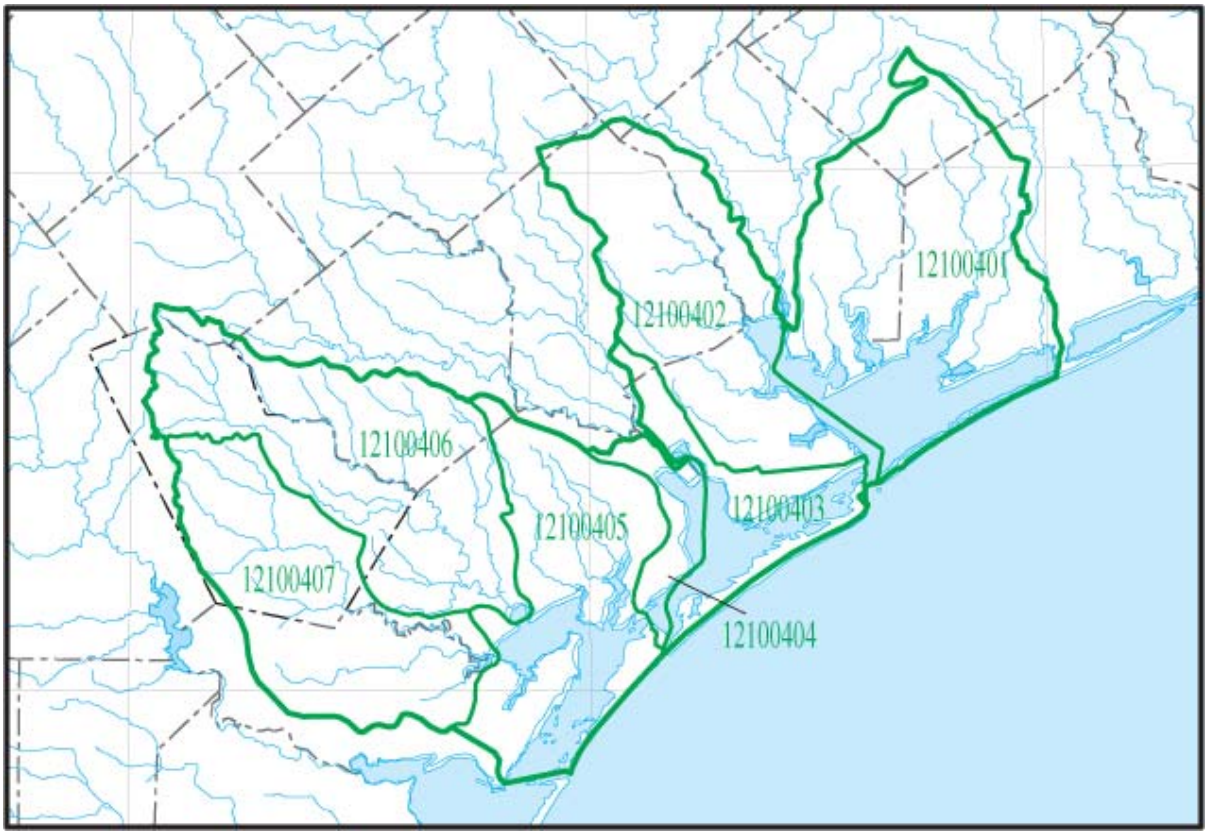


Figure 6. Map showing USGS watershed cataloging units and surface water flow from the Aransas River watershed, the Mission River watershed, and Aransas Bay watershed, into Copano Bay.

Freshwater Inflow

Streamflow of several rivers and creeks, which flow into Copano Bay, is continually monitored at United States Geological Survey (USGS) gaging stations. The following rivers and creeks, which flow in to Copano Bay, have gaging stations. Locations are shown in Figs. 7-9. Historical annual flow data is provided for 1971 to 1999, monthly data is shown for 2000-2003 and weekly flow data is shown for the study period.

Annual average and extremes Mission River, Aransas River, Copano Creek

Flow at USGS gage #08189500 on the Mission River at Refugio for the period from 1971 to 1999, averaged 155.92 cubic feet per second (CFS) annually or 112,881.40 acre-feet per annum (AFA) with extremes ranging from 1.25 CFS in 1989 to 428.15 in 1971 (Fig. 10).

Flow at USGS gage #08189700 on the Aransas River near Skidmore for the same period averaged 32.35 CFS annually or 23,420.43 AFA with extremes ranging from 2.36 CFS in 1989 to 130.77 CFS in 1971.

Flow at USGS gage #08189200 on Copano Creek near Refugio for the same period averaged 44.94 CFS annually or 32,535.21 AFA with extremes ranging from 0 CFS in 1988 to 150.83 CFS in 1981.

46 months before study Mission River, Aransas River, Copano Creek

During the 46 months directly preceding the study period, January 2000 to October 2003, monthly flow at USGS gage # 08189500 on the Mission River at Refugio averaged 187.54 CFS with peaks occurring in September 2001 and November 2001 (2,666.00 and 1,145.00 CFS, respectively) (Fig. 11).

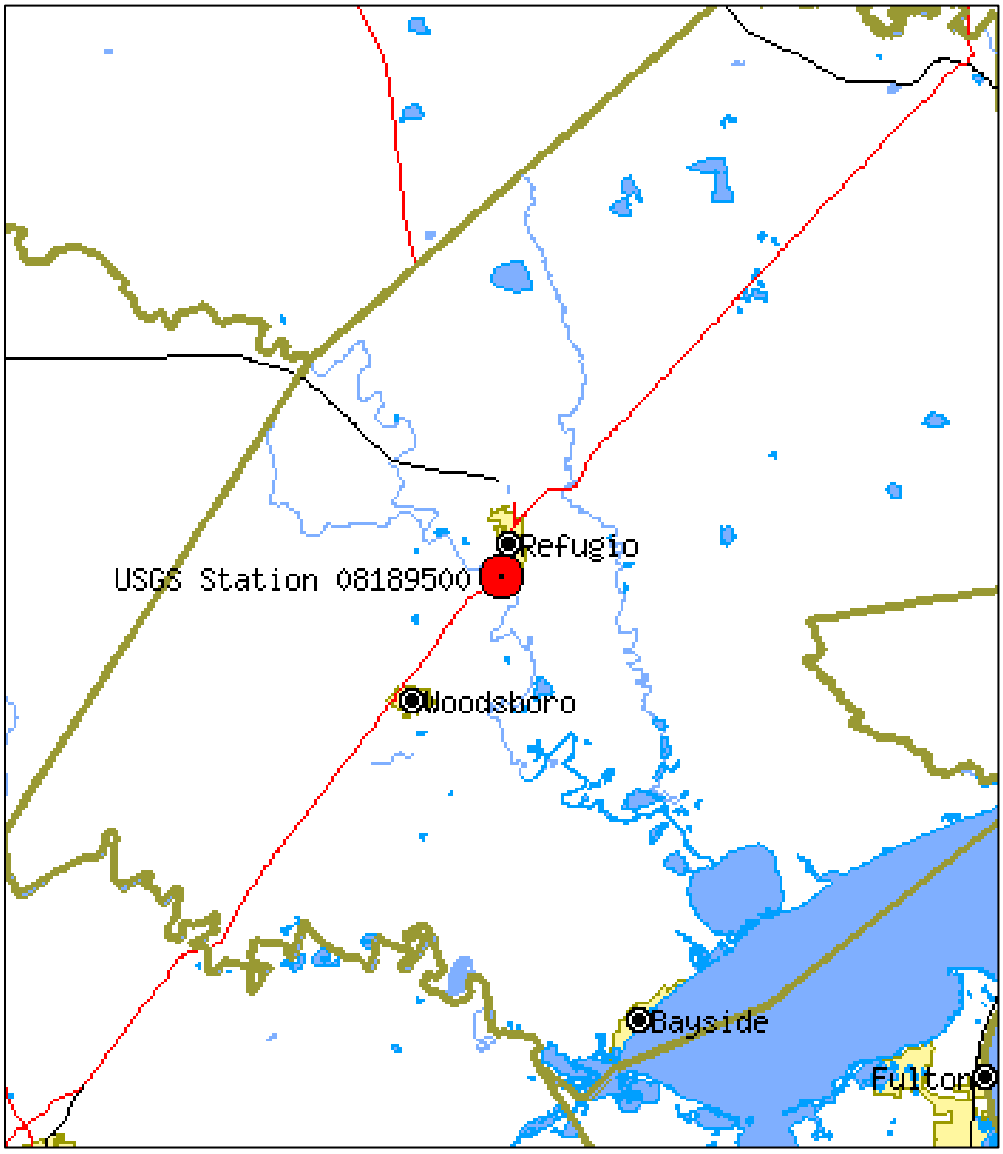


Figure 7. Site map showing USGS gage #08189500 on the Mission River at Refugio, TX.

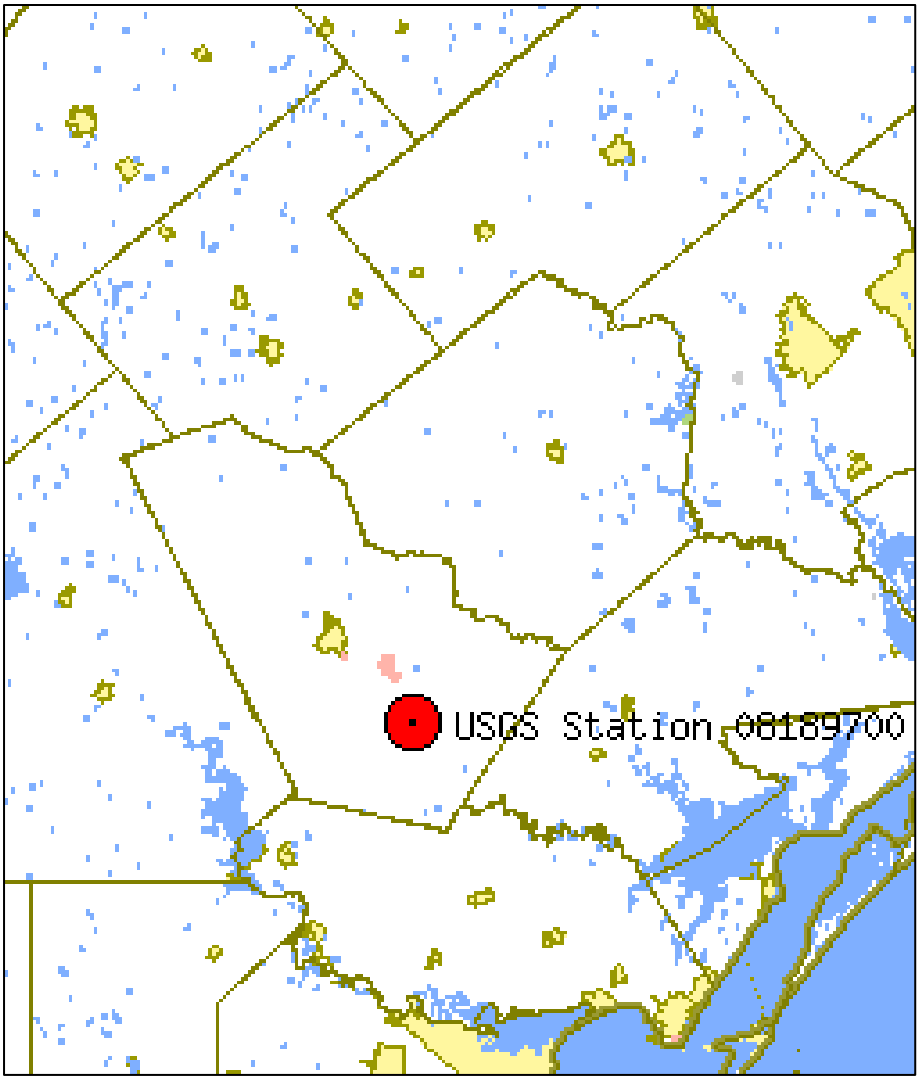


Figure 8. Site map showing USGS gage #08189700 on the Aransas River near Skidmore TX.

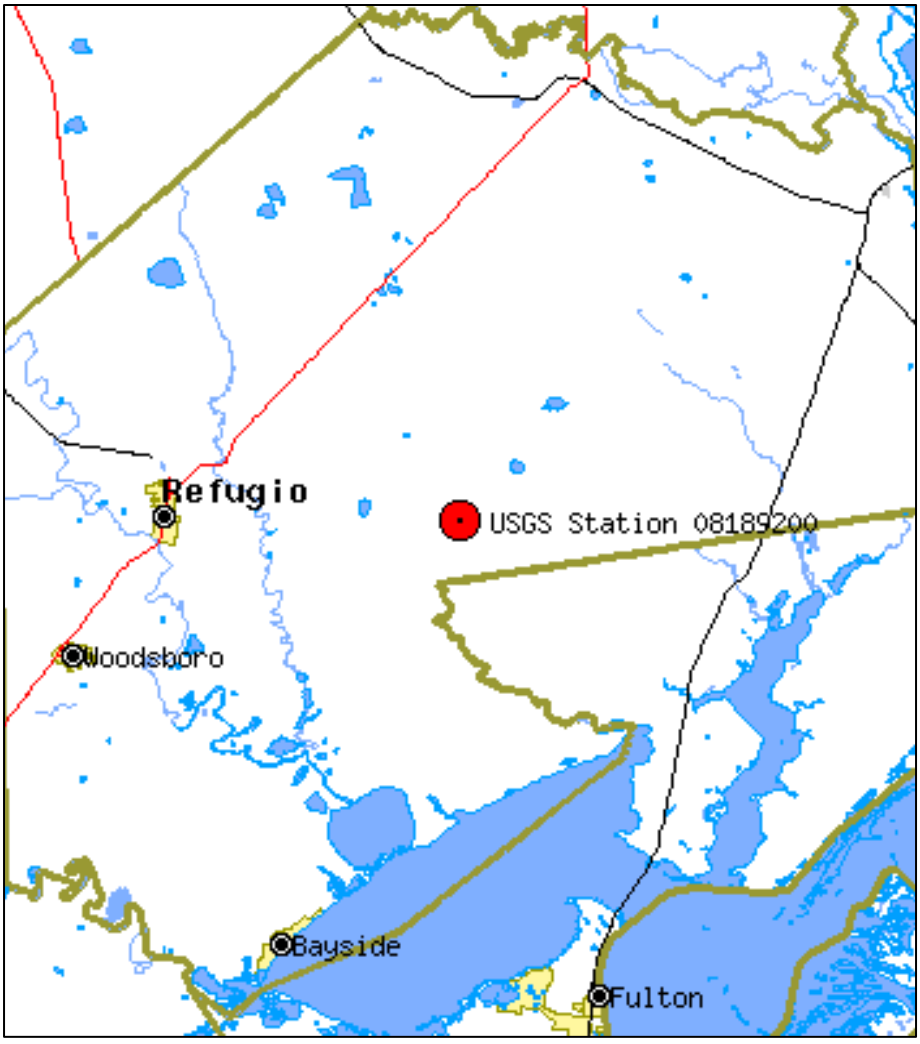


Figure 9. Site map showing USGS gage #08189200 on Copano Creek near Refugio, TX.

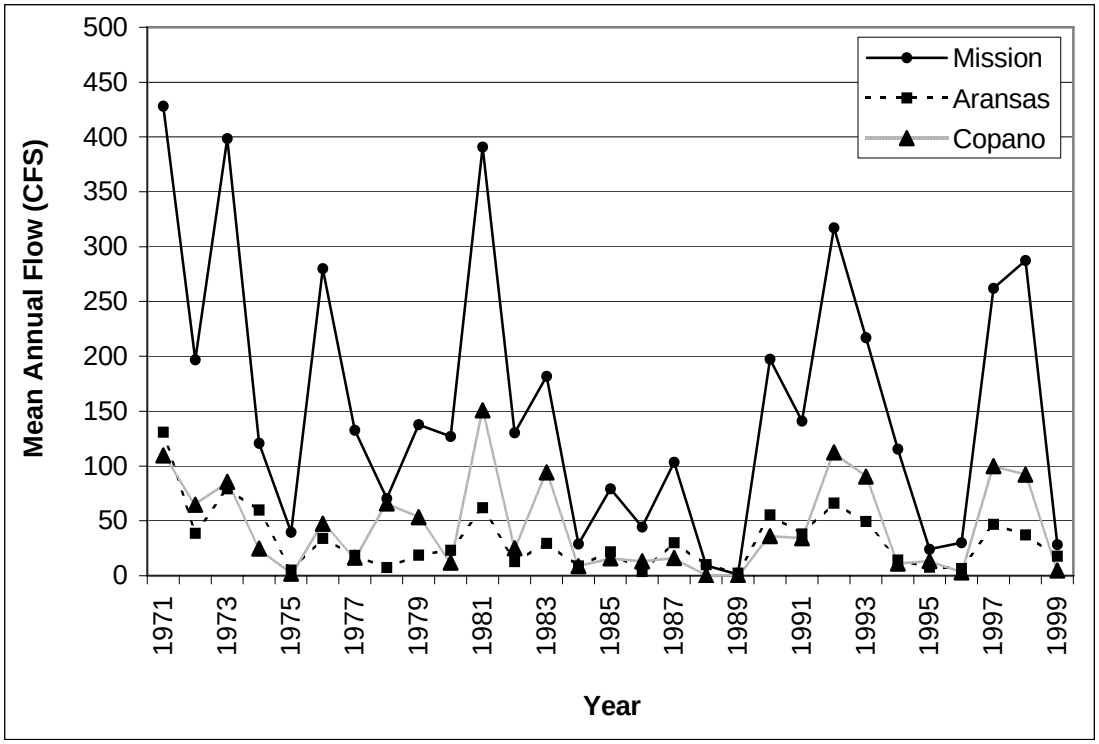


Figure 10. Mean annual flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from 1971 through 1999.

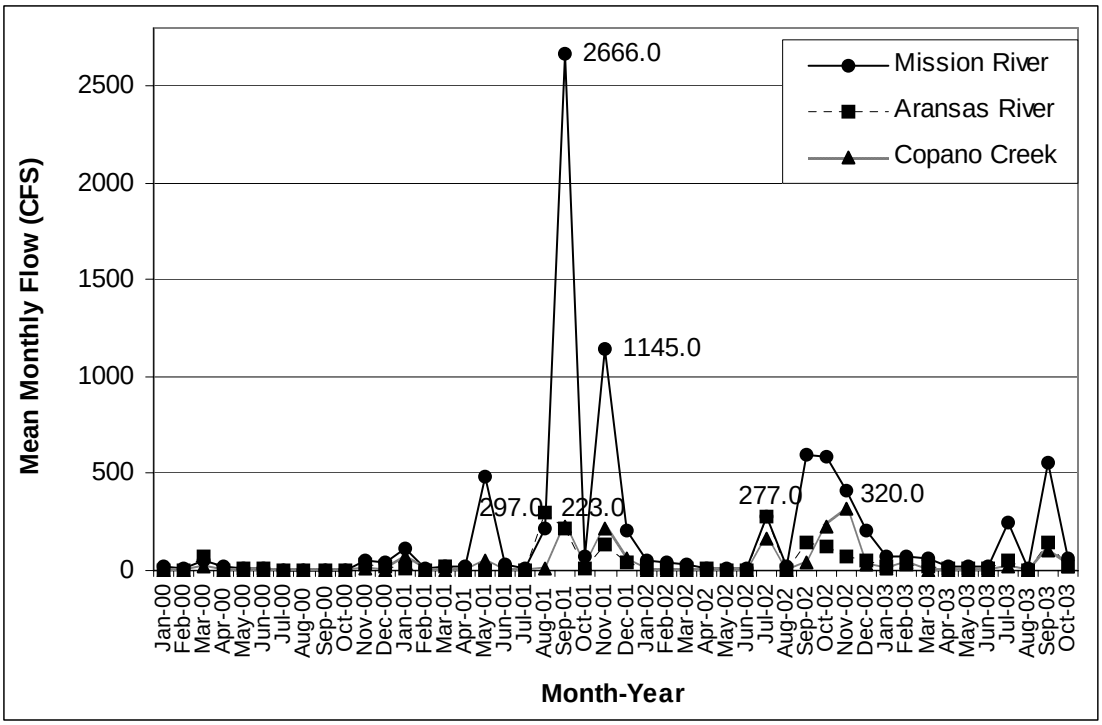


Figure 11. Mean monthly flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from January 2000 through October 2003.

During the 46 months directly proceeding the study period, January 2000 to October 2003, monthly flow at USGS gage # 08189700 on the Aransas River near Skidmore averaged 41.76 CFS with peaks occurring in August 2001 (297.00 CFS) and July 2002 (277.00 CFS).

During the 46 months directly preceding the study period monthly flow at USGS gage # 08189200 on Copano Creek near Refugio averaged 36.38 CFS with peaks occurring in November 2002 (320.00 CFS) and September 2001 (223.00 CFS).

Monthly flow during study Mission, Aransas, Copano Creek

During the study sampling period, October 1, 2003 through April 30, 2004, mean monthly flow at the Mission River gage was 265.62 CFS with a monthly peak of 1553.00 CFS in April 2004 (Fig. 12). Excluding the extreme precipitation events of April 2004, mean monthly flow at the Mission River gage from October 1, 2003 through March 31, 2004 was 45.28 CFS.

Mean monthly flow at the Aransas River gage was 77.16 CFS during the same period with a monthly peak of 466.05 CFS in April 2004. Again, excluding the extreme precipitation events of April 2004, mean monthly flow at the Aransas River gage from October 1, 2003 through March 31, 2004 was 12.72 CFS.

Mean monthly flow at the Copano Creek gage during the study period was 73.32 CFS with a monthly peak of 437.2 CFS in April 2004. Excluding the extreme precipitation events of April 2004, mean monthly flow at the Copano Creek gage from October 1, 2003 through March 31, 2004 was 11.53 CFS.

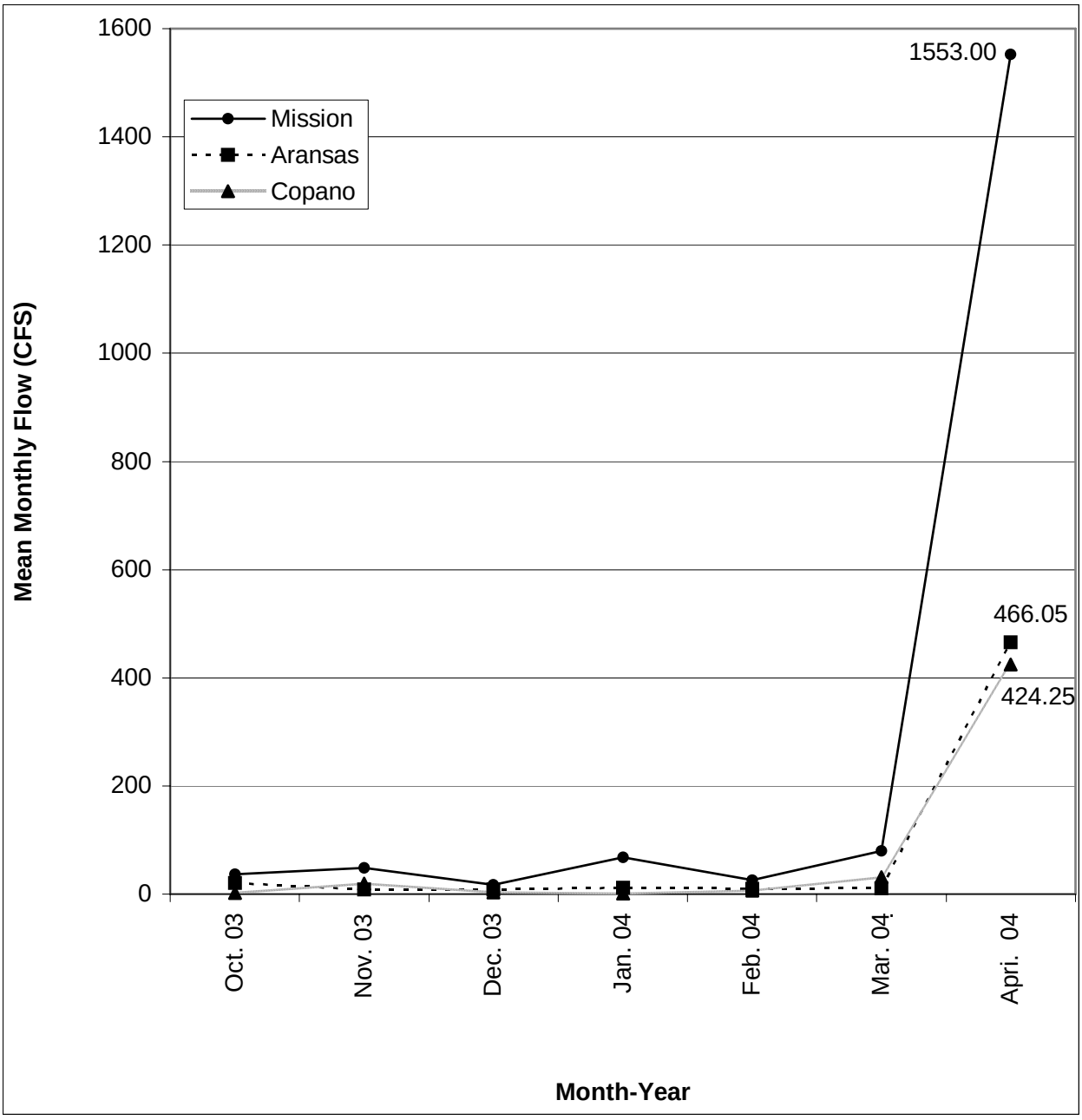


Figure 12. Mean monthly flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from October 2003 through April 2004.

Weekly flow during study MAC

During the study sampling period, mean weekly flow at the Mission River gage was 293.13 CFS with a weekly peak of 3,848.14 CFS during week 24 (April 8-14) (Fig. 13). Excluding the extreme precipitation events of weeks 24 and 25 (April 1-14), mean weekly flow at the Mission River gage from October 1, 2003 through October 31, 2004 was 82.74 CFS. The mean weekly flow rate during weeks 24 and 25 of the sampling period increased to 3,343.79 CFS.

Mean weekly flow at the Aransas River gage during the same period was 85.33 CFS with a weekly peak of 1,643.14 CFS during week 23 (April 1-7). Excluding the extreme precipitation events of weeks 24 and 25 (April 1-14), mean weekly flow at the Aransas River gage from October 1, 2003 through March 31, 2004 was 25.11 CFS. The mean weekly flow rate during weeks 24 and 25 of the sampling period increased to 958.57 CFS.

Mean weekly flow at the Copano Creek gage during the sampling period, was 81.01 CFS with a weekly peak of 956.57 CFS during week 24 (April 8-14). Excluding the extreme precipitation events of weeks 24 and 25 (April 1-14), mean weekly flow at the Copano Creek gage from October 1, 2003 through March 31, 2004 was 45.46 CFS. The mean weekly flow rate during weeks 24 and 25 of the sampling period increased to 596.61 CFS.

Daily flow during study MAC

During the study sampling period, October 1, 2003-April 30, 2004 mean daily flow at the Mission River gage was 292.83 CFS with a daily peak of 9,340.0 CFS on April 8, 2004 (Fig. 14). Mean daily flow at the Aransas River gage during the same period was 86.12 CFS with a daily peak of 5,920.0 CFS on April 5, 2004. Mean daily flow at the Copano Creek

gage during the sampling period was 74.17 CFS with a daily peak of 1,140.0 CFS on April 9, 2004. Mean daily flow is shown for each month during the sampling period in Figs. 15-21.

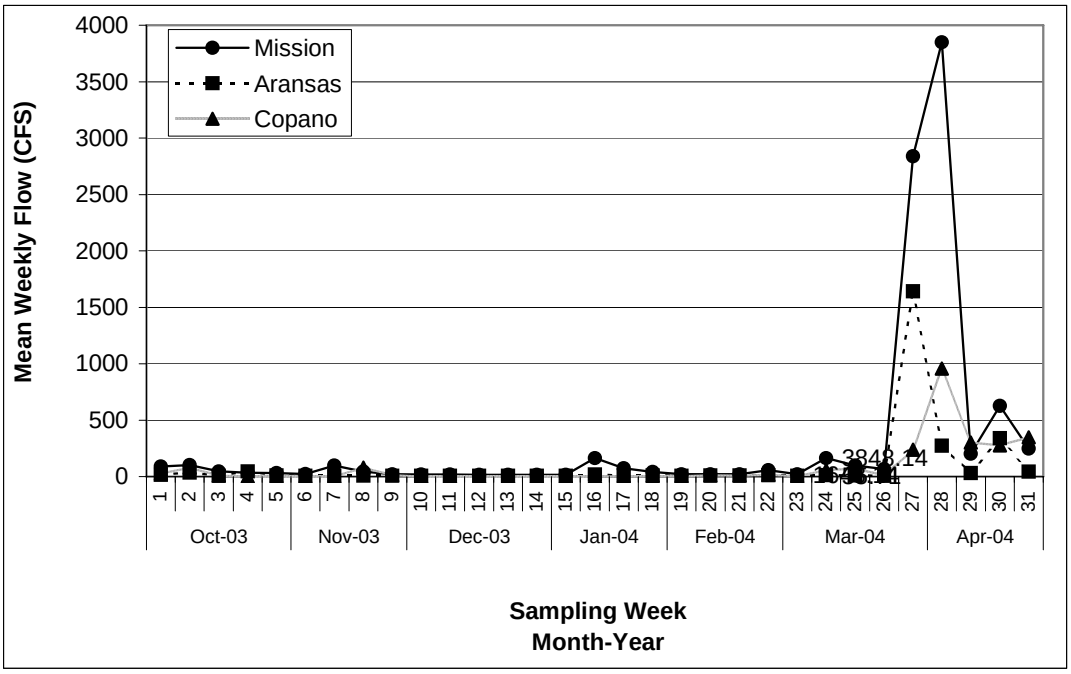


Figure 13. Mean weekly flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from October 2003 through April 2004.

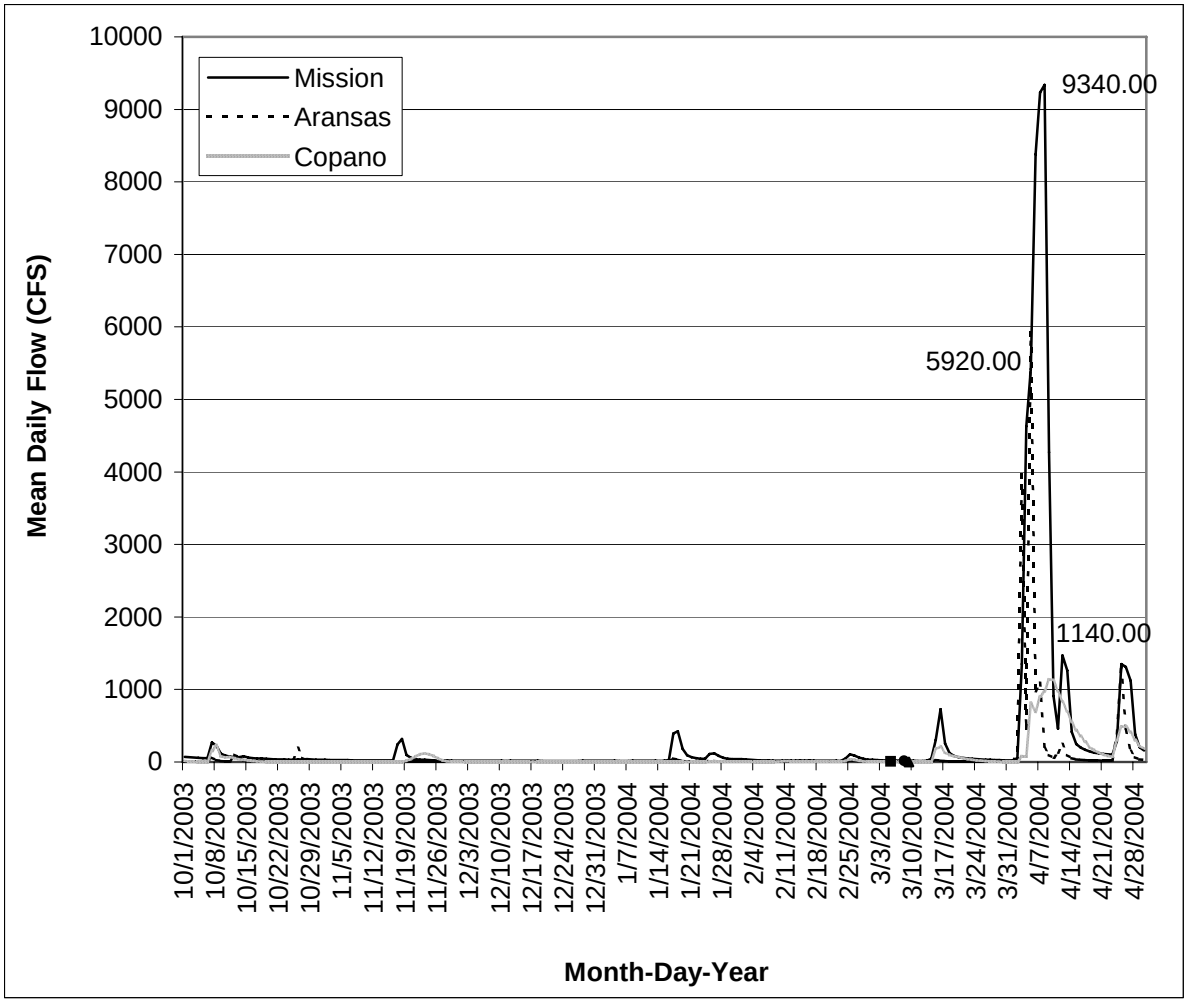


Figure 14. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from October 2003 through April 2004.

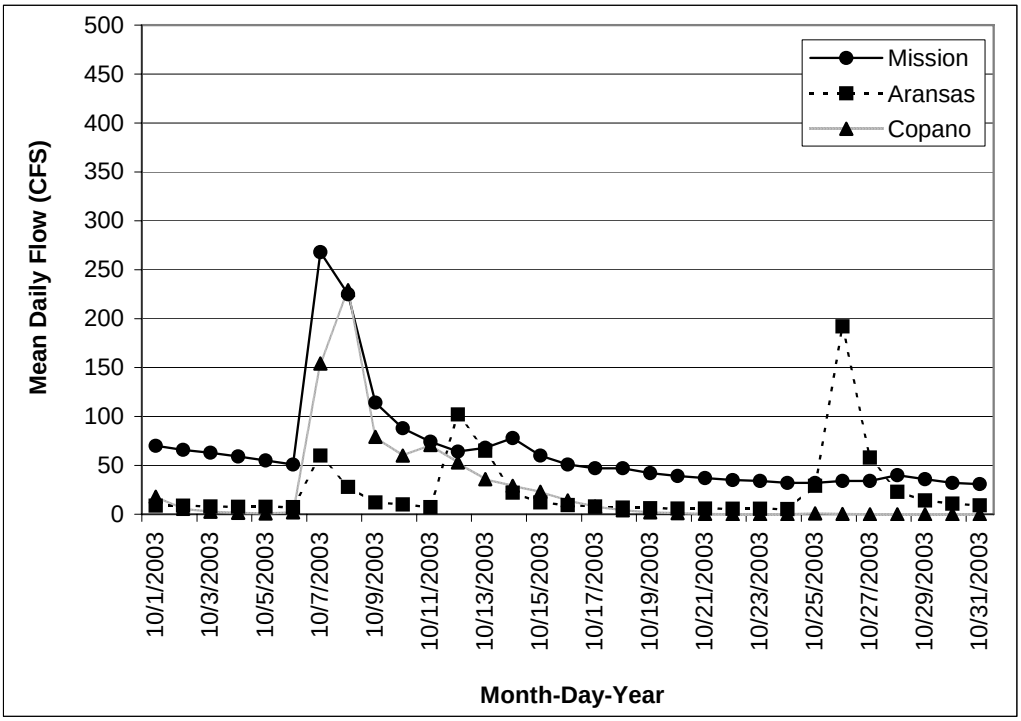


Figure 15. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from Oct. 1, 2003 through Oct. 31, 2003.

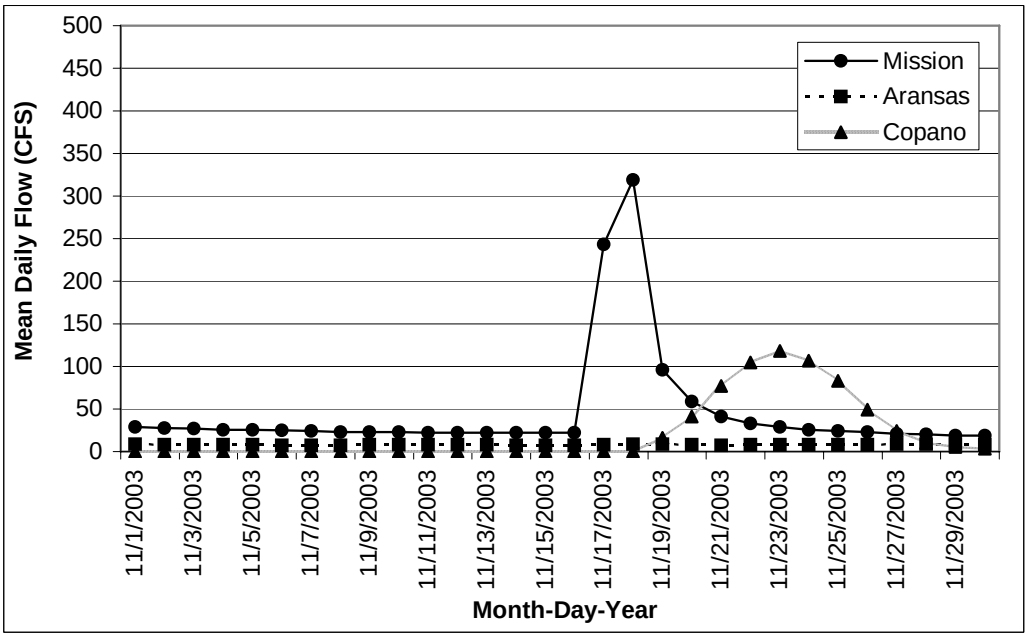


Figure 16. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from Nov. 1, 2003 through Nov. 30, 2003.

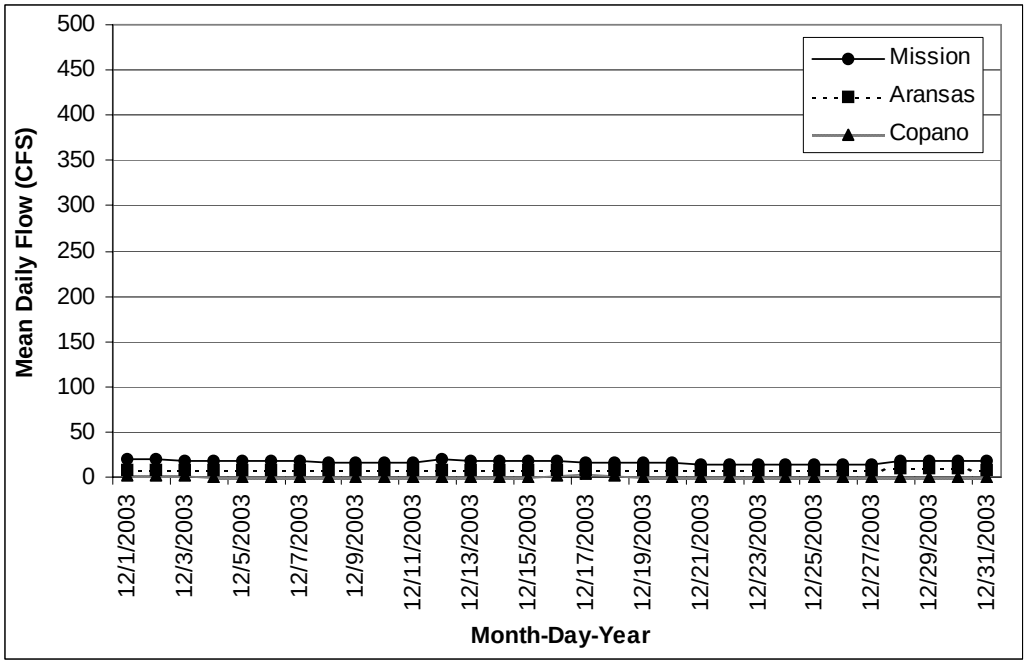


Figure 17. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from Dec. 1, 2003 through Dec. 31, 2003.

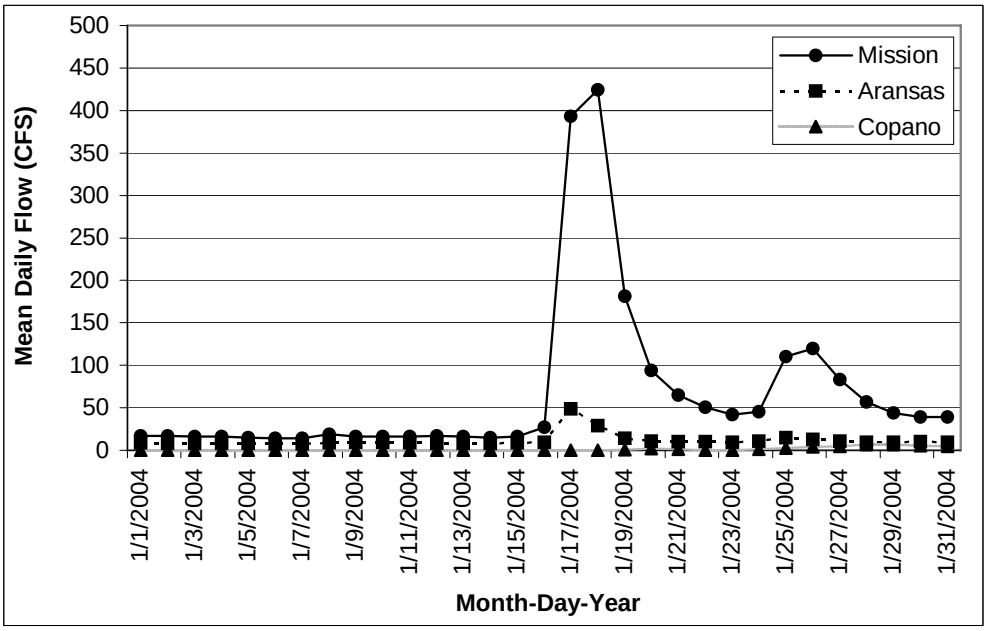


Figure 18. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from January 1, 2004 through January 31, 2004.

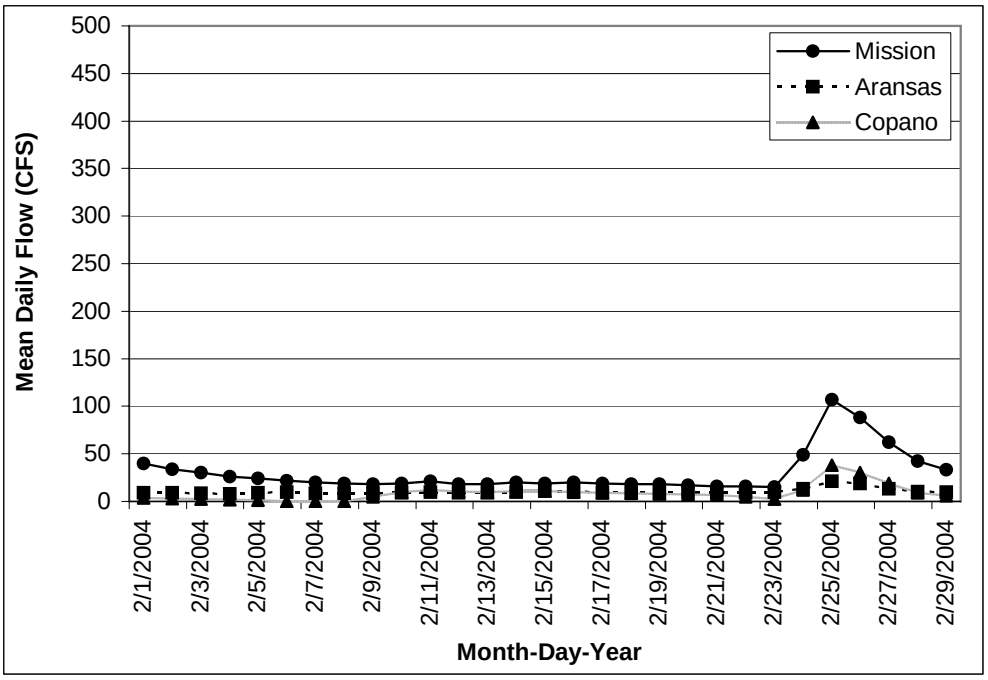


Figure 19. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from Feb. 1, 2004 through Feb. 28, 2004.

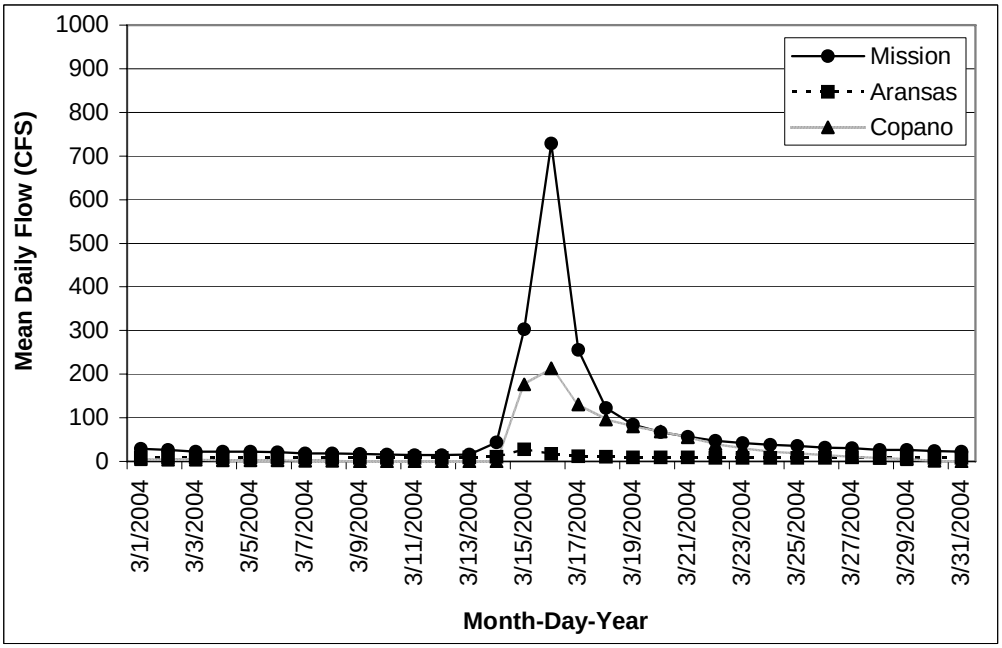


Figure 20. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from March 1, 2004 through March 31, 2004.

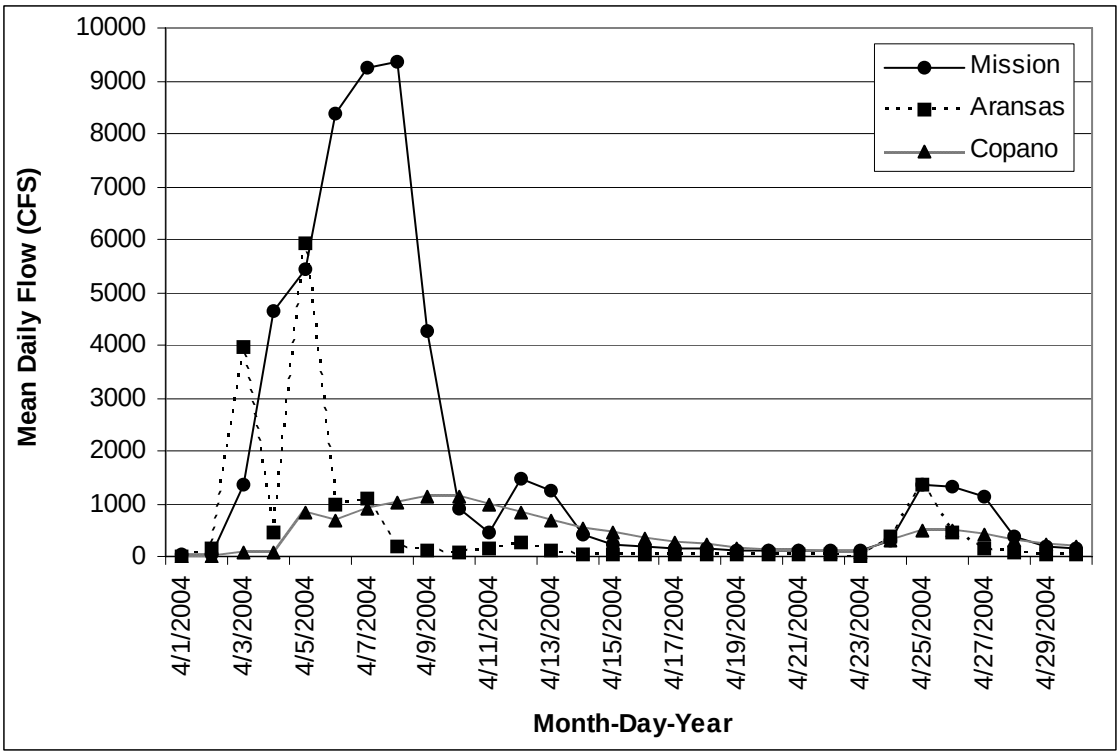


Figure 21. Mean daily flow at USGS gages on the Mission River at Refugio, TX (USGS 08189500), on the Aransas River near Skidmore TX, (USGS 08189700), and on Copano Creek near Refugio, TX (USGS 08189200) from April1, 2004 through April 30, 2004.

METHODS

This project involved the measurement of non-routine parameters. Methods used have been published and/or approved in Quality Assurance Project Plans (QAPPs). The QAPP for this project was reviewed by the TCEQ, TGLO, CBBEP and U.S. EPA to help ensure that data generated for the purposes described herein are scientifically valid and legally defensible and may be used to support decisions related to TMDL development.

The sample design was based on the program requirements of the Total Maximum Daily Load Program. TAMU-CC was tasked with providing data and information to support TMDL data and information needs. The environmental data collected under the QAPP was collected and evaluated with a high degree of confidence that the data are scientifically valid, of known quality, and legally defensible. TAMU-CC coordinated closely with the TCEQ and other TMDL participants to ensure an adequate water monitoring strategy to supply informational needs for modeling, assessment, load allocation, and decision-making.

This data collection effort involved collection of water quality data for the purpose of bacteria source tracking to aid TMDL development. To this end, some general guidelines were followed when selecting sampling sites, as identified below. Overall consideration was given to accessibility and safety. All monitoring activities were developed in coordination with the TCEQ TMDL Project Manager. Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. A sample is in custody if it is in actual physical possession or in a secured area that is restricted to authorized personnel. A Chain of Custody (COC) form was used to document sample handling during transfer from the field to the laboratory and among contractors.

a) Known source samples (fecal samples)

SAMPLE COLLECTION

The TDH Division of Shellfish Sanitation comprehensive sanitary surveys of the shellfish producing waters of Copano Bay (1994, 2000, 2003a draft) were used to determine potential source animals and appropriate locations for sampling. These surveys cite main potential sources of fecal contamination in Copano Bay as human (malfunctioning septic systems, wastewater facilities overloaded after rainfall) and cattle. Lesser sources include sheep, hog and ducks. Geese impact is cited as probably minimal. In order to reflect these sources human samples (volunteers, portable toilets and wastewater), cattle, and duck samples were collected from the Copano Bay watershed. Due to the paucity of sheep and hog found in the watershed area, and the higher incidence of horses, with the approval of the project coordinators, horse fecal samples were collected instead of sheep/hog. General areas from which samples were collected are shown in Table 3. Specific locations and businesses are not identified at the request of owners. Specific locations were documented on field data sheets, stored with the COC Forms at Texas A&M University-Corpus Christi.

The fecal samples from known animal sources were collected by TAMU-CC personnel, under the supervision of the P.I.s on multiple sampling trips in the winter and spring of 2004 to obtain approx. two hundred (200) *E. coli* isolates. An additional set of samples were collected November 22, 2004) (Table 3). A standard approved field data sheet was filled out for each sample with collector signature to include field parameters and date/time collected.

All collection protocols followed those detailed in the Quality Assurance Project Plan for the project (QAPP). Samples from all animal sources were collected in polypropylene,

Table 3. Locations, dates and animal sources for fecal sample collections from the Copano Bay area.

Collection Season	Collection dates	Animal source	Scientific name	Location
Winter/Spring	12/27/03-01/13/04	Wild Duck	*Anas* spp.	Rockport
Winter/Spring	02/07/04-02/10/04	Human	*Homo sapiens*	Beeville WWTP
Winter/Spring	02/23/04-03/8/04	Cow	*Bos taurus*	Sinton/Taft
Winter/Spring	04/28/04	Horse	*Equus caballus*	Beeville/Sinton
Fall	11/22/04	Cow	*Bos taurus*	Sinton/Taft
Fall	11/22/04	Black Bellied Whistling Duck	*Dendrocygna autumnalis*	Goose Island State Park
Fall	11/22/04	Horse	*Equus caballus*	Sinton/Taft
Fall	11/22/04	Human (sewage)	*Homo sapiens*	Rockport Reclamation Plant
Fall	11/22/04	Deer	*Odocoileus virginianus*	Welder Wildlife Refuge
Fall	11/22/04	Coyote	*Canis latrans*	Welder Wildlife Refuge
Fall	11/22/04	Javelina	*Pecari tajacu*	Welder Wildlife Refuge

screw cap, sterile specimen containers or using BD BBL™ EZ culture swabs and/or sample cups. Sterile culture swabs were opened, immediately applied to a fecal sample and returned to the sterile plastic container enclosing the swab. For samples collected in sample cups (for example, samples from wastewater treatment plants), sterile tongue depressors were used to remove the top portion of the fecal sample and a second tongue depressor was utilized to obtain the sample. The sample was then placed immediately into an unopened sample cup and sealed. The duck samples from 2003 were collected via swabbing the cloaca of ducks recently shot by hunters. All sample containers were placed in coolers with ice for transport.

E. coli ISOLATIONS

Samples were transported in coolers with ice to the Environmental Microbiology Research Lab at Texas A&M University-Corpus Christi immediately following field collection. Analysis followed methods described in the QAPP. *Escherichia coli* isolations from fecal samples followed the TAMU-CC SOP (QAPP), in a previous special TNRCC study work plan approved by TNRCC (2000). "Application of antibiotic resistance patterns to differentiate sources of E. *coli* in coastal waters of Texas" (2000), prepared by Dr. Mott for TCEQ.

Fecal samples were swabbed onto mTEC agar plates and incubated first at 35^{O}C for 2hr and than at 44.5^{O}C for 22hr. The filter papers with bacteria colonies were removed from the mTEC plates and placed on absorbent pads saturated with urea for 15 minutes. Yellow colonies were transferred from mTEC plates onto Rainbow® Agar plates (Biolog 1994) and incubated at 35^{O}C for 18-24hr to obtain pure cultures. Rainbow agar was used as it is a selective medium for *E. coli* and differentiates between some strains. Transfers were made as

needed to obtain pure cultures. One to five isolates (colonies showing a colored hue (i.e. blue, purple, magenta) on Rainbow Agar) from each sample, with the exception of a few animal sources, e.g. ducks for which more than 5 were isolated, were swabbed for maximum growth onto Biolog™ Universal Growth plates (BUG-B) and incubated at 35^{O}C for 24h. A turbidity of 61% ± 2 % was achieved before inoculating GN2 Microplates™. The plates were incubated for 24h at 35^{O}C. Each isolate was confirmed as *E. coli* using MicroLog™ Microbial Identification System (Biolog, Inc., 3938 Trust Way, Hayward, CA 94545) following the MicroLog™ System Release 4.0 User Guide (Biolog, 1999). Initial isolates were identified using the Biolog™ MicroLog System (manual readings) (Biolog 1999). The remaining isolates (from the November sampling event) were identified with a semi-automated MicroStation Microbial Identification System (MIS) with MicroLog Software. The upgrade was made possible due to availability of additional funding. The semi-automated system allowed faster processing of isolates as each well reading was not required to be entered manually, but is essentially the same method as confirmations of *E. coli* continue to be based on the well readings. Thus, bias was not introduced and the confirmations should be equivalent. Samples were stored temporarily on Tryptic Soy Agar (TSA) slants, transferred directly from BUG-B plates in order to maintain pure cultures between various analyses. Verified isolates were stored permanently in a -80°C freezer.

Only isolates that were confirmed as *E. coli* (≥90%) were included in the subsets used to develop the ARP (Antibiotic Resistance Profiles) and PFGE (Pulse Field Gel Electrophoresis) libraries. Table 4 shows the number of isolates from each animal analyzed by ARA and PFGE. November collections (11/22/04) from deer, javelina and coyote were

Table 4. Numbers of known source *E. coli* isolates, verified and analyzed for Copano Bay area fecal sample collections (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Animal source	**Collection dates**	**# samples**	**# isolates**	**# verified (Biolog™)**	**# ARP completed**	**# PFGE completed**
Cow	02/23/04-03/08/04	27	105	76	51	45
Duck	12/27/03-01/13/04	8	85	34	34	27
Horse	04/28/04	20	59	37	37	23
Sewage/ Human	02/07/04-02/10/04	5	149	110	99	95
Cow	11/22/04	26	168	85	66	0
Duck	11/22/04	42	214	34	75	0
Horse	11/22/04	24	106	97	79	0
Sewage/ Human	11/22/04	64	208	94	46	0
Deer	11/22/04	9	33	0	0	0
Javelina	11/22/04	3	17	0	0	0
Coyote	11/22/04	8	34	0	0	0

not analyzed by ARA or PFGE, and only a subset of cow, duck, human/sewage and horse isolates were analyzed by ARA as anticipated additional funding was not available. PFGE was only used to analyze a subset of the spring fecal isolates as per the QAPP (due to cost constraints). None of the November isolates were analyzed by PFGE. Wildlife isolates, including deer, coyote and javelina were included in the final library, from an existing TAMU-CC library (see later section). Isolates which were not confirmed by the MicroStation MIS were either closely related species or did not confirm at a ≥ 90%. Sufficient isolates were confirmed to provide a database that exceeded the number of isolates from each animal originally proposed. The most common species identified by the Biolog Microbial Identification System (MIS) other than *E. coli* included *Enterobacter intermedius, Salmonella* spp*., Leclercia adecarboxylata, Buttiauxella izardii, Buttiauxella agrestis, Klebsiella oxytoca, Rahnella aquatilis, Enterobacter aerogenes, Serratia odorifera*, and *Raoutella terrigena*. A sample print out from the MIS is included in the Appendix. The MicroStation MIS hard copies are stored at Texas A&M University-Corpus Christi.

ANTIBIOTIC RESISTANCE ANALYSIS

The analytical procedures for antibiotic resistance profiling followed the standardized Kirby Bauer Disk Diffusion method with a panel of 20 antibiotics (NCCLS 2000, 2002a, 2002b) (Table 5). Performance Standards for Antimicrobial Disc Susceptibility Tests, Approved Standard-Seventh Edition, NCCLS document M2-A7 (2000); NCCLS (2002a) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Approved Standard-Second Edition NCCLS document M31-A2, and NCCLS (2002b) Performance Standards for Antimicrobial Susceptibility Testing, Twelfth

Table 5 Antibiotics used to develop antibiotic resistance profiles for *E. coli* isolates from the Copano Bay, TX watershed 2003-2004.

Antibiotic	**Abbreviation**	**Concentration**
Ampicillin	AMP	10 µg
Augmentin	AMC	30 µg
Cefazolin	CZ	30 µg
Cefotaxime	CTX	30 µg
Ceftazidime	CAZ	30 µg
Ceftriaxone	CRO	30 µg
Chloramphenicol	C	30 µg
Ciprofloxacin	CIP	5 µg
Doxycycline	D	30 mg
Enrofloxacin	ENO	5 µg
Gentamicin	GM	10 µg
Imipenem	IPM	10 µg
Kanamycin	K	30 µg
Nalidixic acid	NA	30 µg
Neomycin	N	30 µg
Spectinomycin	SPT	100 µg
Streptomycin	S	10 µg
Sulfamethoxazole Trimethoprim	SXT	23.75/1.25 µg
Sulfisoxazole	G	0.25 mg
Tetracycline	Te	30 µg

Informational Supplement, and NCCLS document M100-S1, Methodology and Quality Controls. The BIOMIC® system was used for an instantaneous reading of zones of inhibition and interpretation following NCCLS M100 (2002b). This system calculates antibiotic minimum inhibitory concentrations (MICs) and records zone diameters automatically from the standard disk diffusion method. BIOMIC® also determines whether each isolate is resistant, intermediate or susceptible (R-I-S) based on published NCCLS guidelines (Table 6). The automated image analyzer ensured uniformity for future comparisons with *E. coli* isolates from unknown sources as detailed in the TAMU-CC SOP following NCCLS (2002a) as approved in a previous QAPP (2003) "Development of an *E. coli* bacterial source tracking library and assessment of bacterial sources impacting Lake Waco and Lake Belton" prepared for the Texas State Soil and Water Conservation Board by Parsons, Texas A&M El Paso Agricultural Research and Extension Center, TAMU and TAMU-CC.

Duplicates were included for 10% of the isolates. The quality control strains were *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *E. coli* ATCC 25922. Controls were run with each batch of samples or weekly for each new lot number of media or antibiotics. The image analysis system included EXPERT© software which checks quality control, test results and unlikely results. This method has proven to improve reading consistency and speed thereby minimizing technologist variation.

The database is stored in the BIOMIC system computer with back-ups saved in the hard drive and on CD-ROM. A sample print-out showing the results for one isolate is included in the Appendix. The databases are stored on the CD-ROM enclosed with this report. The complete set of print-outs is stored at Texas A&M University-Corpus Christi.

Table 6. Susceptible (S), Intermediate (I), and Resistant (R) ranges (mm) for *E. coli* using the BIOMIC® Microbiology Analyzer System.

Antibiotic	**S**	**I**	**R**
AMP	≥ 17	14-16	≤ 13
AMC	≥ 18	14-17	≤ 13
CZ	≥ 18	15-17	≤ 14
CTX	≥ 23	15-22	≤ 14
CAZ	≥ 18	15-17	≤ 14
CRO	≥ 21	14-20	≤ 13
C	≥ 18	13-17	≤ 12
CIP	≥ 21	16-20	≤ 15
D	≥ 16	13-15	≤ 12
ENO	≥ 21	16-20	≤ 15
GM	≥ 15	13-14	≤ 12
IPM	≥ 16	14-15	≤ 13
K	≥ 18	14-17	≤ 13
NA	≥ 19	14-18	≤ 13
N	≥ 17	13-16	≤ 12
SPT	≥ 18	15-17	≤ 14
S	≥ 15	12-14	≤ 11
SXT	≥ 16	11-15	≤ 10
G	≥ 7	NA	≤ 6
TE	≥ 19	15-18	≤ 14

PULSE FIELD GEL ELECTROPHORESIS

PFGE analysis followed published standard Bio-Rad Methodology and Standards as described in Bio-Rad Laboratories (1995) (CHEF-DR III Pulsed Field Electrophoresis Systems: Instruction Manual and Applications Guide. Hercules, California).

Pulse field gel electrophoresis was used to obtain 'fingerprints' for 190 known source isolates from the first fecal sources collected in the Copano Bay watershed, following the procedure approved in the QAPP.

DNA was extracted, cut with the restriction enzyme *Not* I, embedded in agarose, and fingerprinted. After processing and running the DNA plugs for 20 hours in a CHEF-DRI III Gel Electrophoresis Unit (Bio-Rad, Hercules, CA), the gels were stained with Ethidium Bromide, destained in double deionized distilled water with 1% TBE, and then photographed using the Gel-Doc System (Bio-Rad (Hercules, CA). A minimum of two photographs were printed and digital images for analysis with Quantity One (Bio-Rad, Hercules, CA) were created. For analysis, a copy of the original digital image was created and lanes are established on the image. All samples that yielded distinct bands along with the standard had lane overlays traced on them and were adjusted for any curvatures. The lanes extended from the plug well to the bottom of the gel. The background of each gel was subtracted and all lanes were subjected to a Guassian curve to help establish banding patterns. The gels were imported into the database and a band set was assigned to all of the isolates. The first band in the set was based on the first band of the standard, lambda, and the subsequent bands were based on the software's assignment, there were 60 different band positions determined. All gels and lanes were visually inspected and bands were adjusted to eliminate software errors due to abnormalities and fragments (Duck et al. 2003, McLellan et al. 2003). Once the bands

were assigned a number, they were referenced by the original isolate identification. Each of the unknown isolates was run against the entire database and the known with the most similarity was used to determine the identification of the source of the unknown. The similarity index was automatically calculated by the software as a function of the number of bands the compared isolates had in common divided by the number of bands in each isolate lane (Singer et al. 2004). The percent of similarity ranged from 0 (no bands in common) to 100% (all bands in common). The 100% similarity was from isolates matching itself, although there were isolates from different sampling station and events that were identical. Strains of *E. coli* are known to persist in the environment, so it is not unusual to have the same pattern from different sampling events and stations. The band types were compared as unweighted, as weighting the results compares the relative brightness of the band, which can be highly variable from gel to gel and even among lanes on the same gel. Numerous studies have analyzed their data using unweighted methods (Duffy et al. 2005, Singer et al. 2004). Unweighted band analysis resulted in only the position and number of bands in the lanes being compared to determine their percent similarity. The unknown isolates were classified by identifying which of the known sources shared the most similarity. Isolates which did not show similarity with library isolates were classified as 'no match'. The results of the PFGE were then compared with the results of the ARA analyses.

b) Unknown source samples (water samples)

Proper sample handling and custody procedures ensured the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. A sample is in custody if it is in actual physical possession or in a

secured area that is restricted to authorized personnel. COC forms were used to document sample handling during transfer from the field to the laboratory and among contractors.

Fourteen of the stations in Copano Bay/Mission Bay currently monitored for water quality by TDH were included in the study. These stations were selected by TDH, based on TDH sanitary surveys and historical fecal coliform data (Fig. 22; Table 7).

The water samples were collected by TDH field personnel between October 2003 and May 2004, from fourteen stations, during eight sampling events, dates dependent on factors such as weather (rainfall), following standard TDH procedures as detailed in the TDH SOP (QAPP Appendix). TDH field personnel notified TAMU-CC environmental microbiology personnel prior to each collection. TAMU-CC provided sterilized polypropylene screw cap, 500 ml sterile plastic collection bottles for each event. Samples were collected in immediate succession, at each station, leaving ample air space in each bottle for shaking, in accordance with Section 9000 Standard Methods for the Examination of Water and Wastewater, 20th ed., 1998 (APHA, 1998). One sample was used by TDH for fecal coliform analysis and the second and third (two bottles) were transported to TAMU-CC for bacteria source tracking (BST) analyses. An additional temperature blank was taken to the collection site and transported to the laboratory with the sample water bottles. TDH field staff placed water samples, including the temperature blank, in an ice chest with ice packs and transported samples to the TAMU-CC Environmental Microbiology Laboratory for analyses, immediately after collection (TDH SOP).

A standard TDH water sample collection data sheet was filled out for each station. The date, time and analyst signature were recorded for each sample collection, microbiological isolation and molecular analysis to maintain chain of custody.

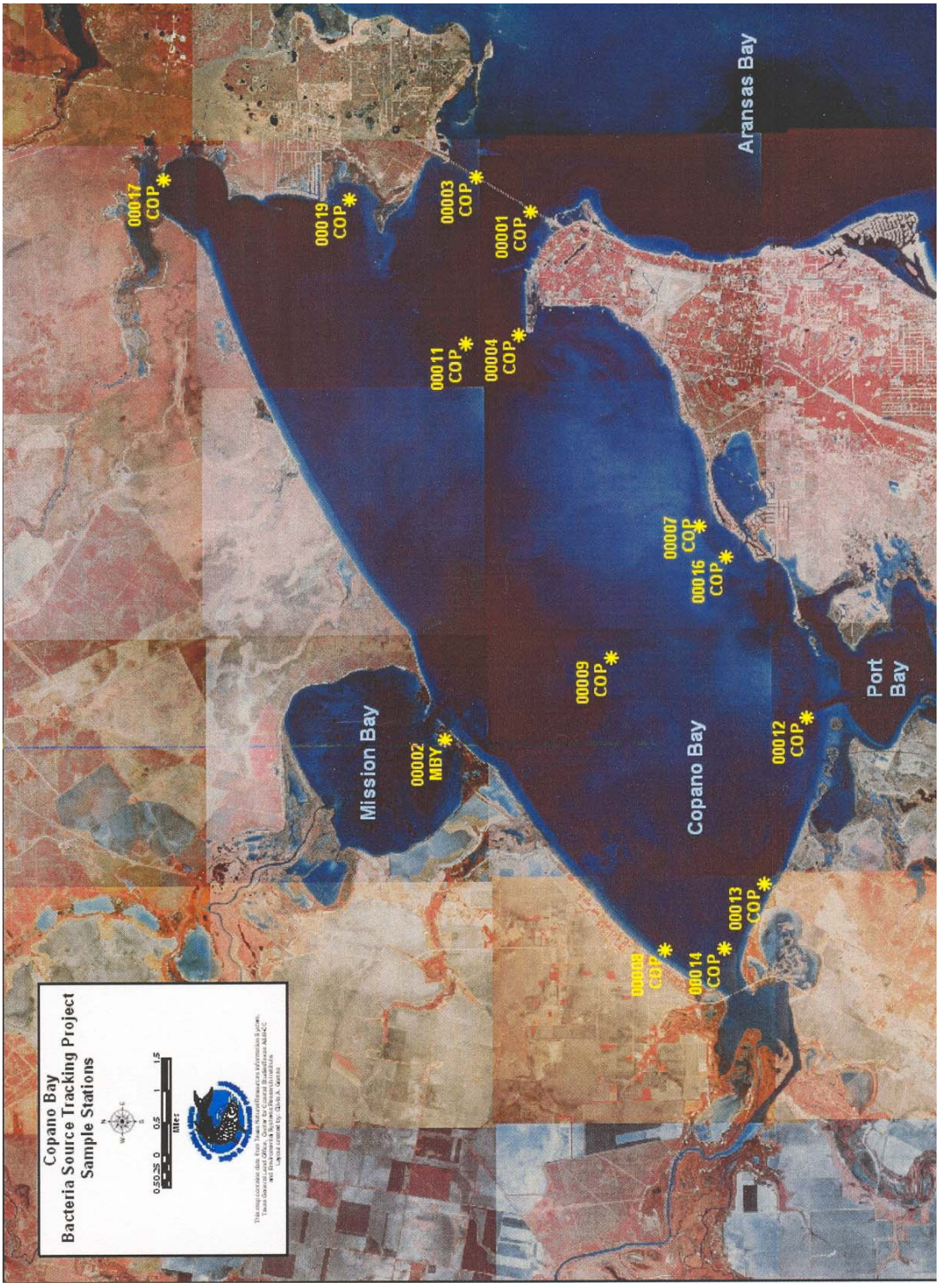


Figure 22. Copano Bay, TX sampling stations (QAPP).

Table 7. Estuarine water quality survey stations sampled for this study. Texas Department of Health, Seafood Safety Division.

Bay Code	Station Number	Latitude	Longitude	Station Descriptions	TNRCC Code
COP	00001	28°06.994	97°01.390	South End of Hwy 35 Causeway	14779
COP	00003	28°07.749	97°00.857	North End of Pier Reef	14780
COP	00004	28° 07.201	97°03.232	Redfish Point	14781
COP	00007	28°04.853	97°06.200	800 Yards Northeast of Salt Lake	14782
COP	00008/	28°05.404	97°12.567	1 Mile East of Bayside	14783
COP	00009	28°06.039	97°08.165	End of Shell Bank Reef	14784
COP	00011	28°07.924	97°03.356	1.5 Miles Northwest of Hwy 35 Causeway	14785
COP	00012	28°03.476	97°09.095	1 Mile West of Rattlesnake Point	14786
COP	00013	28°04.086	97°11.617	1.25 Miles Southeast of Bayside	14787
COP	00014	28°04.621	97°12.579	0.5 Miles South of Bayside	14788
COP	00016	28°04.493	97°06.636	400 Yards North of Lone Tree Point	14790
COP	00017	28°11.874	97°00.811	800 Yards Southeast of Turtle Bay	14792
COP	00019	28°09.420	97°01.120	300 Yards West of Palmetto Point	14793
MBY	00002	28°08.300	97º09.36	South of Mission Bay	14797

The appropriate field data and COC forms were completed prior to samples being returned to the laboratory.

Laboratory analyses commenced immediately once samples were received at the laboratory. The six-hour requirement for quantitative analysis of *E. coli* from time of collection was not always met due to distance from sample stations to analytical laboratory. COC forms included this information. For this study the method was only to be used to obtain isolates, quantification of *E. coli* did not form part of the data. The date, time, and analyst signature was recorded for each sample collection, filtration and colony count to maintain chain of custody. Five hundred ml water from each station was processed immediately on arrival at TAMU-CC laboratory. Surplus water was stored at 4 C, for up to 24 hr. In cases where insufficient (<30) isolates per station were obtained from the 500 ml, some or all of the surplus water was filtered to obtain additional isolates. This was documented.

Water samples were analyzed for *E. coli* using EPA Method 1103.1: the original *E. coli* method (Dufour et al. 1981), introduced by EPA in 1986 (USEPA, 1986) as described in Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli* (2000) EPA/821/R-97/004 and following procedures and quality control methods outlined in Standard Methods for the Examination of Water and Wastewater, 20th ed., 1998. For this project the method was used only to obtain isolates not for quantification of *E. coli* in the water samples, due to the distance between sample collection stations and analytical laboratory.

For each water sample varying volumes (10, 30, and 100 ml) were filtered onto 0.45 micrometer cellulose nitrate filters. As concentrations of bacteria were unknown, different

volumes were utilized to ensure filters with individual colonies were obtained from which isolates could be transferred. Up to 500 ml water was filtered for each water sample, based on specific station historical fecal coliform data. Every effort was made to isolate the required number of isolates. However, it should be noted that in some instances the bacteria were not present in sufficient concentrations to achieve this objective. In such cases, the volume filtered and the number of isolates obtained were recorded and analyses proceeded using those isolates

Filters were placed onto mTEC agar plates, incubated, isolates transferred to Rainbow Agar plates to obtain pure cultures and verified using the MicroLog™ Microbial Identification System as previously described for known source isolates. Cultures were maintained on Tryptic Soy Agar (TSA) slants. Isolates were stored permanently in duplicate at -70^{O}C. MicroLog MIS data is stored as hard copy at TAMU-CC.

ANTIBIOTIC RESISTANCE PROFILING/ PULSE FIELD GEL ELECTROPHORESIS

Water (unknown) isolates were analyzed as described in the preceding section on known source isolates. Originally, a subset of the isolates analyzed by antibiotic resistance was to be analyzed (10 per station per event). However, due to the lack of isolates from some stations/events the design was modified to achieve an overall number equivalent to that originally proposed, but additional isolates were analyzed from events where high numbers of *E. coli* were isolated, to compensate for the reduced numbers from other stations/events.

c) Quality control

Accuracy (a statistical measurement of correctness including components of systemic error) was verified through the analysis of laboratory control standards, and blank samples. These controls are incorporated into each analysis utilized in this study, as per publications cited.

The precision of laboratory data is a measure of the reproducibility of a result when an analysis is repeated. It is strictly defined as a measure of the closeness with which multiple analyses of a given sample agree with each other. Precision is assessed by replicate analyses laboratory control standards or sample/duplicate pairs in the case of bacterial analysis.

A temperature blank was included with each ice chest used in sample collection to check that temperature remained within acceptable range. Field splits were not used for the water samples as this part of the project did not involve quantification. Quality control for *E. coli* isolations followed USEPA. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *E. coli*. EPA-821-R-97-004.

Intralaboratory quality assurance/quality control was based on guidelines in Standard Methods for the Examination of Water and Wastewater, 20th ed., 1998 Section 9020 B (Appendix E). Control cultures were selected from Table 9020:V (APHA 1998) for positive and negative controls. Each medium lot was tested for satisfactory performance using ATCC strains of *E. coli* (positive control). Each medium preparation included testing of the medium using both a positive and a negative control (*E. coli* and *Enterobacter aerogenes*, respectively). A media log sheet showing date, medium, volume, signature and comments was kept for all media prepared. Measurement of method precision was followed as

described in Section 9020 B. 8 Analytical quality control procedures, b. (APHA, 1998). All inoculated plates, tubes, broths etc. were autoclaved in biohazard bags with indicator tape, for at least 30 minutes (121 °C) prior to disposal. Quality control for the ARA and PFGE is detailed in the QAPP as part of the protocol. ARP followed NCCLS Performance Standards (2000, 2002a, 2002b).

Control limits for laboratory control standard/laboratory control standard duplicates are specified in software associated with each technique to be used – MicroLog™ Microbial Identification System provides a % similarity of each isolate with known bacteria in the Biolog database, BIO-MIC® (for ARP analysis) follows NCCLS standards, which includes specifications for duplicate analyses, and PFGE software. A database was created with Diversity Database (Bio-Rad, Hercules, CA) and all samples (both known and unknown) were analyzed based on the standard lambda (Bio-Rad, Hercules, CA). Lambda ladders are frequently used as standards to normalize PFGE patterns for comparison between different gels (Duffy et al. 2005, Lu et al. 2004).

Instrument/equipment was inspected and tested upon receipt and was assured appropriate for use. Initial acceptance occurred at TAMU-CC Central Receiving by a designated employee who receives and signs for the materials. Packages and their contents were reviewed to ensure that the shipment is complete. Items were then delivered to the appropriate analyst or manager. A second inspection was conducted by the NRS or PIs during which the equipment was tested following manufacturer's instructions to ensure equipment meets specifications. All laboratory instruments/equipment used for preparing media and buffered dilution water, sterilization, and incubation was inspected and maintained according to manufacturer specifications and based on Standard Methods Section 9020 B.3

and 9030 B. Equipment includes autoclaves, incubators, refrigerators, freezer, balance, pH meter, membrane filtration equipment, thermometers, double distillation water unit, media dispensing apparatus, centrifuges, safety cabinet, water bath, microscopes, UV lamp, spectrophotometer, Pulsed Gel Electrophoresis Unit, computers, BIO-MIC automated plate reader system, pipettes, bunsen burners, dilution bottles, and sample bottles. Spare parts, such as lamp bulbs, are kept available to prevent downtime.

Instruments requiring calibration were the pH meter, spectrophotometer, Pulsed Field Gel Electrophoresis Unit, incubators, BIO-MIC system, thermometers, pipettes, and balances. The pH meter was calibrated prior to each use using standards at pH 7 and 10. A pH meter calibration log sheet showing date of calibration, standards used and signature of analyst was kept. Instrument technicians on a regular basis checked autoclaves. Autoclave performance was verified monthly following Standard methods 9020 B. Intra-laboratory quality control guidelines (APHA, 1998). Biological safety cabinets are certified annually. The Project Coordinator keeps records of all checks, certifications and performance tests. All incubators were checked daily when in use and log sheets were kept showing time and date, recorded temperature and analyst signature. Spectrophotometer, balances, BIO-MIC and Pulsed Gel Electrophoresis Unit were calibrated prior to each use following manufacturer instructions. All calibration and maintenance activities were recorded on the instrument calibration forms. These sheets are kept on file in the TAMU-CC Environmental Microbiology Laboratory.

Confidence in the comparability of data sets from this project to those for similar uses is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as

described in the QAPP and project SOPs. Comparability is also guaranteed by reporting all data for evaluation by others.

Final acceptance was performed by the PIs. Any results not meeting requirements were omitted from the data analysis and conclusions were not made based on this data. These omissions were documented in the Progress Reports submitted to TGLO and CBBEP Project Managers.

d) Data management and analysis

Data collection began with the collection of field samples. All samples were recorded in field log sheets by hand. Samples analyzed in the laboratory generated the next level of data. This data was recorded on data sheets, taken by hand and proof read. Proof reading in both cases involved a 100% check of each handwritten number. This final report includes the results of the antibiotic resistance and PFGE analysis as Excel or SPSS spreadsheets on a CD-ROM. Statistical analyses are summarized in tables and figures.

Antibiotic resistance profile data was produced as electronic data and printouts from the BIOMIC software. Data was transferred electronically to SPSS spreadsheets for statistical analysis. All transfer of data from one format to another was proof read separately by two lab personnel. Zone diameters were analyzed using discriminant analysis. After considerable assessment and evaluation of the library and potential animal sources in the watershed, known source isolate groupings were developed for use in statistical analysis of the unknown source isolates. The known source isolates were analyzed by two- (human/sewage vs. non-human), four- (human/sewage, cow, horse, wildlife) and six-way (human/sewage, horse, cow, duck, gull, wildlife) analyses. Isolates in existing libraries from

the area considered representative of animal sources in the Copano watershed were added to the library, to provide a larger database, as anticipated funding for additional known source collections were not available. The library was tested for representativeness, cross-validated and challenged with known isolates not included in the library. Unknown source isolates were then compared with the known source library to determine into which known source group each isolate could be classified. Additional assistance in the statistical analyses of the antibiotic resistance profiles was provided by April Judd, University of Northern Colorado.

Pulse Field Gel Electrophoresis database construction began with a single gel image of each selected source. Building of the database continued as each image was evaluated by identifying and matching the unique bands in each sample of that gel called band types. Band types are used to link samples across gels. Each unique band type is defined by its position and molecular weight isoelectric point. Gel images and isolates were added to the database and the list of band types increased. Every band in every gel in the database is identified as a particular band type. Band types are grouped together into band sets; a band set includes all the band types that were created using the same enzyme. This modeling is required of each isolate and each band of the entire database both known and unknowns. The gel images are linked to other gels by band sets within a database file. The database can undergo a variety of searching and population comparison tools to analyze the gel images in detail. The software (Diversity Database) supports single lane and multilane sample definition as well as phylogenetic tree analysis. Each animal has a unique set of bands for each of the lanes of restriction enzyme-cut DNA. The information includes the following:

1. A digital representation of the lane for the source organism with bands indicated as a bar and each numbered from top to bottom.

2. A graphic display that includes the band information with Background Subtracted. Background noise is removed from the lanes by a "Rolling Disk" Method which refers to a hypothetical disk that follows the contour of a lane's profile trace, removing different intensities along the length of the lane. The amount of background removed is determined by the size of the disk chosen. A large disk will follow the profile trace less closely, touching fewer points along she trace and identifying less background. A smaller disk will more closely follow the profile trace, thus identifying more background. When the “Rolling Disk” background subtraction is applied, the lane trace display will change but the image will not reflect the change in background intensity. This is useful when only small amounts of DNA are present in a band and it would otherwise be difficult to discern by the human eye.

3. The Rf (Relative front) method was used for locating the relative positions of bands in lanes. Relative front is calculated by dividing the distance a band has traveled down a lane by the length of lane (Follow Lane). This is useful if the gel image is curved or slanted. Bands in the gel image are marked with a dash at the center of the band. When a band is quantitated, the average intensity value of each horizontal of pixels within the brackets is calculated. Next, the number of pixel rows between the top and bottom brackets is determined. Taken together, these result in an intensity profile for each of the bands.

The 1-D Analysis Report displays all the advanced analysis data (including band types, normalized quantities, etc.) for all the lanes on a gel image. The lanes are ranked in similarity to the lane initially selected to generate the report. A search of isolates in each database was completed using the Jaccard Coefficient Method. Searches use one of two primary Search Strategies: lane similarity or band set membership. Similarity searches allow selection of a lane in a gel and specify the degree of similarity by which other lanes must

match the lane chosen. The Population and Image Report displays a series of lane diagrams of the population, sorted in an order of decreasing similarity from the reference sample for the similarity-searched populations. In addition, a Similarity Matrix can be produced for evaluation. Phylogenetic trees are schematic representations of lane similarity. Cluster analysis produces different varieties of phylogenetic trees that are available in Diversity Database. Phylogenetic trees were computed and the numbers of cluster sets were evaluated. The display is used as a visual indication of the compactness of each cluster and the dissimilarity of each cluster. A Complete Linkage (also known as Furthest Neighbor or Maximum Methods) cladogram using Jaccard Coefficient Method produces good algorithms for indicating outlier clusters. These cladograms were generated for each data set of knowns to determine the number of clusters of closely matching sample bands. A representative isolate was derived from each cluster that resulted from cladistical analysis of each set of known isolates. Each of these isolates was then run against the entire database of unknowns from Copano Bay.

The completeness of the data is basically a relationship of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to accidents, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it was a general goal of the project(s) that 90% data completion was achieved.

Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions. The representativeness of the data is dependent on 1) the sampling locations, 2) the number of samples collected, 3) the number of years and seasons when sampling is performed, 4) the number of depths sampled, and 5) the sampling

procedures. Site selection and sampling of all pertinent media (water, fecal samples) and use of only approved analytical methods will assure that the measurement data represents the conditions at the site. The goal for meeting total representation of the water body is tempered by the availability of time and funding. Representativeness was measured with the completion of samples collected in accordance with the approved QAPP.

RESULTS AND DISCUSSION

A general goal of the project was to achieve 90% completeness. In terms of total numbers of isolates used in the project this goal was met. However, as per quarterly reports, and noted in the methods section, very low numbers of *E. coli* were found at specific stations and for certain events. Thus, the distribution of isolates was not uniform across all stations and events as had been originally proposed. The total number of isolates analyzed exceeded the goal of 90% completeness.

Known source isolates - library development

ANTIBIOTIC RESISTANCE ANALYSIS

The library for this project was developed using a combination of previously characterized *E. coli* isolates from known sources in the Coastal Bend area (608 isolates) with the addition of isolates collected from the Copano Bay watershed during the project (450 isolates). The main goal was to develop a library that included isolates from sources that were considered as potential significant contributors of fecal pollution to the Copano Bay watershed, based on TDH sanitary surveys.

A total of 450 *E. coli* isolates obtained from known sources in the Copano Bay watershed from Spring and Fall (November) 2004 were characterized by their antibiotic resistance profiles. Additional isolates from the November 2004 collection of fecal samples were stored, but were not analyzed due to funding constraints. Zone of inhibition diameters and Susceptible-Intermediate-Resistant values were recorded for each isolate using the BIOMIC image analysis system. The zone diameters were compiled into a library of known

sources to be analyzed using discriminant analysis with SPSS ® Version 12.0 for Windows. The numbers of isolates for each source are shown in Table 4. The isolates from Copano Bay were augmented with isolates from the Coastal Bend area in an existing library at TAMU-CC to obtain a final library of 1,058 isolates as described below.

The human (sewage) isolates from Copano Bay watershed consisted of 145 isolates from both the earlier (spring 2004) and later (November 2004) collections of composite sewage samples. The existing TAMU-CC library isolates from portable toilet and volunteers were not considered as representative of the potential human contamination sources in Copano Bay; additionally their antibiotic resistance profiles differed from the Copano Bay sewage isolates. They were not included in the Copano Bay library. The isolates comprising the human source database for the Copano Bay library were therefore all sewage isolates from the Copano Bay watershed collected during the project period.

The initial known Copano Bay *E. coli* nonhuman isolates (collected spring 2004) consisted of 37 horses, 51 cows, and 34 ducks. November 2004 collections included fecal samples from ducks (different species from earlier collection), cows, sewage, coyotes, deer and javelina (Table 3). As stated above, funding was not available to analyze all these isolates. A total of 220 isolates from November were analyzed (66 cow, 79 horses, 75 duck, but no coyote, deer or javelina) and added to the spring collections database. The non-human source database was expanded with isolates from the existing *E. coli* library at TAMUCC (including deer, javelina and coyote) (Table 8). The additional isolates were carefully evaluated before addition – sources and locations of fecal sample collections were checked for applicability to the Copano Bay watershed area. Cow (119) and gull (110) isolates were added from 2003 collections. Wildlife isolates from an earlier study were grouped to provide

Table 8. Fecal samples included in the Copano Bay study antibiotic resistance profile library, from existing TAMU-CC libraries.

Sampling Date	Source	Geographical Location	# Isolates
8/2000-5/2001	Javelina	Aransas Wildlife Refuge, Victoria Zoo	11
8/2000-5/2001	Opossum	TAMUCC campus, Corpus Christi Animal Control	15
8/2000-5/2001	Bobcat	Welder W.R., Victoria Zoo	10
8/2000-5/2001	Deer	Padre Island National Seashore, Welder WR, Aransas WR, Lake CC	18
8/2000-5/2001	Feral Hog	Lake Corpus Christi, Aransas WR, Welder WR	6
8/2000-5/2001	Gray Fox	TAMUCC campus, Victoria Zoo	6
8/2000-5/2001	Coyote	Welder WR, Padre Island Nation Seashore, Victoria Zoo	17
8/2000-5/2001	Coot	Texas State Aquarium	6
8/2000-5/2001	White Pelican	Corpus Christi	12
8/2000-5/2001	Cormorant	Univ. of Texas, Port Aransas, Laguna Madre	12
8/2000-5/2001	White Ibis	Corpus Christi	6
8/2000-5/2001	Rock Dove	TAMUCC campus, Corpus Christi area	9
8/2000-5/2001	Raccoon	TAMUCC campus, Corpus Christi	18
8/2000-5/2001	Brown Pelican	Port Aransas, Texas St. Ap., Laguna Madre	16
8/2000-5/2001	Skunk	Corpus Christi Animal Control	6
8/2000-5/2001	Gull	Corpus Christi Bay Front, Port Aransas	17
8/2000-5/2001	Duck	Corpus Christi, Corpus Christi Zoo	9
6/17/2004-7/1/2004	Horse	Addicks Reservoir/Buffalo Bayou (Manure Pile), (Grass Feed Lot)/	194
6/17/2004-7/1/2004	Cow	Annaville TX and Macallen TX, Pasture cows –Corpus Christi	119
5/22/2003-11/19/2003	Gull	Sea Wall, Malaquite Beach, Cole Park, Corpus Christi Beach,	110

a wildlife category of 168 isolates from a range of animals including deer, coyote, raccoon, javelina, opossum, feral hogs, various birds etc. Many of these had been collected at the Welder Wildlife Refuge or Aransas Wildlife Refuge, both located in the Copano Bay area. These isolates were added to represent other possible sources that may be contributing to fecal pollution in the Copano Bay watershed. A final evaluation of the database showed a discrepancy in antibiotic resistance between horses from the spring collections and fall collection from the Copano Bay area. The spring horse fecal samples had been collected from local fairgrounds and isolates showed high levels of antibiotic resistance compared with the isolates from horse samples collected in November from rangeland in the Welder Wildlife Refuge. The spring collections horse isolates were not included in the final Copano Bay library. Additional horse isolates from rangeland in the Houston area, part of another TAMU-CC library, were shown to have similar antibiotic resistance profiles to those from the Copano Bay area and were added to the library. Other source isolates from the Houston area differed in profiles from the same animal source isolates in Copano Bay and were not included in the library.

Duck fecal samples were collected in the winter of 2003/2004 from Copano Bay and then the following November. In the two- and four-way analyses of the data an additional nine duck isolates of other species were included from the TAMU-CC library as part of the non-human, or wildlife category. These were not included in the six-way analysis, where ducks were a separate category, as these earlier duck isolates were isolated from unidentified duck species from the local zoo, not from the Copano Bay watershed.

The final library comprised 1,058 isolates (1,067 for two- and four-way analyses) (Table 9), and isolates were grouped as human (sewage), cow, horse, duck, gull and wildlife.

Table 9. Fecal sample isolates included in the final Copano Bay antibiotic resistance profile library.

	Copano Bay winter/ spring	Copano Bay Fall	TAMU-CC	TAMU-CC	TAMU-CC	**2/4-way analysis**	**6- way analysis**
YEAR	2004	2004	2003	2003	2001		
Human	99	46	0	0	0	**145**	**145**
Cow	51	66	0	119	0	**236**	**236**
Horse	0	79	194	0	0	**273**	**273**
Duck	34	75	0	0	(9)	**118**	**109**
Wildlife	0	0	0	0	168	**168**	**168**
Gull	0	0	0	110	17	**127**	**127**
TOTAL	**184**	**266**	**194**	**229**	**(194) 185**	**1067**	**1058**

STATISTICAL ANALYSIS OF THE KNOWN SOURCE LIBRARY

The database forming the Copano Bay library of known sources was analyzed using discriminant analysis with SPSS® Version 12.0 for Windows. The antibiotic resistance profiles (zone diameter) for all isolates were compiled to form databases in SPSS7 for Discriminant Analysis. Discriminant analysis is a multivariate technique that can be used to classify items into categories based on a set of test variables (Huberty, 1994). The rates of correct classification (RCC) for each source can be used to evaluate the predictive capabilities of the database.

The zone diameter database of isolates, with 20 antibiotics was analyzed in a variety of ways, including various groupings of the isolates from different sources. Analysis was conducted on the data from all the antibiotics. Two-way (human vs. non-human), four-way (human vs. cow vs. horse vs. wildlife) and six-way classifications (human vs. cow vs. horse vs. duck vs. wildlife vs. gull) (Tables 10 to 12), were completed to determine average rates of correct classification (ARCC). This is the average of the rates of correct classification (RCC) for each group. As classifications based upon the "cases" (isolates) used to create the model tend to be too "optimistic" in the sense that their classification is inflated, cross-validation was performed to reduce this by classifying each case while leaving it out from the model calculations (leave-one-out method). The closeness of the cross-validation ARCC to the original ARCC provides an indication of the representativeness of the library. The average rate of correct classification (ARCC) for two-way analysis was 72% with 80% of human source isolates correctly classifying.

The cross-validation ARCC was 71%. For four-way classification ARCCs were 64% and 62.1% respectively, with 71% sewage (human) isolates correctly classified. Six-way

Table 10. Discriminant Analysis of the known source *E. coli* isolates in the Copano Bay study library. Two-way classification – Human/sewage vs. Nonhuman (all groups equal). Note: "Original" is the discriminant analysis using all the isolates. "Cross-validated" (also known as resubstitution analysis or leave-one-out method) refers to an analysis performed where each isolate is removed one at a time and classified based on the library of remaining isolates.

Classification Results[b,c]

			Predicted Group Membership		
		Species	Human	Nonhuman	Total
Original	Count	Human	116	29	145
		Nonhuman	269	653	922
	%	Human	80.0	20.0	100.0
		Nonhuman	29.2	70.8	100.0
Cross-validated[a]	Count	Human	112	33	145
		Nonhuman	275	647	922
	%	Human	77.2	22.8	100.0
		Nonhuman	29.8	70.2	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 72.1% of original grouped cases correctly classified.

c. 71.1% of cross-validated grouped cases correctly classified.

Table 11. Discriminant Analysis of the known source *E. coli* isolates in the Copano Bay study library. Four-way classification: Human/sewage vs. Cow vs. Horse vs. Duck (all groups equal).
Note: "Original" is the discriminant analysis using all the isolates. "Cross-validated" (also known as resubstitution analysis or leave-one-out method) refers to an analysis performed where each isolate is removed one at a time and classified based on the library of remaining isolates.

Classification Results[b,c]

			Predicted Group Membership				
		Species	Cow	Horse	Sewage	Wildlife	Total
Original	Count	Cow	118	34	58	26	236
		Horse	23	183	61	6	273
		Sewage	10	27	103	5	145
		Wildlife	33	45	56	279	413
	%	Cow	50.0	14.4	24.6	11.0	100.0
		Horse	8.4	67.0	22.3	2.2	100.0
		Sewage	6.9	18.6	71.0	3.4	100.0
		Wildlife	8.0	10.9	13.6	67.6	100.0
Cross-validated[a]	Count	Cow	111	36	60	29	236
		Horse	23	179	62	9	273
		Sewage	10	29	101	5	145
		Wildlife	35	48	58	272	413
	%	Cow	47.0	15.3	25.4	12.3	100.0
		Horse	8.4	65.6	22.7	3.3	100.0
		Sewage	6.9	20.0	69.7	3.4	100.0
		Wildlife	8.5	11.6	14.0	65.9	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 64.0% of original grouped cases correctly classified.

c. 62.1% of cross-validated grouped cases correctly classified.

Table 12. Discriminant Analysis of the known source *E. coli* isolates in the Copano Bay study library. Six-way classification: Human/sewage vs. Cow vs. Horse vs. Duck vs. Wildlife vs. Gull (all groups equal).
Note: "Original" is the discriminant analysis using all the isolates. "Cross-validated" (also known as resubstitution analysis or leave-one-out method) refers to an analysis performed where each isolate is removed one at a time and classified based on the library of remaining isolates.

Classification Results[b,c]

			Predicted Group Membership						
		Species	Cow	Horse	Sewage	Wildlife	Gull	Duck	Total
Original	Count	Cow	105	31	51	14	11	24	236
		Horse	16	151	47	3	2	54	273
		Sewage	8	21	92	1	4	19	145
		Wildlife	3	1	3	138	18	5	168
		Gulls	3	2	6	28	79	9	127
		Ducks	9	16	22	1	1	60	109
	%	Cow	44.5	13.1	21.6	5.9	4.7	10.2	100.0
		Horse	5.9	55.3	17.2	1.1	.7	19.8	100.0
		Sewage	5.5	14.5	63.4	.7	2.8	13.1	100.0
		Wildlife	1.8	.6	1.8	82.1	10.7	3.0	100.0
		Gulls	2.4	1.6	4.7	22.0	62.2	7.1	100.0
		Ducks	8.3	14.7	20.2	.9	.9	55.0	100.0
Cross-validated[a]	Count	Cow	99	33	52	14	12	26	236
		Horse	17	141	48	3	3	61	273
		Sewage	9	22	90	1	4	19	145
		Wildlife	4	1	3	136	19	5	168
		Gulls	4	3	6	30	75	9	127
		Ducks	10	19	27	1	1	51	109
	%	Cow	41.9	14.0	22.0	5.9	5.1	11.0	100.0
		Horse	6.2	51.6	17.6	1.1	1.1	22.3	100.0
		Sewage	6.2	15.2	62.1	.7	2.8	13.1	100.0
		Wildlife	2.4	.6	1.8	81.0	11.3	3.0	100.0
		Gulls	3.1	2.4	4.7	23.6	59.1	7.1	100.0
		Ducks	9.2	17.4	24.8	.9	.9	46.8	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived f cases other than that case.

b. 59.1% of original grouped cases correctly classified.

c. 56.0% of cross-validated grouped cases correctly classified.

analysis ARCC was 59%, with a cross validated ARCC of 56%. Rates of correct classification for individual sources ranged from 82% (wildlife) to 44.5% (cows). Human (sewage) isolates were correctly classified for 63% of the isolates. Sewage isolates (13-14%) were most frequently misclassified as horse and duck, while 17-20% of horse and duck were classified as human isolates. There was also misclassification of approx. 20% cows as sewage. Gulls and wildlife primarily misclassified as each other, with less than 5% misclassifying as sewage, horse or cow. Based on the distinct groupings of duck isolates compared to wildlife isolates (only 1 duck isolate misclassified as wildlife) (Table 12) and gull isolates compared with either group, and the identification of ducks as a potential source (TDH sanitary surveys) it was decided to use the 6-way analysis for the categorization of unknown isolates.

Pulse field gel electrophoresis was used to characterize known source isolates from the spring 2004 fecal sample collections (194 isolates – human/sewage, cow, horse and duck). The fingerprints generated were used to classify a sub-set of 1100 unknown source isolates (as per the QAPP), and to determine confirmation levels of source identifications for these unknowns as compared to sources identified by antibiotic resistance profile analysis.

Copano Bay sampling events

FIELD PARAMETERS

Field parameters (salinity, air temperature, water temperature, wind direction, wind velocity, specific conductance, rainfall) measured/observed by TDH personnel during sample collection for the eight sampling events are shown in Tables 13 to 19. Weather data (cloudy, foggy, clear etc.) are shown in Table 20.

Table 13. Copano Bay Stations: salinity (ppt). Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	1/8/2004	2/17/2004	2/26/2004	3/2/2004	4/8/2004	Mean
COP-00001	9.7	13.1	11.9	14.4	13.9	10.2	13.9	11.9	12.4
COP-00003	12.9	13.1	10.7	14.8	13.1	9.6	12.5	13.7	12.6
COP-00004	9.1	10.1	11.9	13.7	13.7	10.0	13.7	8.5	11.3
COP-00007	6.5	8.0	10.4	13.2	12.7	8.8	12.9	6.5	9.9
COP-00008	3.8	7.8	8.2	9.7	10.5	9.4	10.8	0.6	7.6
COP-00009	5.3	6.8	8.7	10.5	11.4	10.8	10.7	5.7	8.7
COP-00011	9.3	10.7	11.1	13.6	12.5	9.0	13.1	10.9	11.3
COP-00012	4.9	7.5	7.9	12.5	11.6	8.6	11.2	6.3	8.8
COP-00013	4.2	7.6	7.6	10.3	9.6	7.3	10.7	3.5	7.6
COP-00014	4.1	7.7	7.5	9.7	7.9	8.3	10.6	1.2	7.1
COP-00016	6.3	8.0	10.2	12.4	12.7	8.6	12.2	6.1	9.6
COP-00017	6.6	10.1	10.0	11.7	11.2	7.7	12.3	11.8	10.2
COP-00019	7.8	11.4	9.8	11.3	11.9	8.5	12.7	12.7	10.8
MBY-00002	5.9	7.5	6.6	9.2	10.5	9.8	9.5	0.5	7.4
Mean	**6.9**	**9.2**	**9.5**	**11.9**	**11.7**	**9.0**	**11.9**	**7.1**	

Table 14. Copano Bay Stations: air temperature (Fahrenheit). Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004	Mean
COP-00001	72.0	82	45.0	50.0	60.0	48.0	71.0	71.0	62.4
COP-00003	72.0	82	45.0	50.0	60.0	48.0	71.0	71.0	62.4
COP-00004	75.0	82	45.0	50.0	60.0	50.0	71.0	71.0	63.0
COP-00007	75.0	82	45.0	50.0	60.0	50.0	71.0	71.0	63.0
COP-00008	75.0	82	45.0	50.0	60.0	55.0	71.0	71.0	63.6
COP-00009	75.0	82	45.0	50.0	60.0	55.0	71.0	71.0	63.6
COP-00011	72.0	82	45.0	50.0	60.0	48.0	71.0	71.0	62.4
COP-00012	75.0	82	45.0	50.0	60.0	55.0	71.0	71.0	63.6
COP-00013	75.0	82	45.0	50.0	60.0	55.0	71.0	71.0	63.6
COP-00014	75.0	82	45.0	50.0	60.0	55.0	71.0	71.0	63.6
COP-00016	75.0	82	45.0	50.0	60.0	50.0	71.0	71.0	63.0
COP-00017	75.0	82	45.0	50.0	60.0	50.0	71.0	71.0	63.0
COP-00019	72.0	82	45.0	50.0	60.0	48.0	71.0	71.0	62.4
MBY-00002	75.0	82	45.0	50.0	60.0	55.0	71.0	71.0	63.6
Mean	**74.1**	**82**	**45.0**	**50.0**	**60.0**	**51.6**	**71.0**	**71.0**	

Table 15. Copano Bay Stations: water temperature (Fahrenheit). Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004	Mean
COP-00001	75.0	76.0	55.0	53.0	56.0	65.0	70.0	74.0	65.6
COP-00003	75.0	76.0	55.0	53.0	56.0	65.0	70.0	74.0	65.5
COP-00004	75.0	76.0	55.0	53.0	57.0	65.0	70.0	75.0	65.8
COP-00007	75.0	79.0	55.0	53.0	58.0	65.0	70.0	75.0	66.3
COP-00008	75.0	76.0	55.0	52.0	58.0	65.0	70.0	72.0	65.4
COP-00009	75.0	76.0	55.0	53.0	59.0	63.0	70.0	73.0	65.5
COP-00011	75.0	76.0	55.0	53.0	57.0	65.0	70.0	74.0	65.6
COP-00012	74.0	80.0	55.0	53.0	58.0	65.0	70.0	75.0	66.3
COP-00013	75.0	80.0	55.0	52.0	58.0	65.0	70.0	73.0	66.0
COP-00014	75.0	76.0	55.0	52.0	61.0	65.0	70.0	73.0	65.9
COP-00016	75.0	78.0	55.0	53.0	58.0	65.0	70.0	75.0	66.1
COP-00017	75.0	76.0	55.0	52.0	57.0	65.0	70.0	75.0	65.6
COP-00019	75.0	76.0	55.0	52.0	56.0	65.0	70.0	74.0	65.4
MBY-00002	75.0	76.0	55.0	53.0	58.0	64.0	70.0	75.0	65.3
Mean	**75.0**	**76.9**	**55.0**	**52.6**	**57.6**	**64.8**	**70.0**	**74.1**	

Table 16. Copano Bay Stations: wind direction. Shown in 2 digits in ten degree clockwise increments. Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004
COP-00001	04	13	29	32	28	33	09	06
COP-00003	04	13	29	32	28	33	09	06
COP-00004	04	13	29	32	28	33	12	06
COP-00007	04	13	29	32	28	33	12	06
COP-00008	04	13	29	32	28	33	12	06
COP-00009	04	13	29	32	28	33	12	06
COP-00011	04	13	29	32	28	33	09	06
COP-00012	04	13	29	32	28	33	12	06
COP-00013	04	13	29	32	28	33	12	06
COP-00014	04	13	29	32	28	33	12	06
COP-00016	04	13	29	32	28	33	12	06
COP-00017	04	13	29	32	28	33	12	06
COP-00019	04	13	29	32	28	33	12	06
MBY-00002	04	13	29	32	28	33	12	06
Mean	NE	**SE**	**W/NW**	NW	**W/NW**	**NW**	**SE**	**NE**

Table 17. Copano Bay Stations: wind velocity (MPH). Shown in 2 digits in ten degree clockwise increments. Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004
COP-00001	15	15	10	10	8	15	12	10
COP-00003	10	15	10	10	8	15	12	10
COP-00004	10	30	10	10	5	15	15	15
COP-00007	10	30	10	10	3	15	18	15
COP-00008	10	30	6	10	5	15	20	15
COP-00009	10	30	6	10	5	15	20	15
COP-00011	15	20	10	10	8	15	12	10
COP-00012	10	30	10	10	8	15	20	15
COP-00013	10	30	6	10	3	15	20	15
COP-00014	10	30	6	10	8	15	20	15
COP-00016	10	30	10	10	3	15	18	15
COP-00017	10	30	10	10	5	15	15	10
COP-00019	15	30	10	10	8	15	15	10
MBY-00002	10	30	6	10	10	15	20	15

Table 18. Copano Bay Stations: specific conductance (microromhos). Texas Department of Health, Seafood Safety

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004	Mean
COP-00001	16500	21800	20000	23700	22900	17400	17400	23000	20338
COP-00003	21500	21800	18000	24700	21700	16300	16300	20800	20138
COP-00004	15600	17200	19800	22600	22600	16900	16900	22700	19288
COP-00007	11300	13800	17600	21800	21000	15000	15000	21600	17138
COP-00008	6970	13600	14000	16500	17700	16000	16000	18300	14884
COP-00009	9470	12000	14900	17700	19000	18200	18200	18000	15934
COP-00011	16000	18200	18600	22600	20700	15400	15400	21700	18575
COP-00012	8720	13000	13600	20800	19400	14800	14800	18800	15490
COP-00013	7600	13200	13100	17700	16200	12600	12600	17900	13863
COP-00014	7360	13300	12900	16400	13600	14300	14300	18000	13770
COP-00016	11000	139000	17300	20700	21000	14800	14800	18000	16438
COP-00017	11600	17200	16900	19600	18800	13200	13200	20600	16388
COP-00019	13500	19100	16500	19000	19800	14600	14600	21200	17288
MBY-00002	10300	13000	11400	15600	17800	16500	16500	16300	14675
Mean	**11959**	**15793**	**16043**	**19957**	**19443**	**15429**	**15429**	**19779**	**16729**

Table 19. Copano Bay Stations: average rainfall (inches). Texas Department of Health, Seafood Safety

Rainfall	10/15/200	11/17/200	12/17/200	01/08/200	02/17/200	02/26/200	03/02/200	04/08/200
24 hr	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.001
4 day	0.84	0.00	0.05	0.50	0.35	0.42	0.00	3.56
7 day	1.16	0.05	0.87	0.50	0.73	0.42	0.36	5.99

Table 20. Copano Bay Stations: weather data. CY=Cloudy, CL=Clear, PC=Partly Cloudy, FG=Foggy. Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004
COP-00001	PC	PC	CL	FG	CL	CL	CY	CY
COP-00003	PC	PC	CL	FG	CL	CL	CY	CY
COP-00004	CL	PC	CL	FG	CL	CL	PC	PC
COP-00007	CL	PC	CL	FG	CL	CL	PC	PC
COP-00008	CL	PC	CL	FG	CL	CL	PC	PC
COP-00009	CL	PC	CL	FG	CL	CL	PC	PC
COP-00011	PC	PC	CL	FG	CL	CL	CY	CY
COP-00012	CL	PC	CL	FG	CL	CL	PC	PC
COP-00013	CL	PC	CL	FG	CL	CL	PC	PC
COP-00014	CL	PC	CL	FG	CL	CL	PC	PC
COP-00016	CL	PC	CL	FG	CL	CL	PC	PC
COP-00017	CL	PC	CL	FG	CL	CL	CY	CY
COP-00019	PC	PC	CL	FG	CL	CL	CY	CY
MBY-00002	CL	PC	CL	FG	CL	CL	PC	PC

Water samples were collected between 9 am and 1.00 pm, generally within a 2 hour timeframe (Table 21). Salinity means over the collection period ranged from 6.9-11.9 ppt. Salinities were lower when fecal bacterial levels (fecal coliform MPNs) were high, especially for the 4/8/04 sampling event where salinities at stations COP-00008, 00013, 00014 and MBY-00002 were 3.5 or less.

Fecal coliform data as MPN/100 ml (analysis by Corpus Christi-Nueces County Public Health District Laboratory) are shown in Table 22**.** The Texas Administrative Code (2000) states that "The indicator bacteria for suitability for oyster waters is fecal coliform". The fecal coliform criterion for oyster waters is 14 colonies per 100 ml and no more than 10% >43 MPN as specified in §307.7(b)(3)(B)". Based on the data from TDH, water samples from stations COP 00001, 00003, 00004, 00011, 00012 did not exceed the criteria over the study period. Stations 00007, 00008, 00009, 00016, MBY0002 only exceeded the criteria on 4/8/04. Stations 00013, 00014 and 00017 exceeded the criteria on 2/26/04 and 4/8/04. Station 00019 exceeded the criteria on 1/8/04 and Station 000017 on 12/17/03.

Station classifications provided by TDH are shown in Table 23 for each sampling event. Stations 00001, 00003, 00004, 00007, 00009, 00011, 00016, 00017 were classified as "Approved" throughout the study period. Stations 00008, 00013, 00014, and MBY-00002 were "Restricted" throughout the study period. Stations 00012 and 00017 were "Restricted" only on 3/2/04.

The colonies obtained during *E. coli* isolations showed similar trends to the MPN data (counts shown in Table 24) but are only estimates, as the six hour holding time was exceeded in some cases. Enumerations of *E. coli* were not part of the project analysis, as explained in

Table 21. Copano Bay Stations: sampling times. Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004
COP-00001	1033	1030	0908	1110	1142	0935	1033	1111
COP-00003	1038	1036	0913	1116	1146	0942	1040	1116
COP-00004	1111	1309	0945	1154	1217	1021	1122	1146
COP-00007	1119	1259	0953	1203	1227	1029	1129	1152
COP-00008	1149	1221	1016	1227	1252	1058	1151	1219
COP-00009	1156	1210	1022	1234	1301	1107	1047	1231
COP-00011	1044	1046	0920	1124	1152	0952	1047	1122
COP-00012	1129	1245	1002	1213	1239	1041	1139	1202
COP-00013	1139	1234	1009	1220	1245	1050	1145	1210
COP-00014	1144	1226	1012	1224	1248	1054	1148	1215
COP-00016	1123	1253	0956	1207	1232	1034	1133	1156
COP-00017	1100	1108	0934	1139	1206	1009	1108	1135
COP-00019	1053	1100	0928	1133	1201	1001	1100	1130
MBY-00002	1211	1145	1034	1243	1308	1114	1214	1241

Table 22. Fecal coliform data for Copano Bay Stations (MPN/100 ml). Corpus Christi-Nueces County Public Health District Laboratory.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004	Mean
COP-00001	1.8	1.8	1.8	1.8	1.8	4.5	2.0	1.8	**2.2**
COP-00003	1.8	1.8	1.8	2.0	1.8	1.8	7.8	1.8	**2.6**
COP-00004	1.8	1.8	4.5	1.8	1.8	6.8	1.8	13.0	**4.2**
COP-00007	2.0	1.8	1.8	1.8	1.8	1.8	2.0	64.0	**9.6**
COP-00008	2.0	1.8	1.8	1.8	1.8	1.8	1.8	1600.0	**201.6**
COP-00009	1.8	1.8	1.8	1.8	1.8	1.8	1.8	33.0	**5.7**
COP-00011	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	**108**
COP-00012	2.0	1.8	11.0	4.5	1.8	13.0	1.8	13.0	**6.1**
COP-00013	7.8	2.0	4.0	4.5	1.8	33.0	1.8	130.0	**23.1**
COP-00014	1.8	2.0	7.8	6.8	2.0	33.0	1.8	540.0	**74.4**
COP-00016	2.0	2.0	1.8	1.8	1.8	1.8	13.0	46.0	**8.8**
COP-00017	2.0	1.8	33.0	1.8	4.5	1600.0	4.5	110.0	**219.7**
COP-00019	2.0	13.0	13.0	49.0	1.8	12.0	13.0	4.5	**13.5**
MBY-00002	2.0	1.8	4.5	13.0	1.8	1.8	4.5	240.0	**33.9**
Mean	2.5	2.6	6.5	6.7	2.0	122.5	4.2	199.9	

Table 23. Station classification. AP=Approved, CA=Conditionally Approved, R=Restricted, PR=Prohibited. Texas Department of Health, Seafood Safety Division.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004
COP-00001	AP	AP	AP	AP	AP	AP	AP	AP
COP-00003	AP	AP	AP	AP	AP	AP	AP	AP
COP-00004	AP	AP	AP	AP	AP	AP	AP	AP
COP-00007	AP	AP	AP	AP	AP	AP	AP	AP
COP-00008	RS	RS	RS	RS	RS	RS	RS	RS
COP-00009	AP	AP	AP	AP	AP	AP	AP	AP
COP-00011	AP	AP	AP	AP	AP	AP	AP	AP
COP-00012	AP	AP	AP	AP	AP	AP	RS	AP
COP-00013	RS	RS	RS	RS	RS	RS	RS	RS
COP-00014	RS	RS	RS	RS	RS	RS	RS	RS
COP-00016	AP	AP	AP	AP	AP	AP	AP	AP
COP-00017	AP	AP	AP	AP	AP	AP	AP	AP
COP-00019	AP	AP	AP	AP	AP	AP	RS	AP
MBY-00002	RS	RS	RS	RS	RS	RS	RS	RS

Table 24. Number of presumptive *E. coli* (colony forming units) isolated, on mTEC agar plates, from Copano Bay, over the period of the study.

Station #'s	10/15/2003	11/17/2003	12/17/2003	01/08/2004	02/17/2004	02/26/2004	03/02/2004	04/08/2004	Total
COP-00001	1	0	0	18	0	3	1	13	36
COP-00003	0	0	0	24	0	12	40	0	76
COP-00004	5	0	6	1	0	8	0	28	48
COP-00007	0	2	2	4	0	6	13	90	117
COP-00008	15	5	0	8	1	2	3	1635	1669
COP-00009	5	13	10	3	0	2	1	188	222
COP-00011	10	1	3	6	0	1	1	16	38
COP-00012	16	3	30	4	0	42	108	154	357
COP-00013	14	10	28	32	2	230	54	296	666
COP-00014	9	0	43	18	3	212	4	879	1168
COP-00016	1	20	16	5	0	67	78	85	272
COP-00017	14	0	174	13	26	1193	3	150	1573
COP-00019	2	61	26	192	0	24	10	31	346
MBY-00002	8	6	19	6	0	2	18	640	699
Total #'s	**100**	**121**	**258**	**306**	**30**	**1593**	**334**	**4205**	**6947**

the "Methods" section. However the similarity is useful in supporting the analysis of higher numbers of *E. coli* isolates during higher fecal coliform events for the individual stations.

Unknown (water sample) isolates

ANTIIOTIC RESISTANCE PROFILES

Over the course of this study (10/15/03-04/08/04) more than 6,900 colonies were isolated on mTEC medium from water samples collected during eight sampling events, from fourteen stations in Copano Bay. Of this number 3,381 isolates were verified as *E. coli* using the Biolog™ MicroLog System. Antibiotic Resistance Profiles (ARP) were developed for 2,811 of the verified *E. coli* isolates (Table 25). Numbers of *E. coli* isolates confirmed as *E. coli* by the Biolog Microbial Identification System, and analyzed for antibiotic resistance and PFGE for each sampling event are shown in Tables 26 to 33. The zone diameters produced during antibiotic resistance testing were compiled into an unknown *E. coli* isolate database (saved on CD-ROM included with report). These zone diameters were analyzed statistically against zone diameters from the known source *E. coli* isolate database, which represented *E. coli* from possible contamination sources in the Copano Bay watershed.

Discriminant analysis with SPSS ® Version 12.0 for Windows was used to classify the unknown water *E. coli* isolates into predicted groups of known sources based upon zone diameters. All the unknown source isolates (2,811 isolates) from all stations for the sampling events were classified by source using the library of known source isolates (Tables 34 to 36). In two-way analysis, 42% of the isolates were classified as human (sewage) and 58% were classified as non-human. In four-way this was reduced to 29%, with some isolates classified as human in two-way being identified as horse or cow. Six-way analysis, used for the

Table 25. Numbers of *E. coli* isolates analyzed using antibiotic resistance profiling for each station, for all events.

Station #'s	**10/15/2003**	**11/17/2003**	**12/17/2003**	**01/08/2004**	**02/17/2004**	**02/26/2004**	**03/02/2004**	**04/08/2004**	**Total**
	COP A	**COP B**	**COP C**	**COPD**	**COP F**	**COP E**	**COP G**	**COP H**	
COP-00001	1	0	0	11	0	1	1	11	25
COP-00003	0	0	0	23	0	9	33	0	65
COP-00004	4	0	1	1	0	6	0	25	37
COP-00007	0	2	1	4	0	1	10	54	72
COP-00008	13	5	0	7	1	1	3	252	282
COP-00009	3	12	3	3	0	2	1	138	162
COP-00011	9	0	1	6	0	1	0	14	31
COP-00012	14	3	15	4	0	25	88	131	280
COP-00013	3	9	18	26	0	160	27	165	408
COP-00014	4	0	20	10	2	112	4	156	308
COP-00016	0	19	11	4	0	55	66	65	220
COP-00017	8	0	89	10	22	204	2	93	428
COP-00019	1	46	7	159	0	15	9	28	265
MBY-00002	6	6	12	3	0	2	13	186	228
Total #s	**66**	**102**	**178**	**271**	**25**	**594**	**257**	**1318**	**2811**

Table 26. Numbers of unknown source *E. coli* isolates verified, and analyzed for October 15, 2003 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	1	1	1	1
COP-00003	0	0	0	0
COP-00004	5	4	4	1
COP-00007	0	0	0	0
COP-00008	15	13	13	10
COP-00009	5	3	3	2
COP-00011	10	9	9	7
COP-00012	16	13	13	12
COP-00013	14	3	3	3
COP-00014	9	4	4	3
COP-00016	1	0	0	0
COP-00017	14	8	8	7
COP-00019	2	1	1	1
MBY-00002	8	6	6	2
TOTAL	100	65	65	49

Table 27. Numbers of unknown source *E. coli* isolates verified, and analyzed for November 17, 2003 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis).

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	0	0	0	0
COP-00003	0	0	0	0
COP-00004	0	0	0	0
COP-00007	2	2	2	2
COP-00008	5	5	5	4
COP-00009	13	14	12	12
COP-00011	1	0	0	0
COP-00012	3	3	3	2
COP-00013	10	9	9	7
COP-00014	0	0	0	0
COP-00016	20	20	19	5
COP-00017	0	0	0	0
COP-00019	61	46	46	36
MBY-00002	6	6	6	6
TOTAL	121	105	102	74

Table 28. Numbers of unknown source *E. coli* isolates verified, and analyzed for December 17, 2003 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	0	0	0	0
COP-00003	0	0	0	0
COP-00004	6	1	1	1
COP-00007	2	2	1	1
COP-00008	0	0	0	0
COP-00009	10	4	3	3
COP-00011	3	1	1	1
COP-00012	30	17	15	14
COP-00013	28	25	18	15
COP-00014	43	29	20	16
COP-00016	16	13	11	8
COP-00017	174	98	89	31
COP-00019	26	7	7	4
MBY-00002	19	12	12	9
TOTAL	258	209	178	103

Table 29. Numbers of unknown source *E. coli* isolates verified, and analyzed for January 8, 2004 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	12	11	11	8
COP-00003	25	23	23	18
COP-00004	1	1	1	1
COP-00007	4	4	4	2
COP-00008	8	7	7	6
COP-00009	3	3	3	1
COP-00011	6	6	6	3
COP-00012	4	4	4	3
COP-00013	31	27	26	21
COP-00014	10	10	10	7
COP-00016	5	4	4	3
COP-00017	13	10	10	7
COP-00019	180	165	159	31
MBY-00002	4	3	3	3
TOTAL	306	278	271	114

Table 30. Numbers of unknown source *E. coli* isolates verified, and analyzed for February 17, 2004 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	0	0	0	0
COP-00003	0	0	0	0
COP-00004	0	0	0	0
COP-00007	0	0	0	0
COP-00008	1	1	1	1
COP-00009	0	0	0	0
COP-00011	0	0	0	0
COP-00012	0	0	0	0
COP-00013	2	0	0	0
COP-00014	3	3	2	1
COP-00016	0	0	0	0
COP-00017	24	22	22	17
COP-00019	0	0	0	0
MBY-00002	0	0	0	0
TOTAL	30	26	25	19

Table 31. Numbers of unknown source *E. coli* isolates verified, and analyzed for February 26, 2004 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	3	1	1	0
COP-00003	12	10	9	7
COP-00004	7	6	6	5
COP-00007	5	1	1	1
COP-00008	2	1	1	1
COP-00009	2	2	2	2
COP-00011	1	1	1	1
COP-00012	42	27	25	18
COP-00013	218	172	160	28
COP-00014	204	119	112	50
COP-00016	63	56	55	31
COP-00017	1017	453	204	31
COP-00019	24	14	15	12
MBY-00002	2	2	2	1
TOTAL	1602	865	594	189

Table 32. Numbers of unknown source *E. coli* isolates verified, and analyzed for March 2, 2004 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	1	1	1	0
COP-00003	40	37	33	22
COP-00004	0	0	0	0
COP-00007	12	10	10	3
COP-00008	3	3	3	2
COP-00009	1	1	1	1
COP-00011	1	0	0	0
COP-00012	108	88	88	26
COP-00013	54	32	27	13
COP-00014	4	4	4	2
COP-00016	78	67	66	34
COP-00017	3	2	2	2
COP-00019	10	9	9	6
MBY-00002	18	15	13	8
TOTAL	333	269	257	119

Table 33. Numbers of unknown source *E. coli* isolates verified, and analyzed for April 8, 2004 Copano Bay sampling event (ARP = Antibiotic Resistance Profiles, PFGE = Pulse Field Gel Electrophoresis)

Station	# of isolates	# verified (Biolog™)	# ARP completed	# PFGE completed
COP-00001	13	11	11	9
COP-00003	0	0	0	0
COP-00004	26	26	25	20
COP-00007	60	57	54	48
COP-00008	393	274	252	83
COP-00009	161	145	138	95
COP-00011	16	16	14	12
COP-00012	143	139	131	26
COP-00013	216	203	165	20
COP-00014	216	179	156	36
COP-00016	76	72	65	33
COP-00017	127	119	93	41
COP-00019	29	28	28	26
MBY-00002	400	294	186	78
TOTAL	1876	1563	1318	527

Table 34. Discriminant analysis of the unknown source *E. coli* isolates with the Copano Bay study library isolates. Two-way classification – Human/sewage vs. Non-human (all groups equal).
Note: "Cases Selected" are the library isolates, "Cases Not Selected" are the water sample (unknown source) isolates. "Original" is the discriminant analysis using all the isolates. "Cross-validated" (also known as resubstitution analysis or leave-one-out method) refers to an analysis performed where each isolate is removed one at a time and classified based on the library of remaining isolates.

Classification Results[b,c,d]

				Predicted Group Membership		
			Species	Human	Nonhuman	Total
Cases Selected	Original	Count	Human	116	29	145
			Nonhuman	269	653	922
		%	Human	80.0	20.0	100.0
			Nonhuman	29.2	70.8	100.0
	Cross-validated[a]	Count	Human	112	33	145
			Nonhuman	275	647	922
		%	Human	77.2	22.8	100.0
			Nonhuman	29.8	70.2	100.0
Cases Not Selected	Original	Count	Human	0	0	0
			Nonhuman	0	0	0
			Unknowns	1189	1622	2811
		%	Human	.0	.0	100.0
			Nonhuman	.0	.0	100.0
			Unknowns	42.3	57.7	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 72.1% of selected original grouped cases correctly classified.

c. .0% of unselected original grouped cases correctly classified.

d. 71.1% of selected cross-validated grouped cases correctly classified.

Table 35. Discriminant Analysis of the unknown source *E. coli* isolates in the Copano Bay study library. Four-way classification: Human/sewage vs. Cow vs. Horse vs. Wildlife (all groups equal).
Note: "Cases Selected" are the library isolates, "Cases Not Selected" are the water sample (unknown source) isolates. "Original" is the discriminant analysis using all the isolates. "Cross-validated" (also known as resubstitution analysis or leave-one-out method) refers to an analysis performed where each isolate is removed one at a time and classified based on the library of remaining isolates.

Classification Results[b,c]

			Predicted Group Membership				
		Species	Cow	Horse	Sewage	Wildlife	Total
Original	Count	Cow	118	34	58	26	236
		Horse	23	183	61	6	273
		Sewage	10	27	103	5	145
		Wildlife	33	45	56	279	413
		Unknowns	688	1276	808	39	2811
	%	Cow	50.0	14.4	24.6	11.0	100.0
		Horse	8.4	67.0	22.3	2.2	100.0
		Sewage	6.9	18.6	71.0	3.4	100.0
		Wildlife	8.0	10.9	13.6	67.6	100.0
		Unknowns	24.5	45.4	28.7	1.4	100.0
Cross-validated[a]	Count	Cow	111	36	60	29	236
		Horse	23	179	62	9	273
		Sewage	10	29	101	5	145
		Wildlife	35	48	58	272	413
	%	Cow	47.0	15.3	25.4	12.3	100.0
		Horse	8.4	65.6	22.7	3.3	100.0
		Sewage	6.9	20.0	69.7	3.4	100.0
		Wildlife	8.5	11.6	14.0	65.9	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 64.0% of original grouped cases correctly classified.

c. 62.1% of cross-validated grouped cases correctly classified.

Table 36. Discriminant Analysis of the unknown source *E. coli* isolates with the Copano Bay study library isolates. Six-way classification: Human/sewage vs. Cow vs. Horse vs. Duck vs. Gull vs. Wildlife (all groups equal). (See Notes on Table 34, 35 for additional information)

Classification Results[b,c,d]

				Predicted Group Membership						
			Species	Cow	Horse	Sewage	Wildlife	Gull	Duck	Total
Cases Selected	Original	Count	Cow	105	31	51	14	11	24	236
			Horse	16	151	47	3	2	54	273
			Sewage	8	21	92	1	4	19	145
			Wildlife	3	1	3	138	18	5	168
			Gull	3	2	6	28	79	9	127
			Duck	9	16	22	1	1	60	109
		%	Cow	44.5	13.1	21.6	5.9	4.7	10.2	100.0
			Horse	5.9	55.3	17.2	1.1	.7	19.8	100.0
			Sewage	5.5	14.5	63.4	.7	2.8	13.1	100.0
			Wildlife	1.8	.6	1.8	82.1	10.7	3.0	100.0
			Gull	2.4	1.6	4.7	22.0	62.2	7.1	100.0
			Duck	8.3	14.7	20.2	.9	.9	55.0	100.0
	Cross-validate[a]	Count	Cow	99	33	52	14	12	26	236
			Horse	17	141	48	3	3	61	273
			Sewage	9	22	90	1	4	19	145
			Wildlife	4	1	3	136	19	5	168
			Gull	4	3	6	30	75	9	127
			Duck	10	19	27	1	1	51	109
		%	Cow	41.9	14.0	22.0	5.9	5.1	11.0	100.0
			Horse	6.2	51.6	17.6	1.1	1.1	22.3	100.0
			Sewage	6.2	15.2	62.1	.7	2.8	13.1	100.0
			Wildlife	2.4	.6	1.8	81.0	11.3	3.0	100.0
			Gull	3.1	2.4	4.7	23.6	59.1	7.1	100.0
			Duck	9.2	17.4	24.8	.9	.9	46.8	100.0
Cases Not Select	Original	Count	Cow	0	0	0	0	0	0	0
			Horse	0	0	0	0	0	0	0
			Sewage	0	0	0	0	0	0	0
			Wildlife	0	0	0	0	0	0	0
			Gull	0	0	0	0	0	0	0
			Duck	0	0	0	0	0	0	0
			Unknowns	564	996	621	6	24	600	2811
		%	Cow	.0	.0	.0	.0	.0	.0	100.0
			Horse	.0	.0	.0	.0	.0	.0	100.0
			Sewage	.0	.0	.0	.0	.0	.0	100.0
			Wildlife	.0	.0	.0	.0	.0	.0	100.0
			Gull	.0	.0	.0	.0	.0	.0	100.0
			Duck	.0	.0	.0	.0	.0	.0	100.0
			Unknowns	20.1	35.4	22.1	.2	.9	21.3	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived f case.

b. 59.1% of selected original grouped cases correctly classified.

c. .0% of unselected original grouped cases correctly classified.

d. 56.0% of selected cross-validated grouped cases correctly classified.

majority of the discussion, showed 22% human (sewage), with 20-35% as each of cow, horse and duck. Few isolates were identified as wildlife or gulls.

The six-way (all groups equal) discriminant analysis (human sewage vs. cow vs. horse vs. wildlife vs. duck vs. gull) was used to classify unknown *E. coli* isolates as human/sewage, cow, horse, wildlife, duck, or gull *E. coli* isolates. This decision was made based on the sanitary survey which specifically identified ducks as a potential source. Thus, they were separated from wildlife in order to classify isolates as duck rather than just wildlife. Gulls formed a distinct group based on ARPs and were therefore kept as a separate classification category.

PULSE FIELD GEL ELECTROPHORESIS

A subset of the unknown source isolates analyzed for antibiotic resistance were also analyzed using PFGE. The PFGE results were compared to the results of antibiotic resistance analysis (Tables 37, 38 and 39). Isolates that both ARP and PFGE identified as from the same known source (Sewage, Cow, Horse, and Duck) were confirmed as being from that known source. The isolates were evaluated by sampling event (10/15/03-04/08/04) and across the stations that were sampled. There were 1,077 isolates that were analyzed by both methods and that identified with known sources from the database. Of the 250 isolates classified as sewage using antibiotic profiling for all sampling events, 159 were confirmed as sewage by PFGE, at a rate of 63.60%. The sampling events ranged from 46.67% of the isolates being confirmed as sewage for 01/08/04 to 100% of the isolates for 02/17/04. For non-human isolates 52.72% were confirmed by PFGE as non-human. For cow, of 213 isolates in common that classified as cow using antibiotic resistance profiling, PFGE confirmed 60 of those isolates, with an average rate of 28.17%, ranging from no confirmation

Table 37. Comparison of PFGE and ARP human/sewage vs. Nonhuman source identifications for isolates analyzed by both techniques.

		Sewage/Human			Non-Human		
Event	**Total**	**# ARA**	**# PFGE**	**%**	**# ARA**	**# PFGE**	**%**
10/15/03	042	031	022	70.97	11	5	45.45
11/17/03	048	026	013	50.00	22	12	54.54
12/17/03	090	019	012	63.16	71	37	52.11
01/08/04	112	015	007	46.67	97	64	65.98
02/17/04	019	003	003	100.00	16	7	43.75
02/26/04	182	029	017	58.62	153	71	46.40
03/02/04	102	029	021	72.41	73	38	52.05
04/08/04	482	098	064	65.31	384	202	52.06
Total	1,077	250	159	63.60	827	436	52.72
		Sewage/Human			**Non-Human**		
Site	**Total**	**# ARA**	**# PFGE**	**%**	**# ARA**	**# PFGE**	**%**
COP 00001	016	005	002	40.00	11	10	90.90
COP 00003	036	007	005	71.14	29	15	51.72
COP 00004	024	005	002	40.00	19	6	31.58
COP 00007	054	017	003	17.65	37	22	59.45
COP 00008	096	025	020	83.00	71	34	47.88
COP 00009	106	026	017	65.53	80	40	50.00
COP 000011	022	010	009	90.00	12	5	41.66
COP 000012	091	031	026	83.87	60	36	60.00
COP 000013	094	020	013	65.00	74	40	54.05
COP 000014	108	018	011	61.11	90	40	44.44
COP 000016	115	019	013	68.42	96	51	53.13
COP 000017	124	025	014	56.00	99	49	49.49
COP 000019	099	024	011	45.83	75	46	61.33
MBY 00002	092	018	013	72.22	74	42	56.75
Total	1,077	250	159	63.60	827	436	52.72

Table 38. Comparison of PFGE and ARP source identifications for isolates analyzed by both techniques, for each sampling event.

		SEWAGE			COW			HORSE			DUCK		
EVENT	**Total**	**ARP**	**PFGE**	**%**	**ARP**	**PFGE**	**%**	**ARP**	**PFGE**	**%**	**ARP**	**PFGE**	**%**
10/15/2003	042	031	022	70.97	001	01	100.00	004	00	00.00	006	00	00.00
11/17/2003	048	026	013	550.00	001	01	100.00	006	00	00.00	014	01	07.14
12/17/2003	090	019	012	63.16	005	02	40.00	034	06	17.65	032	01	03.13
01/08/2004	112	015	007	46.67	034	08	23.53	052	08	15.55	007	03	42.85
02/17/2004	019	003	003	100.00	004	00	00.00	007	02	28.57	005	00	00.00
02/26/2004	182	029	017	58.62	053	12	22.64	070	13	18.57	024	01	04.17
03/02/2004	102	029	021	72.41	017	05	29.41	034	06	17.65	022	02	09.09
04/08/2004	482	098	064	65.31	098	31	31.63	189	35	18.51	093	10	10.75
TOTAL	1077	250	159	63.86	213	60	27.17	396	70	17.68	203	18	08.90

Table 39. Comparison of PFGE and ARP source identifications for isolates analyzed by both techniques, for each station.

		SEWAGE			COW			HORSE			DUCK		
STATION	**Total**	**ARP**	**PFGE**	**%**	**ARP**	**PFGE**	**%**	**ARP**	**PFGE**	**%**	**ARP**	**PFGE**	**%**
COP 00001	016	005	002	40.00	005	00	00.00	004	00	00.00	002	01	50.00
COP 00003	036	007	005	71.43	007	03	42.86	011	02	18.01	009	00	00.00
COP 00004	024	005	002	40.00	010	02	20.00	008	02	25.00	001	00	00.00
COP 00007	054	017	004	23.52	008	04	50.00	018	04	22.22	010	02	20.00
COP 00008	096	025	020	80.00	014	04	28.57	036	07	19.44	019	00	00.00
COP 00009	106	026	017	65.38	020	04	20.00	042	06	14.29	018	02	11.11
COP 000011	022	010	009	90.00	005	00	00.00	006	00	00.00	002	00	00.00
COP 000012	091	031	026	83.87	020	05	25.00	028	04	14.28	010	00	00.00
COP 000013	094	020	013	65.00	021	04	19.05	031	06	19.35	020	01	05.00
COP 000014	108	018	011	61.11	023	04	17.39	039	07	17.95	026	01	03.85
COP 000016	115	019	013	68.42	025	11	44.00	049	09	18.37	022	03	13.64
COP 000017	124	026	014	56.00	017	02	11.76	046	09	19.57	036	02	05.56
COP 000019	099	025	011	45.83	021	10	47.62	037	04	10.81	013	05	38.46
MBY 00002	092	018	013	72.22	017	07	41.18	042	10	23.80	015	01	06.67
TOTAL	1077	250	159	63.60	213	60	28.17	396	70	17.68	203	18	08.87

from February 17, 2004 to 100% of the isolates identified by ARA as cow in October and November 2003. ARP classified 396 isolates as horse, of which 70 isolates were confirmed by PFGE for a rate of only 17.68%. The isolates that ARP identified as horse were usually identified with PFGE as human; this may be due to the fact that PFGE horse isolates came from horses that were at a county fair in the first set of fecal sample collections, instead of horses on pasture or in the watershed area of the Copano Bay. Of 203 isolates classified as duck using ARP only 18 being confirmed with PFGE, a rate of 8.90%. The majority of duck isolates from PFGE were very similar to each other (from the winter duck collection only), which may account for the low confirmation of ARP duck classifications. A more diverse database of ducks was used for the antibiotic resistance database which probably explains the discrepancy in results between the two techniques.

Antibiotic resistance profile analysis

Unknown source isolates were separated by station and event to evaluate possible animal sources of the *E. coli*, so that sources of fecal contamination in Copano Bay could be identified. These are shown by table and pie-chart for each sampling event (Tables 40 to 47; Figs. 23 to 30) and for each station (Tables 48 to 61; Figs 31 to 44). For stations with more than 25 isolates for a sampling event individual station and event tables and charts are shown individually Tables 62 to 75; Figs. 45 to 56). While percentage of isolates classified as each source are shown, it should be stressed that this is only based on comparison with the Copano Bay library developed for the study. A certain level of misclassification between groups must be assumed. PFGE confirmations, especially for human/sewage source isolates, provide some additional level of confidence. Each station is discussed below, following the sequence

Table 40. Source identification for unknown *E. coli* isolates from sampling event 10/15/03 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	1	0	0	0	0	0	1
COP-00003	0	0	0	0	0	0	0
COP-00004	0	0	0	4	0	0	4
COP-00007	0	0	0	0	0	0	0
COP-00008	1	1	0	10	1	0	13
COP-00009	0	0	0	3	0	0	3
COP-00011	0	0	0	7	2	0	9
COP-00012	0	0	0	11	3	0	14
COP-00013	0	0	0	2	1	0	3
COP-00014	0	0	0	3	1	0	4
COP-00016	0	0	0	0	0	0	0
COP-00017	1	0	0	6	0	1	8
COP-00019	1	0	0	0	0	0	1
MBY-00002	1	0	0	5	0	0	6
TOTAL #'s	5	1	0	51	8	1	66
%	7.6%	1.5%	0.0%	77.3%	12.1%	1.5%	100.0%

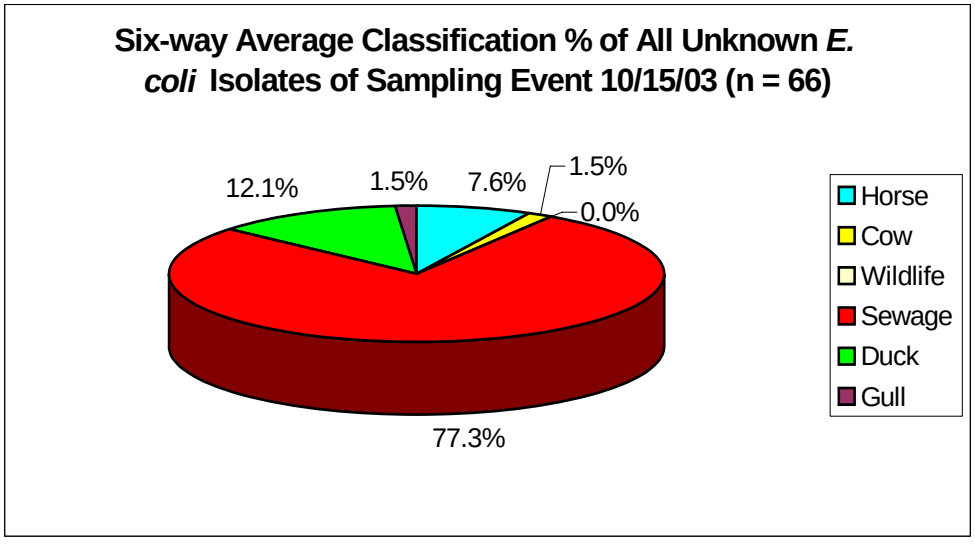


Figure. 23. Proportion of unknown isolates classified as each source for sampling event 10/15/03 for all stations.

Table 41. Source identification for unknown *E. coli* isolates from sampling event 11/17/03 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	0	0	0	0	0	0	0
COP-00003	0	0	0	0	0	0	0
COP-00004	0	0	0	0	0	0	0
COP-00007	0	0	0	1	1	0	2
COP-00008	0	0	0	2	2	1	5
COP-00009	0	0	0	6	6	0	12
COP-00011	0	0	0	0	0	0	0
COP-00012	0	0	0	2	1	0	3
COP-00013	0	0	0	3	5	1	9
COP-00014	0	0	0	0	0	0	0
COP-00016	3	1	0	11	4	0	19
COP-00017	0	0	0	0	0	0	0
COP-00019	7	1	0	33	5	0	46
MBY-00002	0	0	0	5	1	0	6
TOTAL #'s	10	2	0	63	25	2	102
%	9.8%	2.0%	0.0%	61.8%	24.5%	2.0%	100.0%

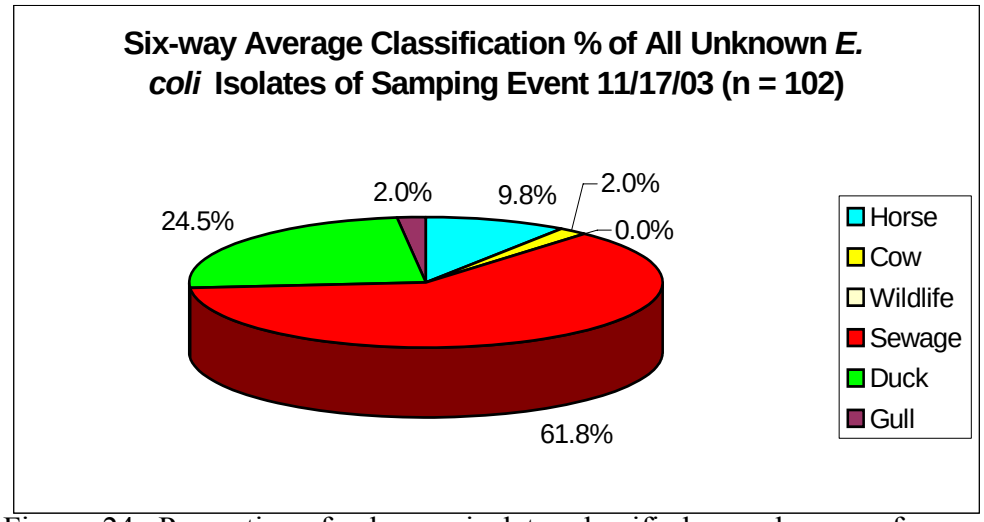


Figure. 24. Proportion of unknown isolates classified as each source for sampling event 11/17/03 for all stations.

Table 42. Source identification for unknown *E. coli* isolates from sampling event 12/17/03 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	0	0	0	0	0	0	0
COP-00003	0	0	0	0	0	0	0
COP-00004	0	0	0	1	0	0	1
COP-00007	0	0	0	1	0	0	1
COP-00008	0	0	0	0	0	0	0
COP-00009	0	0	0	2	1	0	3
COP-00011	0	0	0	1	0	0	1
COP-00012	6	2	0	6	1	0	15
COP-00013	4	2	0	10	2	0	18
COP-00014	6	0	0	0	14	0	20
COP-00016	7	0	0	0	4	0	11
COP-00017	33	2	0	6	48	0	89
COP-00019	3	0	0	1	3	0	7
MBY-00002	7	2	0	1	2	0	12
TOTAL #'s	66	8	0	29	75	0	178
%	37.1%	4.5%	0.0%	16.3%	42.1%	0.0%	100.0%

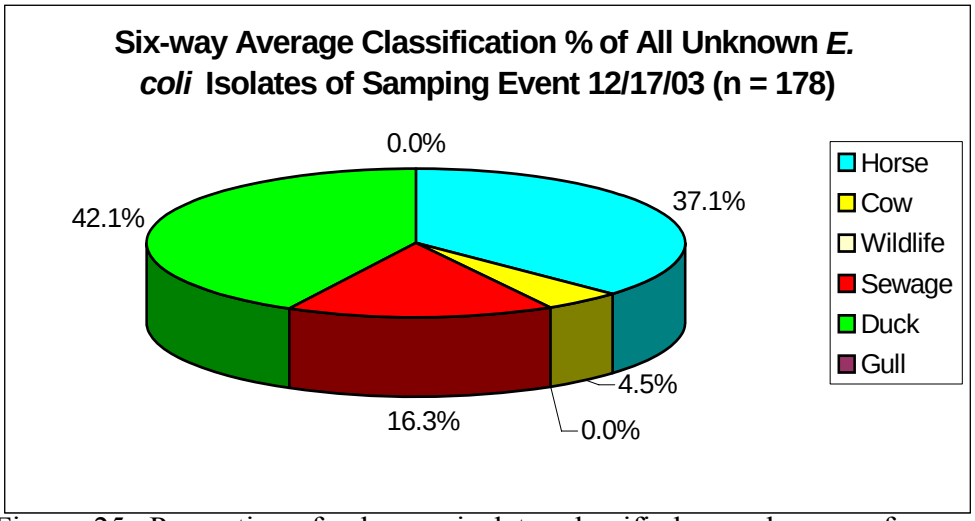


Figure. 25. Proportion of unknown isolates classified as each source for sampling event 12/17/03 for all stations.

Table 43. Source identification for unknown *E. coli* isolates from sampling event 01/08/04 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	Cow	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	5	1	0	4	1	0	11
COP-00003	8	9	0	4	1	1	23
COP-00004	1	0	0	0	0	0	1
COP-00007	2	1	0	0	1	0	4
COP-00008	1	3	0	2	0	1	7
COP-00009	2	0	0	1	0	0	3
COP-00011	3	3	0	0	0	0	6
COP-00012	2	2	0	0	0	0	4
COP-00013	10	11	0	3	2	0	26
COP-00014	9	1	0	0	0	0	10
COP-00016	3	1	0	0	0	0	4
COP-00017	5	5	0	0	0	0	10
COP-00019	57	56	0	25	14	7	159
MBY-00002	0	3	0	0	0	0	3
TOTAL #'s	108	96	0	39	19	9	271
%	39.9%	35.4%	0.0%	14.4%	7.0%	3.3%	100.0%

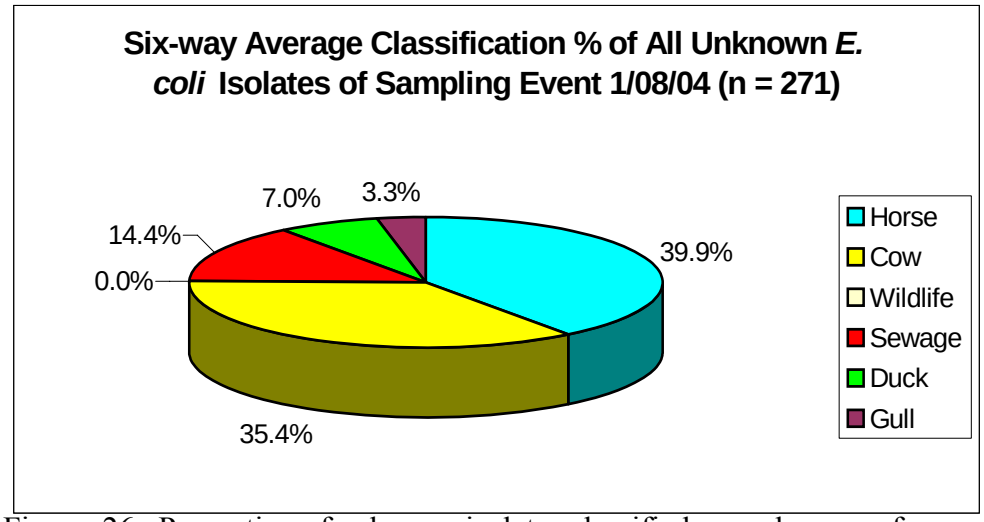


Figure. 26. Proportion of unknown isolates classified as each source for sampling event 01/08/04 for all stations.

Table 44. Source identification for unknown *E. coli* isolates from sampling event 02/17/04 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	0	0	0	0	0	0	0
COP-00003	0	0	0	0	0	0	0
COP-00004	0	0	0	0	0	0	0
COP-00007	0	0	0	0	0	0	0
COP-00008	0	0	0	1	0	0	1
COP-00009	0	0	0	0	0	0	0
COP-00011	0	0	0	0	0	0	0
COP-00012	0	0	0	0	0	0	0
COP-00013	0	0	0	0	0	0	0
COP-00014	0	0	0	1	1	0	2
COP-00016	0	0	0	0	0	0	0
COP-00017	8	5	0	3	6	0	22
COP-00019	0	0	0	0	0	0	0
MBY-00002	0	0	0	0	0	0	0
TOTAL #'s	8	5	0	5	7	0	25
%	32.0%	20.0%	0.0%	20.0%	28.0%	0.0%	100.0%

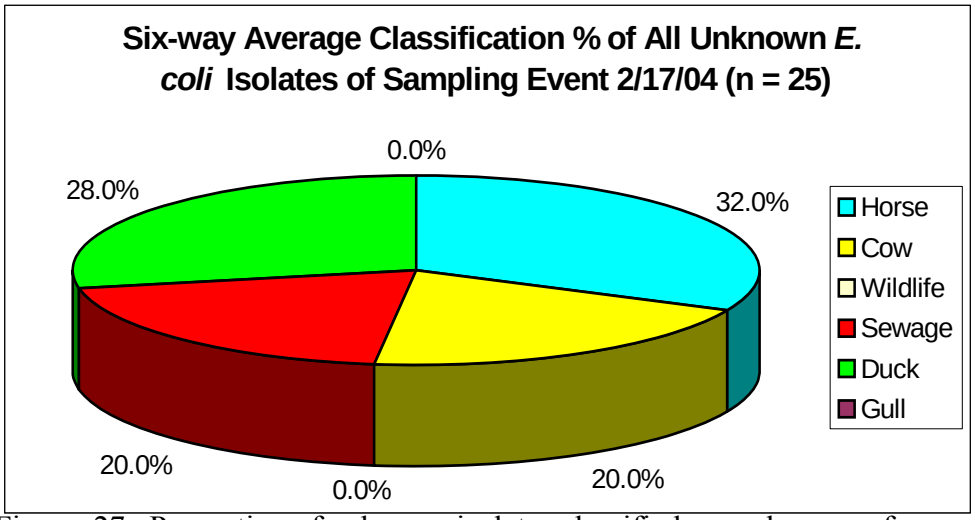


Figure. 27. Proportion of unknown isolates classified as each source for sampling event 02/17/04 for all stations.

Table 45. Source identification for unknown *E. coli* isolates from sampling event 02/26/04 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	0	0	0	0	1	0	1
COP-00003	0	3	0	2	3	1	9
COP-00004	1	5	0	0	0	0	6
COP-00007	1	0	0	0	0	0	1
COP-00008	1	0	0	0	0	0	1
COP-00009	2	0	0	0	0	0	2
COP-00011	0	1	0	0	0	0	1
COP-00012	9	10	0	1	3	2	25
COP-00013	80	30	0	17	31	2	160
COP-00014	42	42	0	19	9	0	112
COP-00016	26	9	0	5	15	0	55
COP-00017	49	36	0	56	63	0	204
COP-00019	3	7	0	2	1	2	15
MBY-00002	0	0	0	2	0	0	2
TOTAL #'s	214	143	0	104	126	7	594
%	36.0%	24.1%	0.0%	17.5%	21.2%	1.2%	100.0%

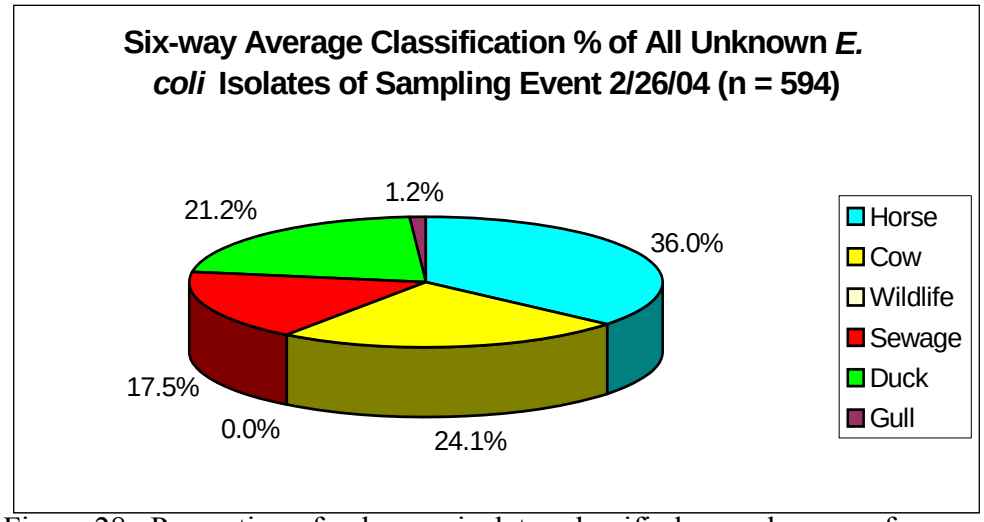


Figure 28. Proportion of unknown isolates classified as each source for sampling event 02/26/04 for all stations.

Table 46. Source identification for unknown *E. coli* isolates from sampling event 03/02/04 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	1	0	0	0	0	0	1
COP-00003	8	4	0	6	15	0	33
COP-00004	0	0	0	0	0	0	0
COP-00007	4	0	0	1	5	0	10
COP-00008	3	0	0	0	0	0	3
COP-00009	0	0	0	0	1	0	1
COP-00011	0	0	0	0	0	0	0
COP-00012	16	20	0	43	9	0	88
COP-00013	9	3	0	9	6	0	27
COP-00014	2	1	0	1	0	0	4
COP-00016	32	5	0	16	13	0	66
COP-00017	0	2	0	0	0	0	2
COP-00019	0	9	0	0	0	0	9
MBY-00002	3	5	0	3	2	0	13
TOTAL #'s	78	49	0	79	51	0	257
%	30.4%	19.1%	0.0%	30.7%	19.8%	0.0%	100.0%

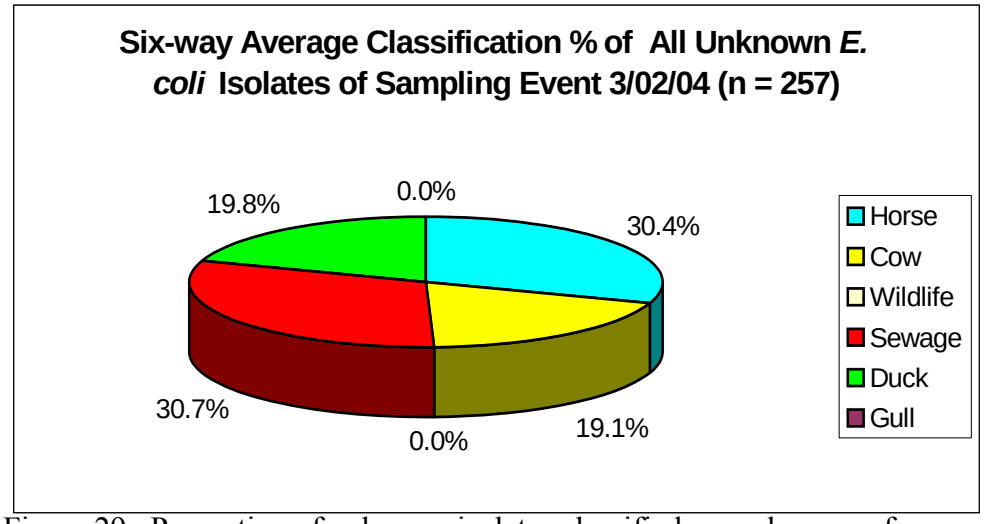


Figure 29. Proportion of unknown isolates classified as each source for sampling event 03/02/04 for all stations.

Table 47. Source identification for unknown *E. coli* isolates from sampling event 04/08/04 using SPSS Discriminant analysis six-way classification (all groups equal).

# of *E. coli* Isolates							
Station I.D.	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
COP-00001	2	5	0	2	2	0	11
COP-00003	0	0	0	0	0	0	0
COP-00004	9	10	0	4	2	0	25
COP-00007	17	10	0	18	8	1	54
COP-00008	100	46	2	61	42	1	252
COP-00009	64	25	0	27	21	1	138
COP-00011	5	4	0	4	1	0	14
COP-00012	60	15	0	33	23	0	131
COP-00013	51	32	2	32	48	0	165
COP-00014	50	14	1	15	74	2	156
COP-00016	19	29	0	9	8	0	65
COP-00017	35	22	0	20	16	0	93
COP-00019	9	6	0	8	5	0	28
MBY-00002	86	42	1	18	39	0	186
TOTAL #'s	507	260	6	251	289	5	1318
%	38.5%	19.7%	0.5%	19.0%	21.9%	0.4%	100.0%

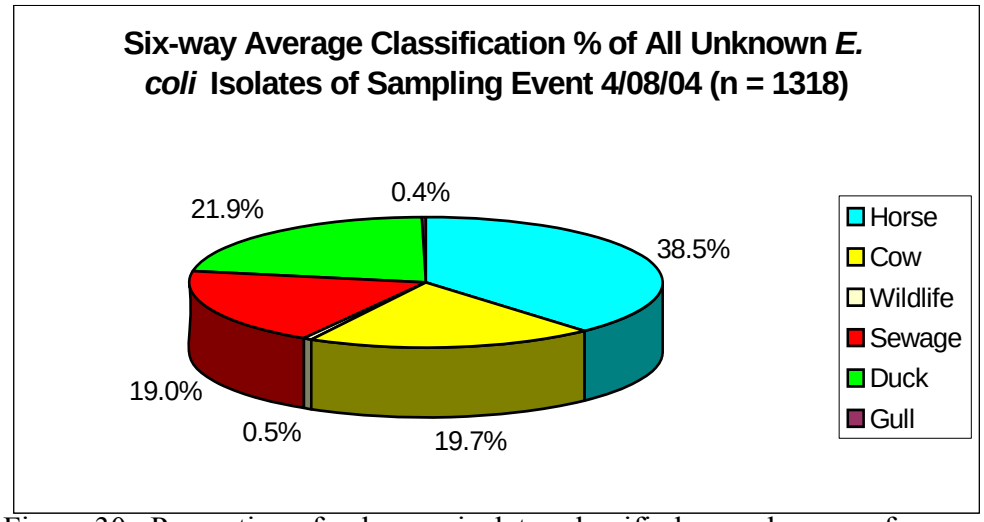


Figure 30. Proportion of unknown isolates classified as each source for sampling event 04/08/04 for all stations.

Table 48: Source identification for unknown isolates from station COP 00001 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	1	0	0	0	0	0	1
11/17/2003	0	0	0	0	0	0	0
12/17/2003	0	0	0	0	0	0	0
01/08/2004	5	1	0	4	1	0	11
02/17/2004	0	0	0	0	0	0	0
02/26/2004	0	0	0	0	1	0	1
03/02/2004	1	0	0	0	0	0	1
04/08/2004	2	5	0	2	2	0	11
Total	9	6	0	6	4	0	25
%	36.0%	24.0%	0.0%	24.0%	16.0%	0.0%	100.0%

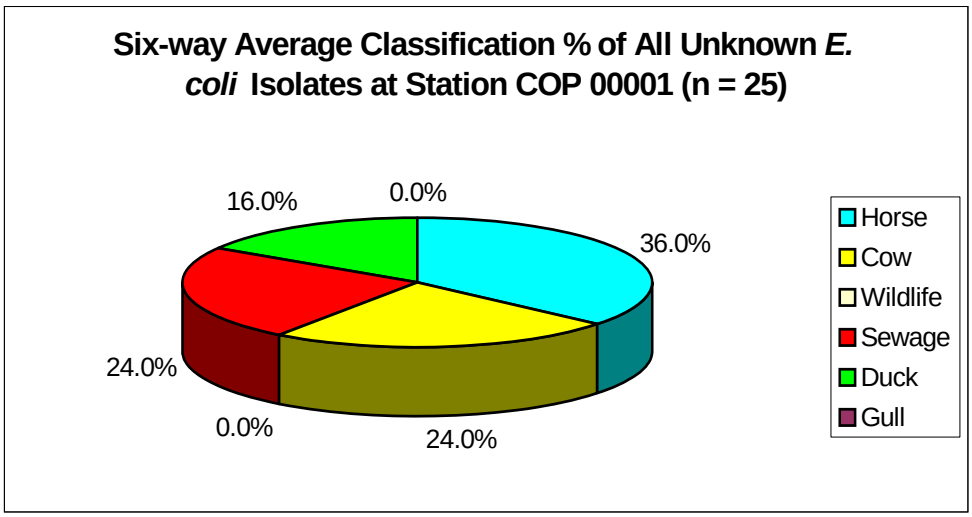


Figure 31. Proportion of unknown isolates classified as each source for station COP 00001 over all sampling events.

Table 49. Source identification for unknown isolates from station COP 00003 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	0	0	0	0
11/17/2003	0	0	0	0	0	0	0
12/17/2003	0	0	0	0	0	0	0
01/08/2004	8	9	0	4	1	1	23
02/17/2004	0	0	0	0	0	0	0
02/26/2004	0	3	0	2	3	1	9
03/02/2004	8	4	0	6	15	0	33
04/08/2004	0	0	0	0	0	0	0
Total	16	16	0	12	19	2	65
%	24.6%	24.6%	0.0%	18.5%	29.2%	3.1%	100.0%

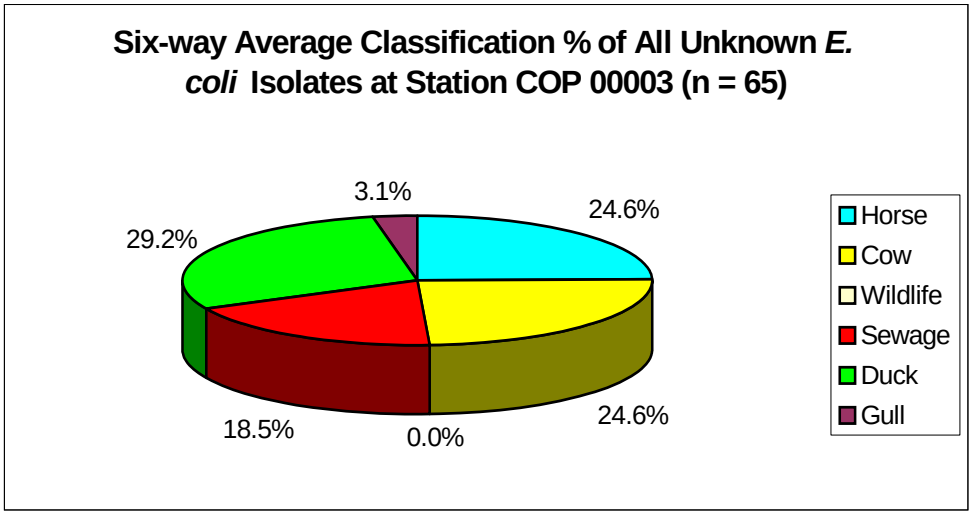


Figure 32. Proportion of unknown isolates classified as each source for station COP 00003 over all sampling events.

Table 50. Source identification for unknown isolates from station COP 00004 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	4	0	0	4
11/17/2003	0	0	0	0	0	0	0
12/17/2003	0	0	0	1	0	0	1
01/08/2004	1	0	0	0	0	0	1
02/17/2004	0	0	0	0	0	0	0
02/26/2004	1	5	0	0	0	0	6
03/02/2004	0	0	0	0	0	0	0
04/08/2004	9	10	0	4	2	0	25
Total	11	15	0	9	2	0	37
%	29.7%	40.5%	0.0%	24.3%	5.4%	0.0%	100.0%

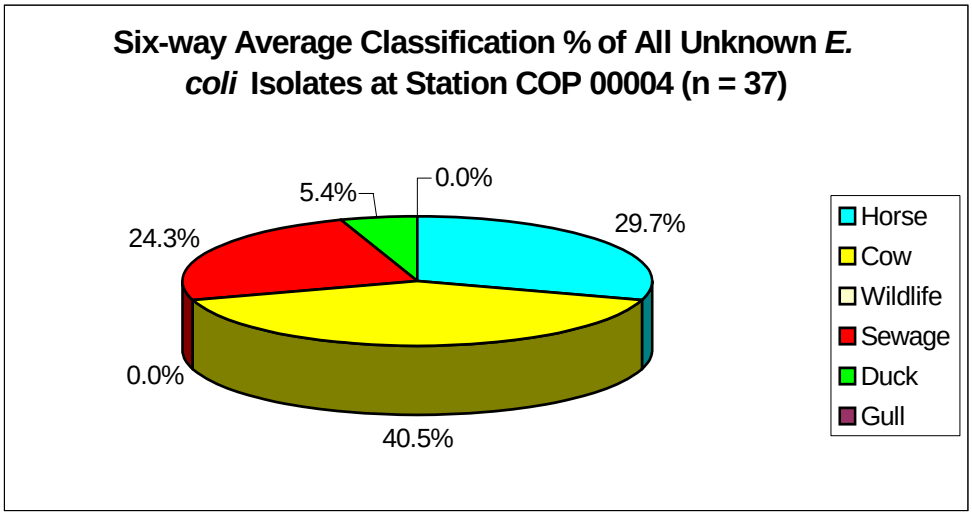


Figure 33. Proportion of unknown isolates classified as each source for station COP 00004 over all sampling events.

Table 51. Source identification for unknown isolates from station COP 00007 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	0	0	0	0
11/17/2003	0	0	0	1	1	0	2
12/17/2003	0	0	0	1	0	0	1
01/08/2004	2	1	0	0	1	0	4
02/17/2004	0	0	0	0	0	0	0
02/26/2004	1	0	0	0	0	0	1
03/02/2004	4	0	0	1	5	0	10
04/08/2004	17	10	0	18	8	1	54
Total	24	11	0	21	15	1	72
%	33.3%	15.3%	0.0%	29.2%	20.8%	1.4%	100.0%

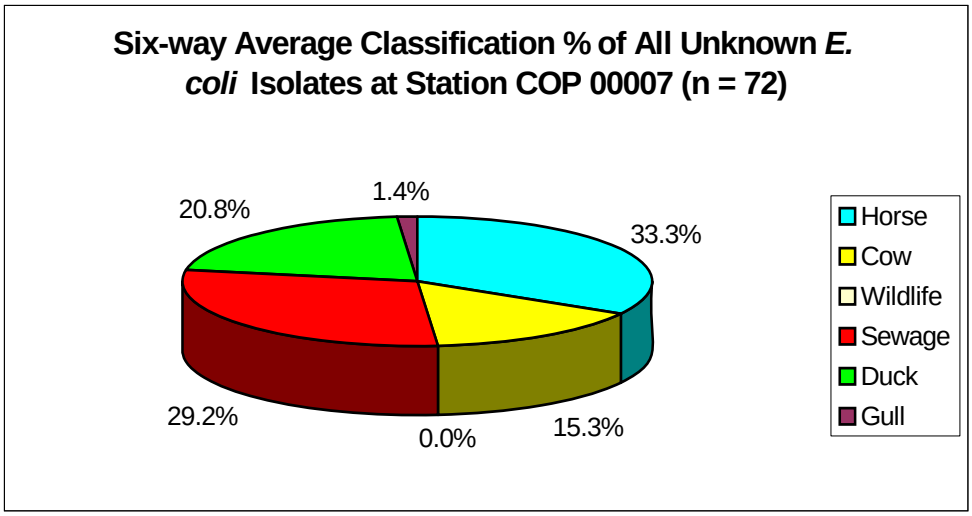


Figure 34. Proportion of unknown isolates classified as each source for station COP 00007 over all sampling events.

Table 52. Source identification for unknown isolates from station COP 00008 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	1	1	0	10	1	0	13
11/17/2003	0	0	0	2	2	1	5
12/17/2003	0	0	0	0	0	0	0
01/08/2004	1	3	0	2	0	1	7
02/17/2004	0	0	0	1	0	0	1
02/26/2004	1	0	0	0	0	0	1
03/02/2004	3	0	0	0	0	0	3
04/08/2004	100	46	2	61	42	1	252
Total	106	50	2	76	45	3	282
%	37.6%	17.7%	0.7%	27.0%	16.0%	1.1%	100.0%

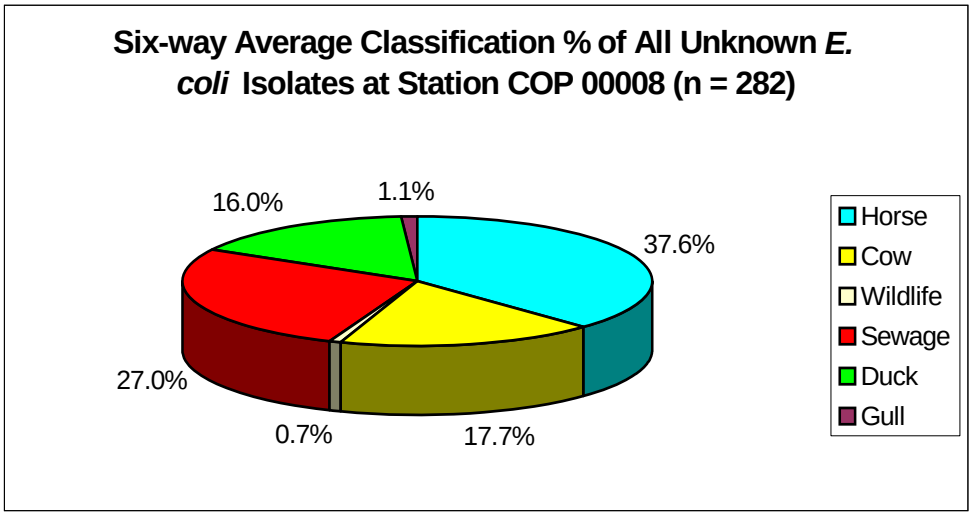


Figure 35. Proportion of unknown isolates classified as each source for station COP 00008 over all sampling events.

Table 53. Source identification for unknown isolates from station COP 00009 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	3	0	0	3
11/17/2003	0	0	0	6	6	0	12
12/17/2003	0	0	0	2	1	0	3
01/08/2004	2	0	0	1	0	0	3
02/17/2004	0	0	0	0	0	0	0
02/26/2004	2	0	0	0	0	0	2
03/02/2004	0	0	0	0	1	0	1
04/08/2004	64	25	0	27	21	1	138
Total	68	25	0	39	29	1	162
%	42.0%	15.4%	0.0%	24.1%	17.9%	0.6%	100.0%

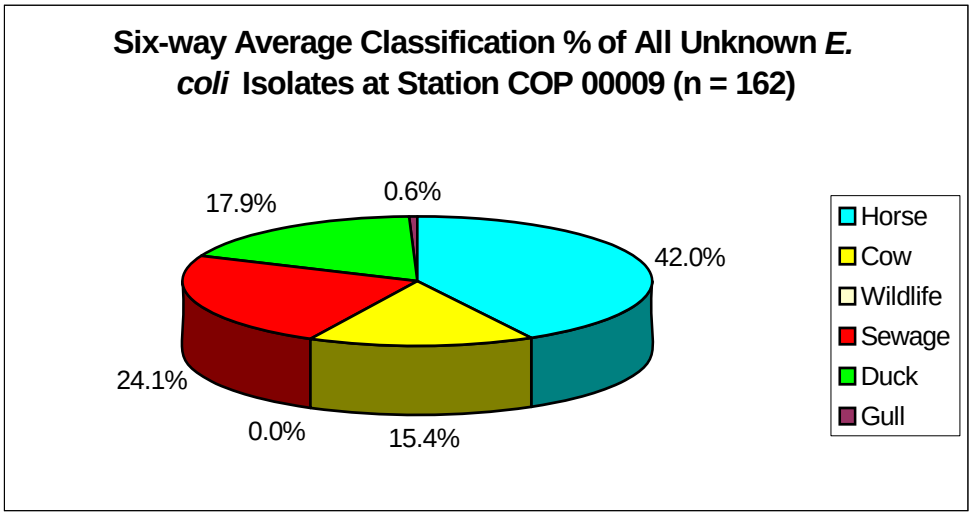


Figure 36. Proportion of unknown isolates classified as each source for station COP 00009 over all sampling events.

Table 54. Source identification for unknown isolates from station COP 00011 for each sampling event. SPSS Discriminant analysis Six-way Classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	7	2	0	9
11/17/2003	0	0	0	0	0	0	0
12/17/2003	0	0	0	1	0	0	1
01/08/2004	3	3	0	0	0	0	6
02/17/2004	0	0	0	0	0	0	0
02/26/2004	0	1	0	0	0	0	1
03/02/2004	0	0	0	0	0	0	0
04/08/2004	5	4	0	4	1	0	14
Total	8	8	0	0	12	3	31
%	25.8%	25.8%	0.0%	0.0%	38.7%	9.7%	100.0%

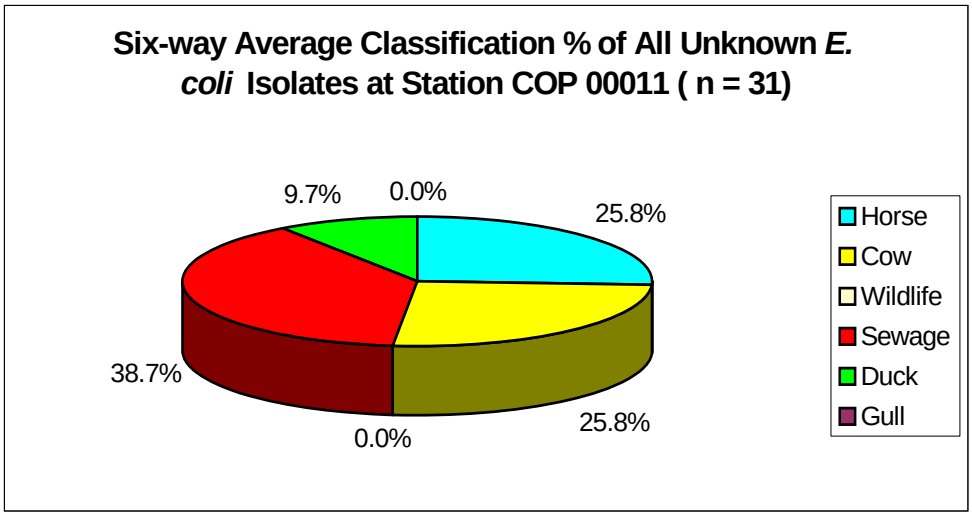


Figure 37. Proportion of unknown isolates classified as each source for station COP 00011 over all sampling events.

Table 55. Source identification for unknown isolates from station COP 00012 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	11	3	0	14
11/17/2003	0	0	0	2	1	0	3
12/17/2003	6	2	0	6	1	0	15
01/08/2004	2	2	0	0	0	0	4
02/17/2004	0	0	0	0	0	0	0
02/26/2004	9	10	0	1	3	2	25
03/02/2004	16	20	0	43	9	0	88
04/08/2004	60	15	0	33	23	0	131
Total	93	49	0	96	40	2	280
%	33.2%	17.5%	0.0%	34.3%	14.3%	0.7%	100.0%

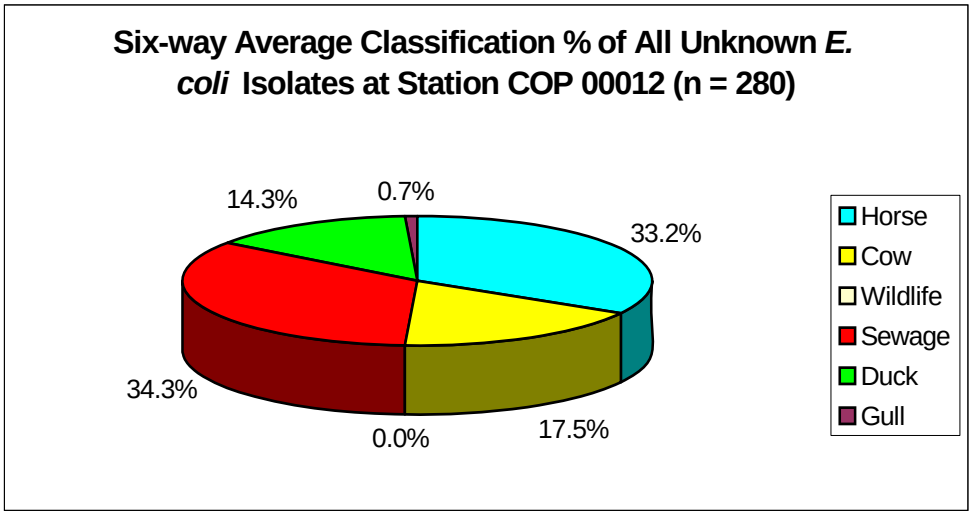


Figure 38. Proportion of unknown isolates classified as each source for station COP 00012 over all sampling events.

Table 56. Source identification for unknown isolates from station COP 00013 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	2	1	0	3
11/17/2003	0	0	0	3	5	1	9
12/17/2003	4	2	0	10	2	0	18
01/08/2004	10	11	0	3	2	0	26
02/17/2004	0	0	0	0	0	0	0
02/26/2004	80	30	0	17	31	2	160
03/02/2004	9	3	0	9	6	0	27
04/08/2004	51	32	2	32	48	0	165
Total	154	78	2	76	95	3	408
%	37.7%	19.1%	0.5%	18.6%	23.3%	0.7%	100.0%

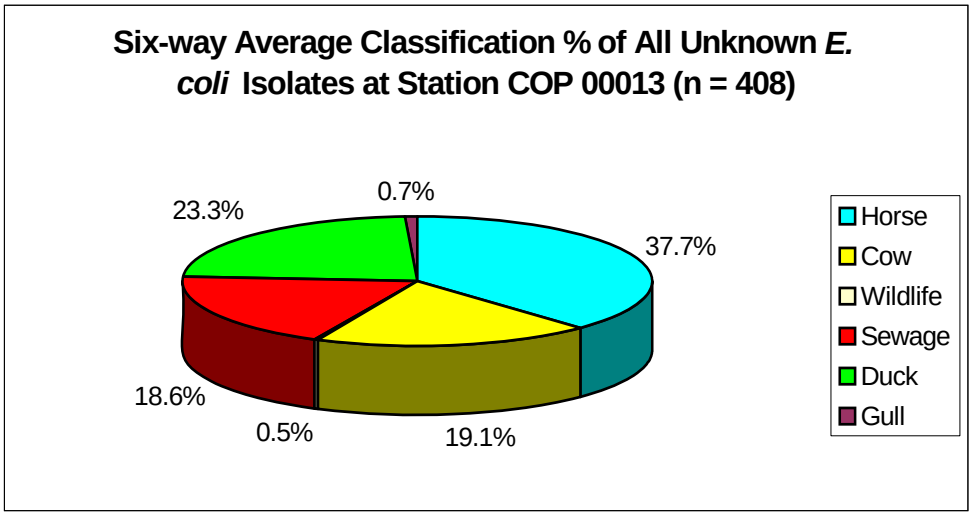


Figure 39. Proportion of unknown isolates classified as each source for station COP 00013 over all sampling events.

Table 57. Source identification for unknown isolates from station COP 00014 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	3	1	0	4
11/17/2003	0	0	0	0	0	0	0
12/17/2003	6	0	0	0	14	0	20
01/08/2004	9	1	0	0	0	0	10
02/17/2004	0	0	0	1	1	0	2
02/26/2004	42	42	0	19	9	0	112
03/02/2004	2	1	0	1	0	0	4
04/08/2004	50	14	1	15	74	2	156
Total	109	58	1	39	99	2	308
%	35.4%	18.8%	0.3%	12.7%	32.1%	0.6%	100.0%

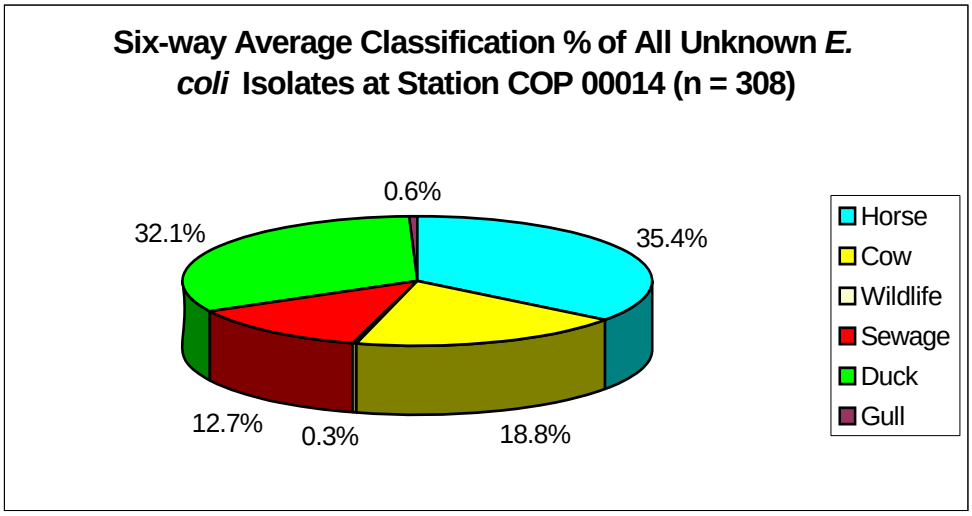


Figure 40. Proportion of unknown isolates classified as each source for station COP 00014 over all sampling events.

Table 58. Source identification for unknown isolates from station COP 00016 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	0	0	0	0	0	0	0
11/17/2003	3	1	0	11	4	0	19
12/17/2003	7	0	0	0	4	0	11
01/08/2004	3	1	0	0	0	0	4
02/17/2004	0	0	0	0	0	0	0
02/26/2004	26	9	0	5	15	0	55
03/02/2004	32	5	0	16	13	0	66
04/08/2004	19	29	0	9	8	0	65
Total	90	45	0	41	44	0	220
%	40.9%	20.5%	0.0%	18.6%	20.0%	0.0%	100.0%

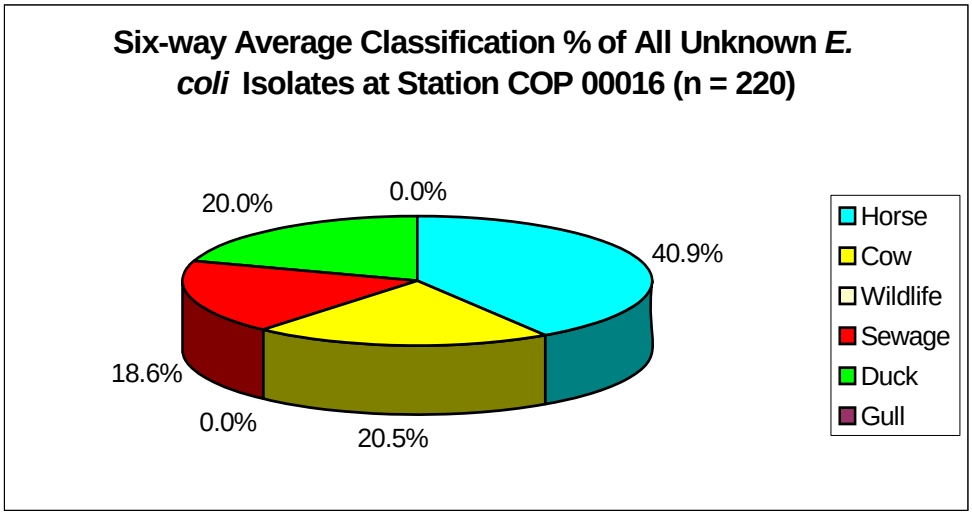


Figure 41. Proportion of unknown isolates classified as each source for station COP 00016 over all sampling events.

Table 59. Source identification for unknown isolates from sStation COP 00017 for each sampling event. SPSS Discriminant Analysis Six-way Classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	1	0	0	6	0	1	8
11/17/2003	0	0	0	0	0	0	0
12/17/2003	33	2	0	6	48	0	89
01/08/2004	5	5	0	0	0	0	10
02/17/2004	8	5	0	3	6	0	22
02/26/2004	49	36	0	56	63	0	204
03/02/2004	0	2	0	0	0	0	2
04/08/2004	35	22	0	20	16	0	93
Total	131	72	0	91	133	1	428
%	30.6%	16.8%	0.0%	21.3%	31.1%	0.2%	100.0%

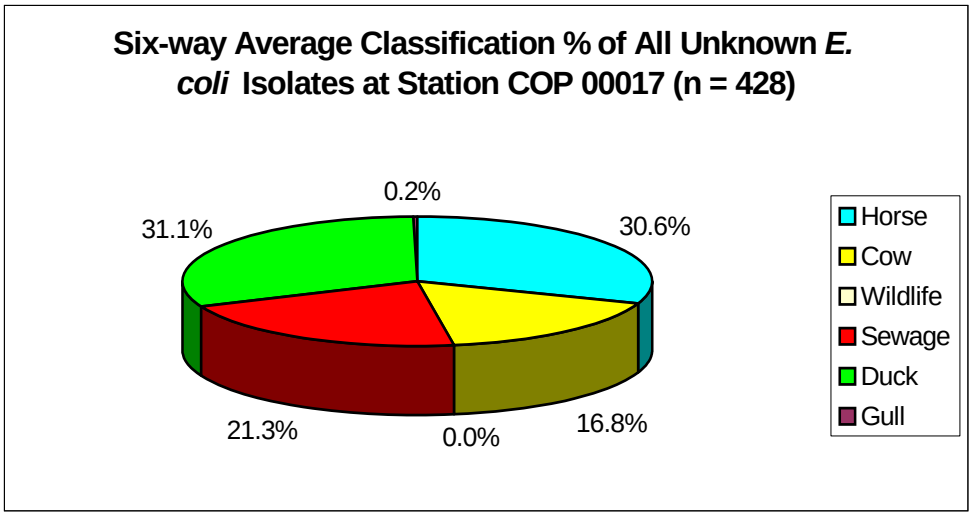


Figure 42. Proportion of unknown isolates classified as each source for station COP 00017 over all sampling events.

Table 60. Source identification for unknown isolates from station COP 00019 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	Cow	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	1	0	0	0	0	0	1
11/17/2003	7	1	0	33	5	0	46
12/17/2003	3	0	0	1	3	0	7
01/08/2004	57	56	0	25	14	7	159
02/17/2004	0	0	0	0	0	0	0
02/26/2004	3	7	0	2	1	2	15
03/02/2004	0	9	0	0	0	0	9
04/08/2004	9	6	0	8	5	0	28
Total	80	79	0	69	28	9	265
%	30.2%	29.8%	0.0%	26.0%	10.6%	3.4%	100.0%

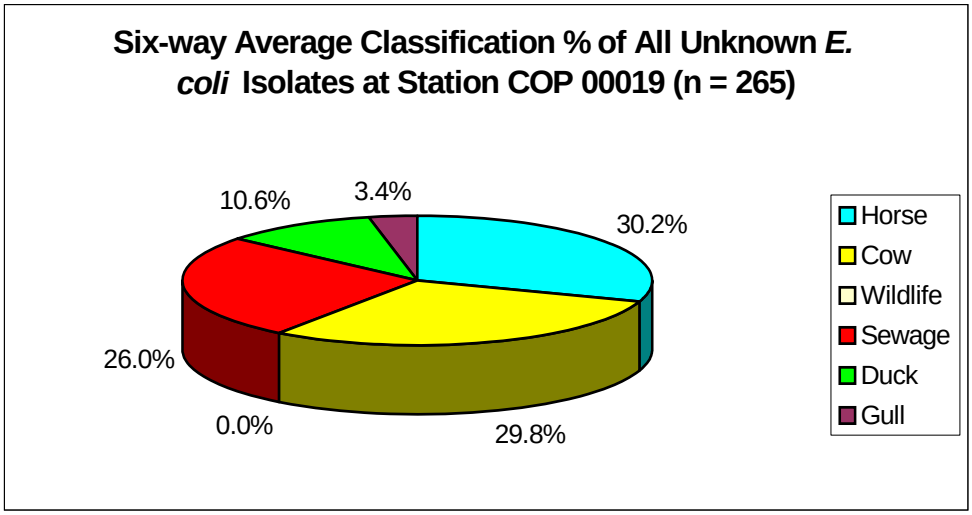


Figure 43. Proportion of unknown isolates classified as each source for station COP 00019 over all sampling events.

Table 61. Source identification for unknown isolates from station MYB 00002 for each sampling event. SPSS Discriminant analysis six-way classification (all groups equal).

Number of *E. coli* Isolates							
Sampling Event	**Horse**	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total**
10/15/2003	1	0	0	5	0	0	6
11/17/2003	0	0	0	5	1	0	6
12/17/2003	7	2	0	1	2	0	12
01/08/2004	0	3	0	0	0	0	3
02/17/2004	0	0	0	0	0	0	0
02/26/2004	0	0	0	2	0	0	2
03/02/2004	3	5	0	3	2	0	13
04/08/2004	86	42	1	18	39	0	186
Total	97	52	1	34	44	0	228
%	42.5%	22.8%	0.4%	14.9%	19.3%	0.0%	100.0%

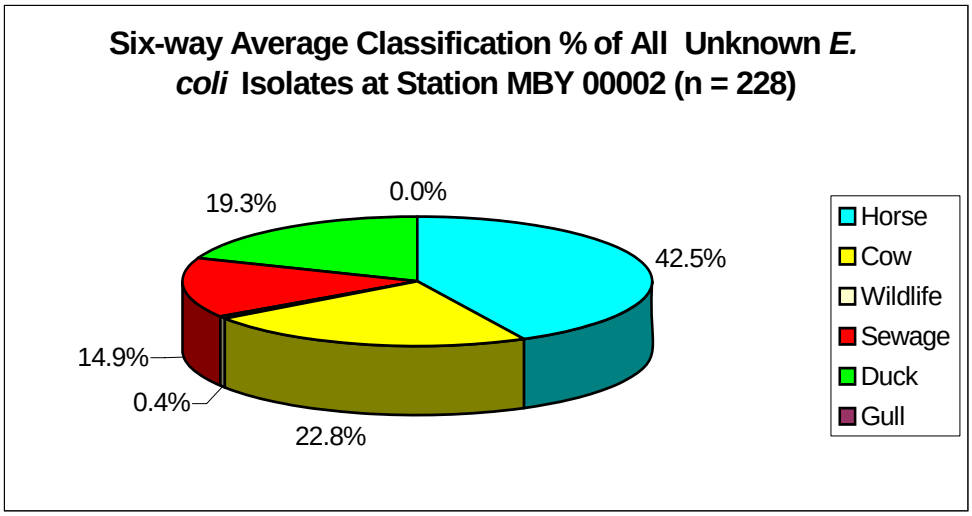


Figure 44. Proportion of unknown isolates classified as each source for station MYB 00002 over all sampling events.

Table 62. Percent classification of *E. coli* isolates for each sampling event from station COP 00001 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	100.0	0.0	0.0	0.0	0.0	0.0	1
11/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
12/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
01/08/2004	45.5	9.1	0.0	36.4	9.1	0.0	11
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	0.0	0.0	0.0	0.0	100.0	0.0	1
03/02/2004	100.0	0.0	0.0	0.0	0.0	0.0	1
04/08/2004	18.2	45.5	0.0	18.2	18.2	0.0	11
Total							25

Table 63. Percent classification of *E. coli* isolates for each sampling event from station COP 00003 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total Isolates**
10/15/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
11/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
12/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
01/08/2004	34.8	39.1	0.0	17.4	4.3	4.3	23
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	0.0	33.3	0.0	22.2	33.3	11.1	9
03/02/2004	24.2	12.1	0.0	18.2	45.5	0.0	33
04/08/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
Total							65

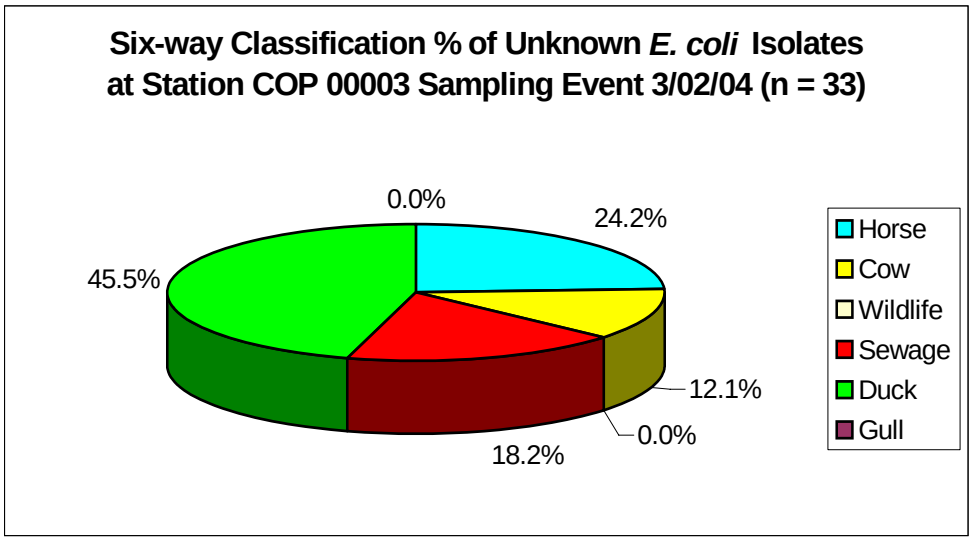


Figure 45. Proportion of unknown isolates classified as each source for station COP 00003 for sampling event 03/02/04.

Table 64. Percent classification of *E. coli* isolates for each sampling event from station COP 00004 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	0.0	0.0	0.0	100.0	0.0	0.0	4
11/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
12/17/2003	0.0	0.0	0.0	100.0	0.0	0.0	1
01/08/2004	100.0	0.0	0.0	0.0	0.0	0.0	1
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	16.7	83.3	0.0	0.0	0.0	0.0	6
03/02/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
04/08/2004	36.0	40.0	0.0	16.0	8.0	0.0	25
Total							37

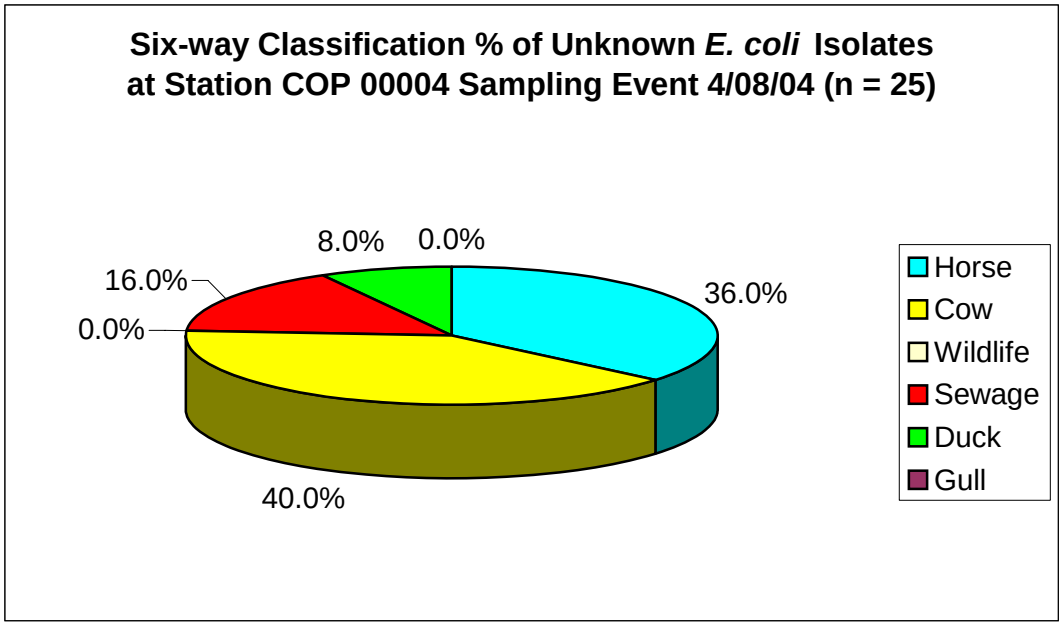


Figure 46. Proportion of unknown isolates classified as each source for station COP 00004 for sampling event 04/08/04.

Table 65. Percent classification of *E. coli* isolates for each sampling event from station COP 00007 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
11/17/2003	0.0	0.0	0.0	50.0	50.0	0.0	2
12/17/2003	0.0	0.0	0.0	100.0	0.0	0.0	1
01/08/2004	50.0	25.0	0.0	0.0	25.0	0.0	4
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	100.0	0.0	0.0	0.0	0.0	0.0	1
03/02/2004	40.0	0.0	0.0	10.0	50.0	0.0	10
04/08/2004	31.5	18.5	0.0	33.3	14.8	2.0	54
Total							72

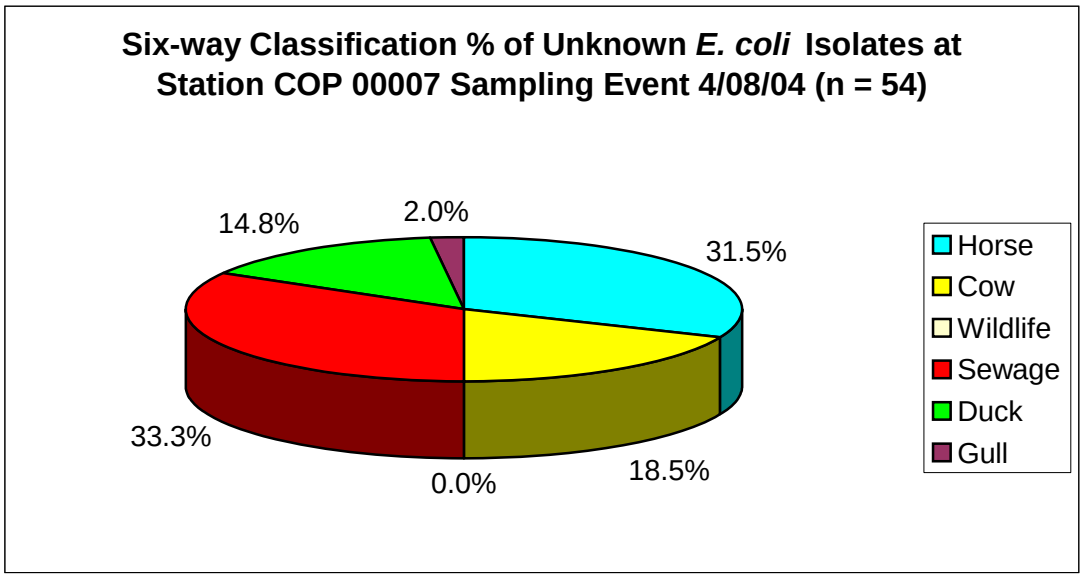


Figure 47. Proportion of unknown isolates classified as each source for station COP 00007 for sampling event 04/08/04.

Table 66. Percent classification of *E. coli* isolates for each sampling event from station COP 00008 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	7.7	7.7	0.0	76.9	7.7	0.0	13
11/17/2003	0.0	0.0	0.0	40.0	40.0	20.0	5
12/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
01/08/2004	14.3	42.9	0.0	28.6	0.0	14.3	7
02/17/2004	0.0	0.0	0.0	100.0	0.0	0.0	1
02/26/2004	100.0	0.0	0.0	0.0	0.0	0.0	1
03/02/2004	100.0	0.0	0.0	0.0	0.0	0.0	3
04/08/2004	39.7	18.3	0.8	24.2	16.7	0.4	252
Total							282

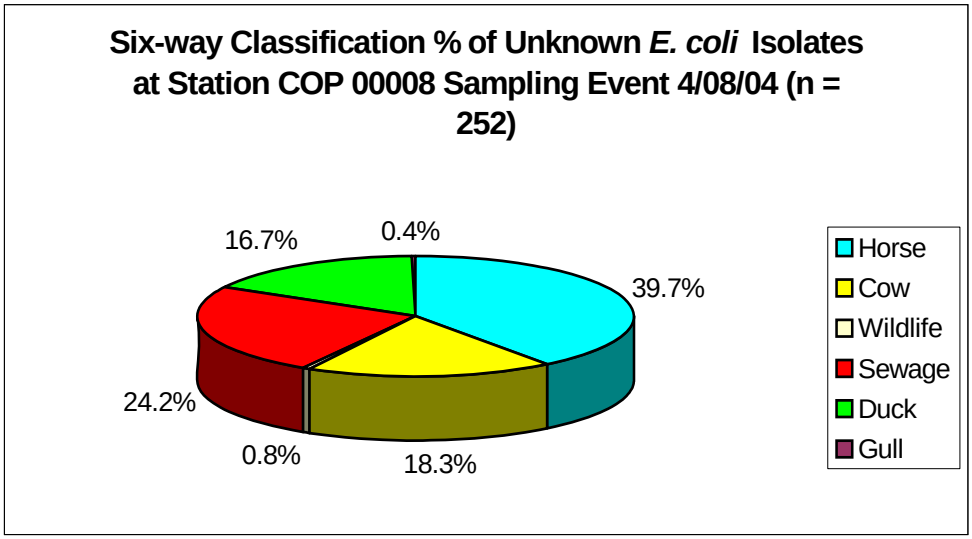


Figure 48. Proportion of unknown isolates classified as each source for station COP 00008 for sampling event 04/08/04.

Table 67. Percent classification of *E. coli* isolates for each sampling event from station COP 00009 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	0.0	0.0	0.0	100.0	0.0	0.0	3
11/17/2003	0.0	0.0	0.0	50.0	50.0	0.0	12
12/17/2003	0.0	0.0	0.0	66.7	33.3	0.0	3
01/08/2004	66.7	0.0	0.0	33.3	0.0	0.0	3
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	100.0	0.0	0.0	0.0	0.0	0.0	2
03/02/2004	0.0	0.0	0.0	0.0	100.0	0.0	1
04/08/2004	46.4	18.1	0.0	19.6	15.2	0.7	138
Total							162

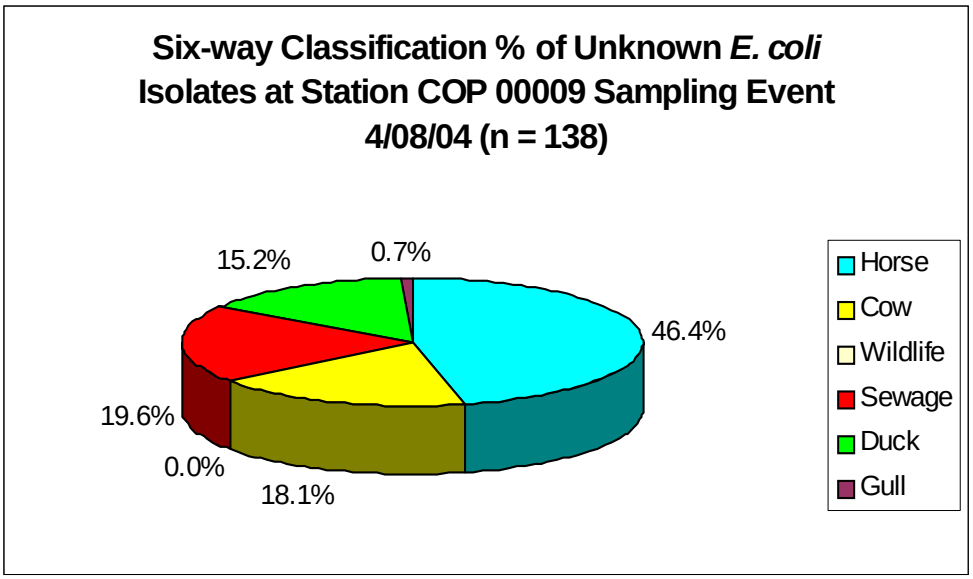


Figure 49. Proportion of unknown isolates classified as each source for station COP 00009 for sampling event 04/08/04.

Table 68. Percent classification of *E. coli* isolates for each sampling event from station COP 00011 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	0.0	0.0	0.0	77.8	22.2	0.0	9
11/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
12/17/2003	0.0	0.0	0.0	100.0	0.0	0.0	1
01/08/2004	50.0	50.0	0.0	0.0	0.0	0.0	6
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	0.0	100.0	0.0	0.0	0.0	0.0	1
03/02/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
04/08/2004	35.7	28.6	0.0	28.6	7.1	0.0	14
Total							31

Table 69. Percent classification of *E. coli* isolates for each sampling event from station COP 00012 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total Isolates**
10/15/2003	0.0	0.0	0.0	78.6	21.4	0.0	14
11/17/2003	0.0	0.0	0.0	66.7	33.3	0.0	3
12/17/2003	40.0	13.3	0.0	40.0	6.7	0.0	15
01/08/2004	50.0	50.0	0.0	0.0	0.0	0.0	4
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	36.0	40.0	0.0	4.0	12.0	8.0	25
03/02/2004	18.2	22.7	0.0	48.9	10.2	0.0	88
04/08/2004	45.8	11.5	0.0	25.2	17.6	0.0	131
Total							280

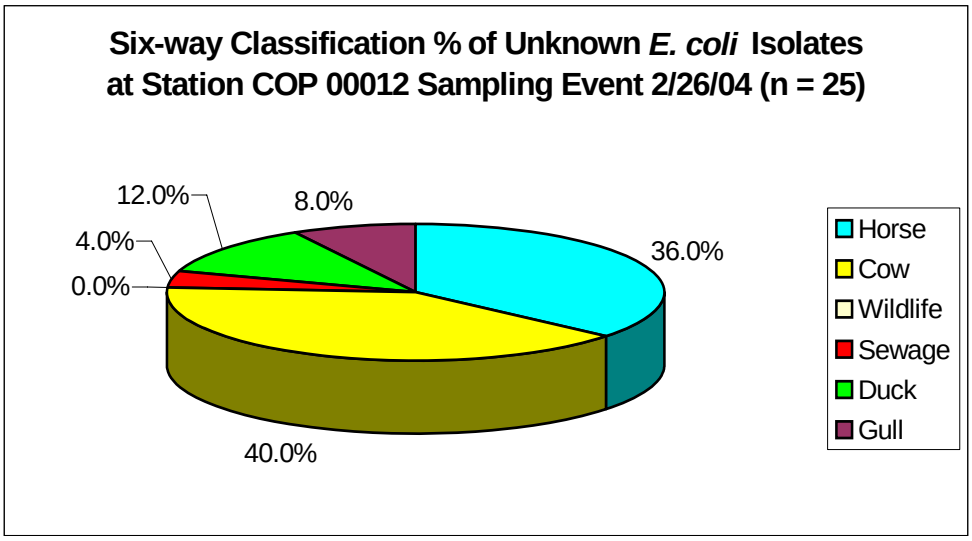


Figure 50a. Proportion of unknown isolates classified as each source for station COP 00012 for sampling event 2/26/04.

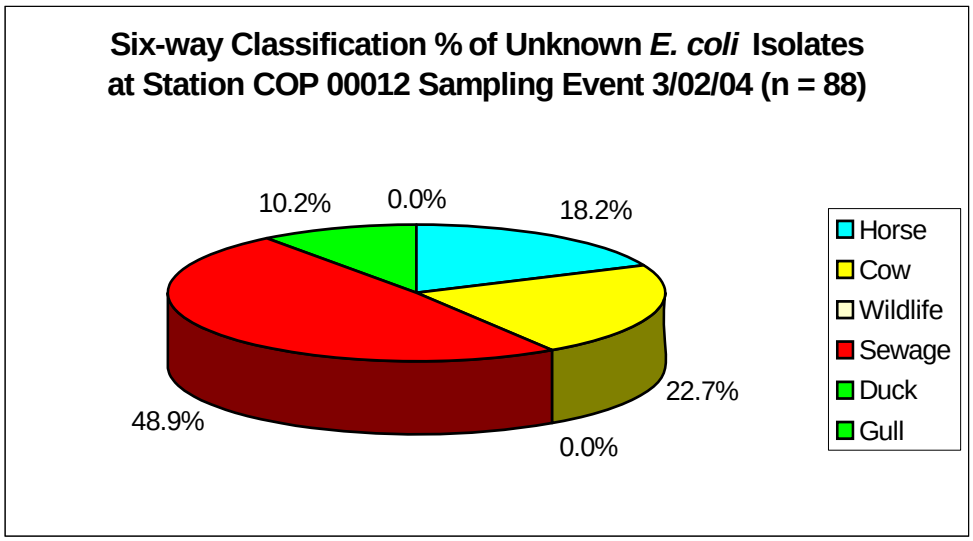


Figure 50b. Proportion of unknown isolates classified as each source for station COP 00012 for sampling event 03/02/04.

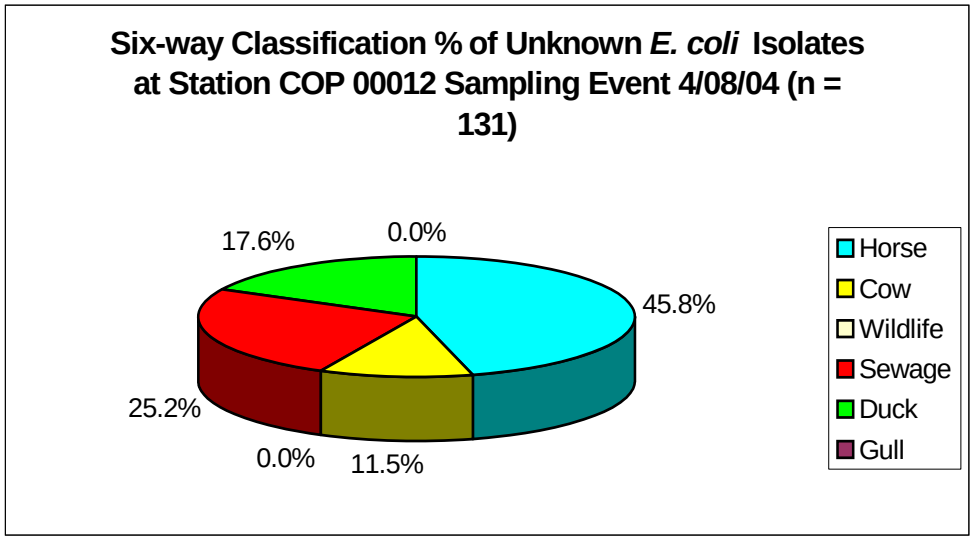


Figure 50c. Proportion of unknown isolates classified as each source for station COP 00012 for sampling event 04/08/04.

Table 70. Percent classification of *E. coli* isolates for each sampling event from station COP 00013 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	0.0	0.0	0.0	66.7	33.3	0.0	3
11/17/2003	0.0	0.0	0.0	33.3	55.6	11.1	9
12/17/2003	22.2	11.1	0.0	55.6	11.1	0.0	18
01/08/2004	38.5	42.3	0.0	11.5	7.7	0.0	26
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	50.0	18.8	0.0	10.6	19.4	1.3	160
03/02/2004	33.3	11.1	0.0	33.3	22.2	0.0	27
04/08/2004	30.9	19.4	1.2	19.4	29.1	0.0	165
Total							408

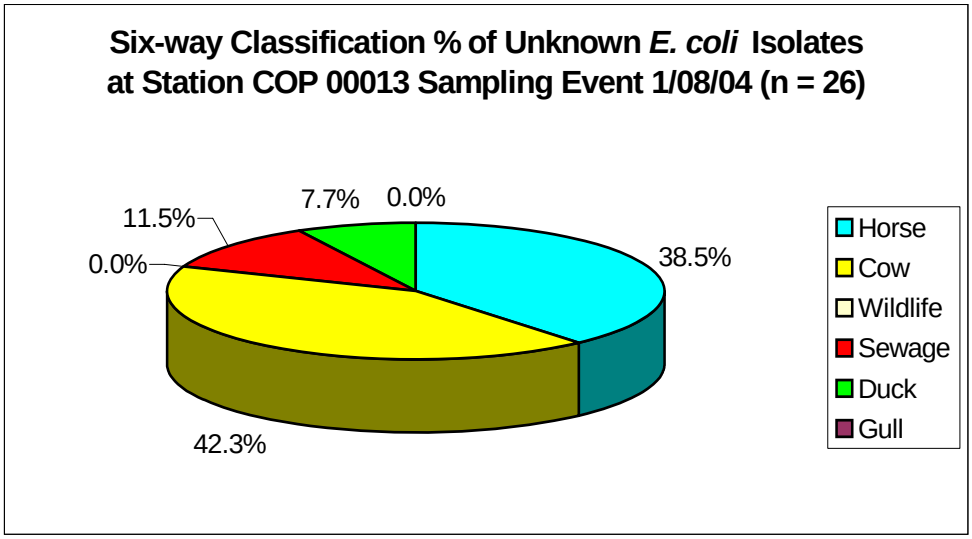


Figure 51a. Proportion of unknown isolates classified as each source for station COP 00013 for sampling event 01/08/04.

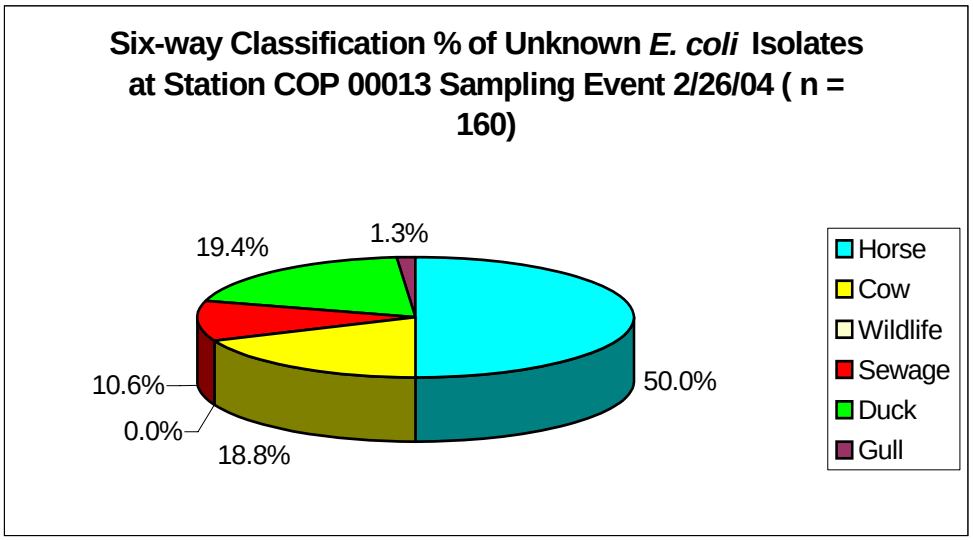


Figure 51b. Proportion of unknown isolates classified as each source for station COP 00013 for sampling event 02/26/04.

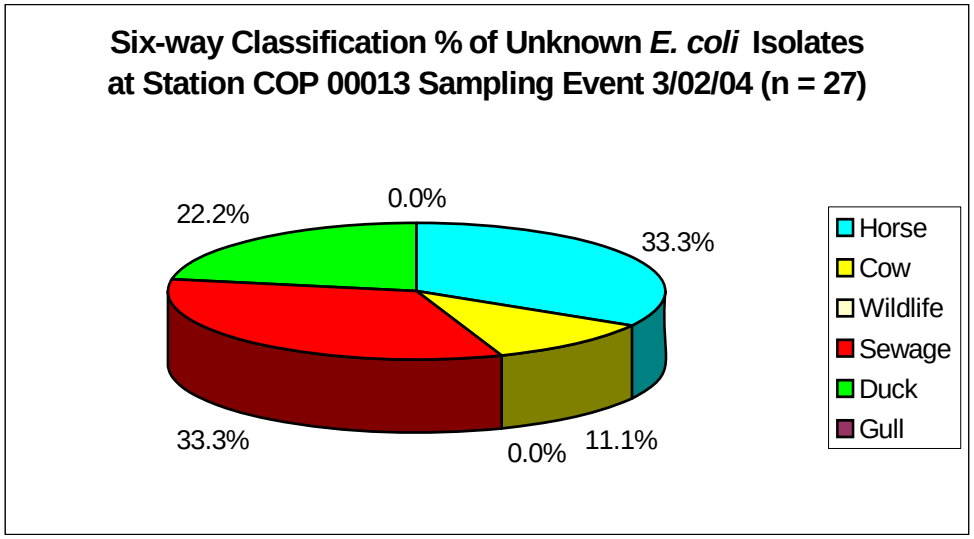


Figure 51c. Proportion of unknown isolates classified as each source for station COP 00013 for sampling event 03/02/04.

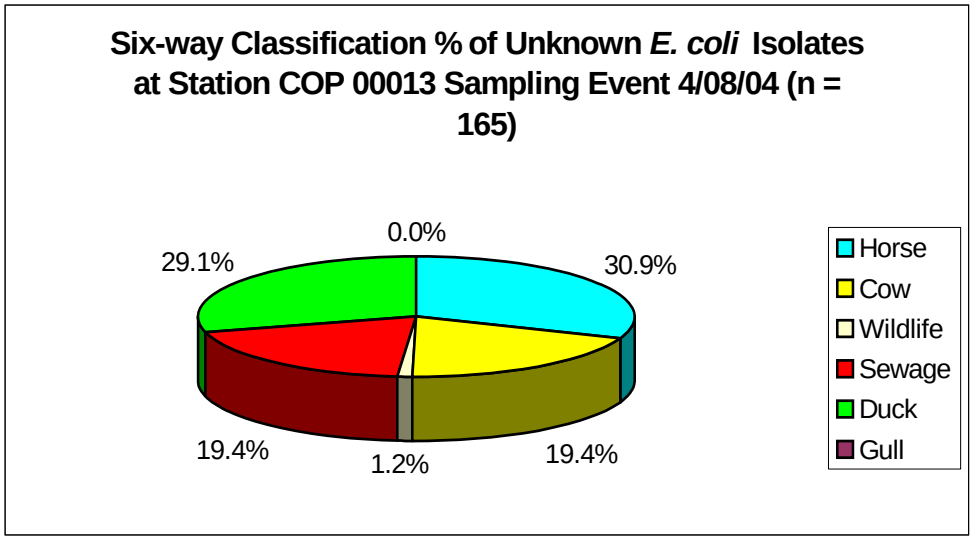


Figure 51d. Proportion of unknown isolates classified as each source for station COP 00013 for sampling event 04/08/04.

Table 71. Percent classification of *E. coli* isolates for each sampling event from station COP 00014 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	**Cow**	**Wildlife**	**Sewage**	**Duck**	**Gull**	**Total Isolates**
10/15/2003	0.0	0.0	0.0	75.0	25.0	0.0	4
11/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
12/17/2003	30.0	0.0	0.0	0.0	70.0	0.0	20
01/08/2004	90.0	10.0	0.0	0.0	0.0	0.0	10
02/17/2004	0.0	0.0	0.0	50.0	50.0	0.0	2
02/26/2004	37.5	37.5	0.0	17.0	8.0	0.0	112
03/02/2004	50.0	25.0	0.0	25.0	0.0	0.0	4
04/08/2004	32.1	9.0	0.6	9.6	47.4	1.3	156
Total							308

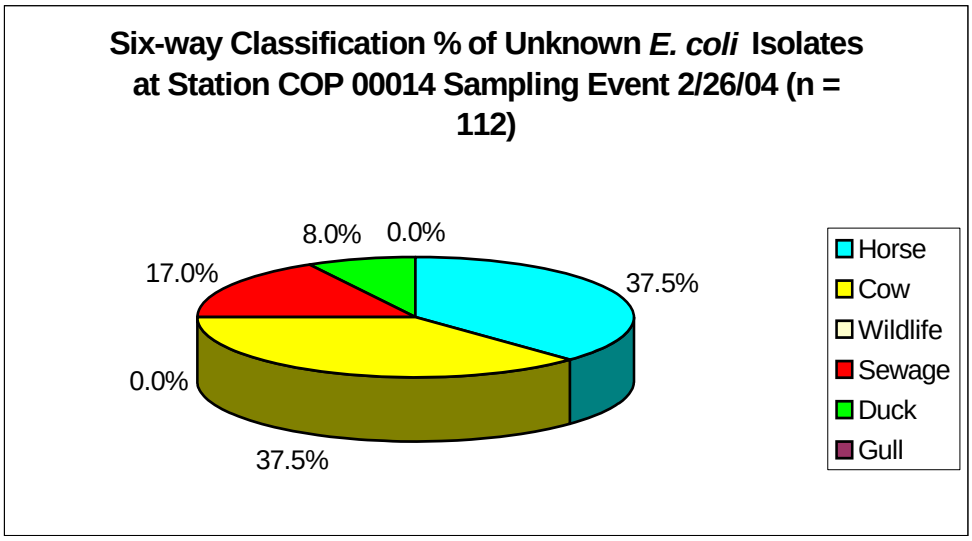


Figure 52a. Proportion of unknown isolates classified as each source for station COP 00014 for sampling event 02/26/04.

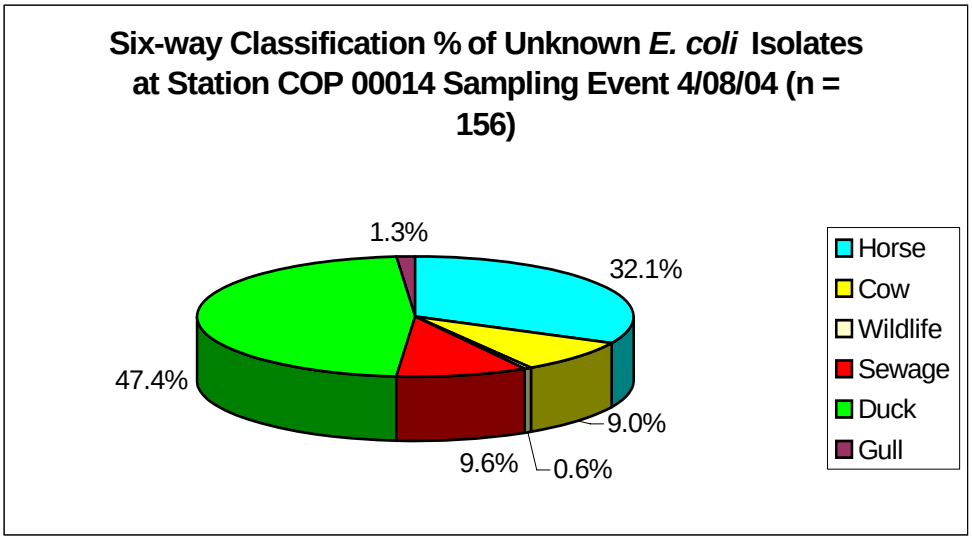


Figure 52b. Proportion of unknown isolates classified as each source for station COP 00014 for sampling event 04/08/04.

Table 72. Percent classification of *E. coli* isolates for each sampling event from station COP 00016 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
11/17/2003	15.8	5.3	0.0	57.9	21.1	0.0	19
12/17/2003	63.6	0.0	0.0	0.0	36.4	0.0	11
01/08/2004	75.0	25.0	0.0	0.0	0.0	0.0	4
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	47.3	16.4	0.0	9.1	27.3	0.0	55
03/02/2004	48.5	7.6	0.0	24.2	19.7	0.0	66
04/08/2004	29.2	44.6	0.0	13.8	12.3	0.0	65
Total							220

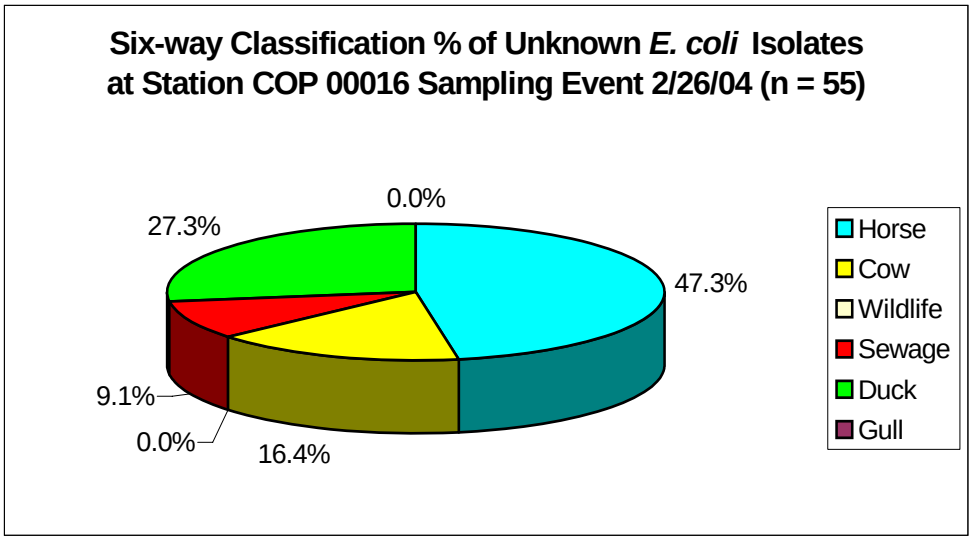


Figure 53a. Proportion of unknown isolates classified as each source for station COP 00016 for sampling event 02/26/04.

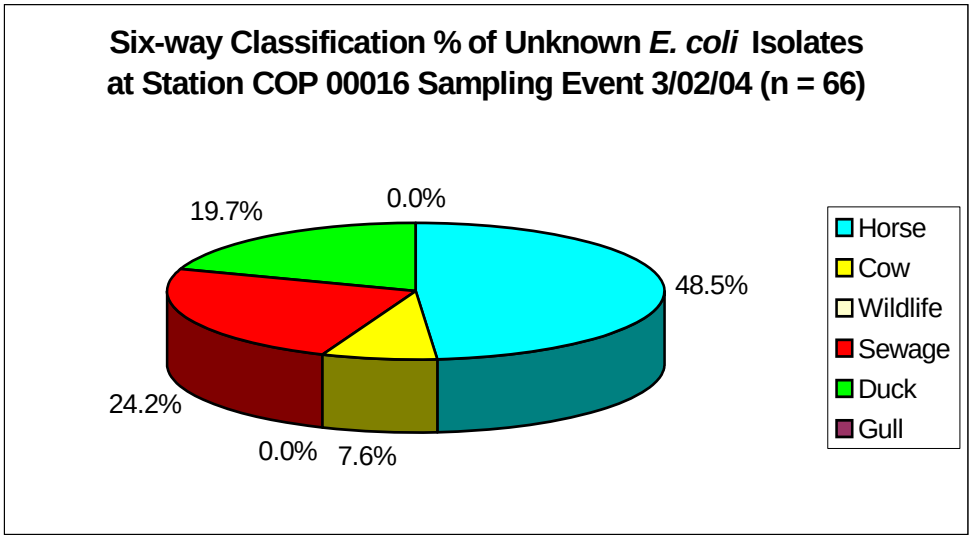


Figure 53b. Proportion of unknown isolates classified as each source for station COP 00016 for sampling event 03/02/04.

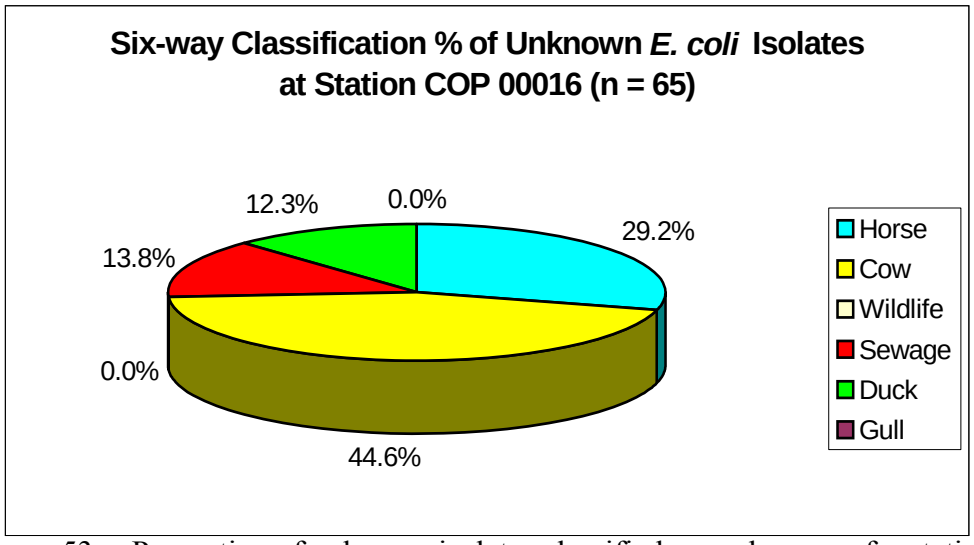


Figure 53c. Proportion of unknown isolates classified as each source for station COP 00016 for sampling event 04/08/04.

Table 73. Percent classification of *E. coli* isolates for each sampling event from station COP 00017 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	12.5	0.0	0.0	75.0	0.0	12.5	8
11/17/2003	0.0	0.0	0.0	0.0	0.0	0.0	0
12/17/2003	37.1	2.2	0.0	6.7	53.9	0.0	89
01/08/2004	50.0	50.0	0.0	0.0	0.0	0.0	10
02/17/2004	36.4	22.7	0.0	13.6	27.3	0.0	22
02/26/2004	24.0	17.6	0.0	27.5	30.9	0.0	204
03/02/2004	0.0	100.0	0.0	0.0	0.0	0.0	2
04/08/2004	37.6	23.7	0.0	21.5	17.2	0.0	93
Total							428

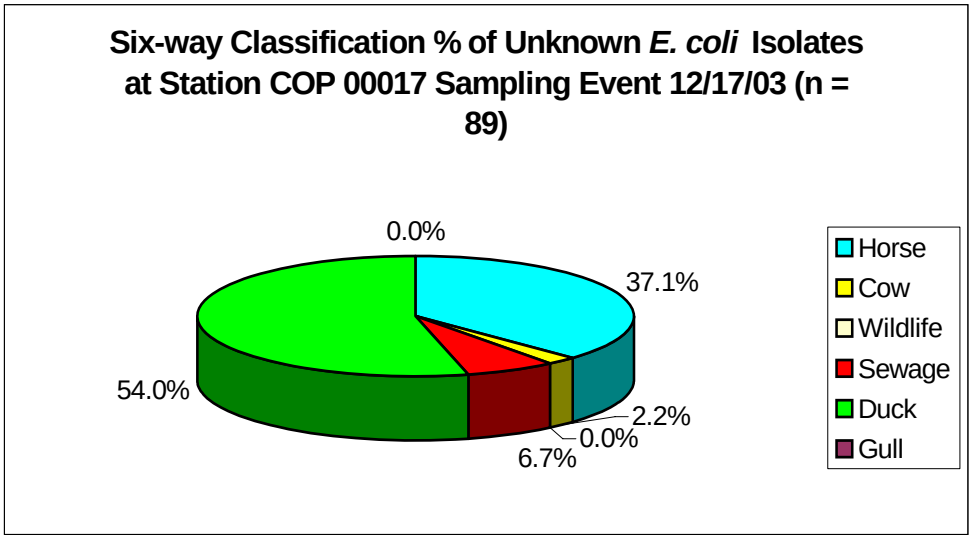


Figure 54a. Proportion of unknown isolates classified as each source for station COP 00017 for sampling event 12/17/03.

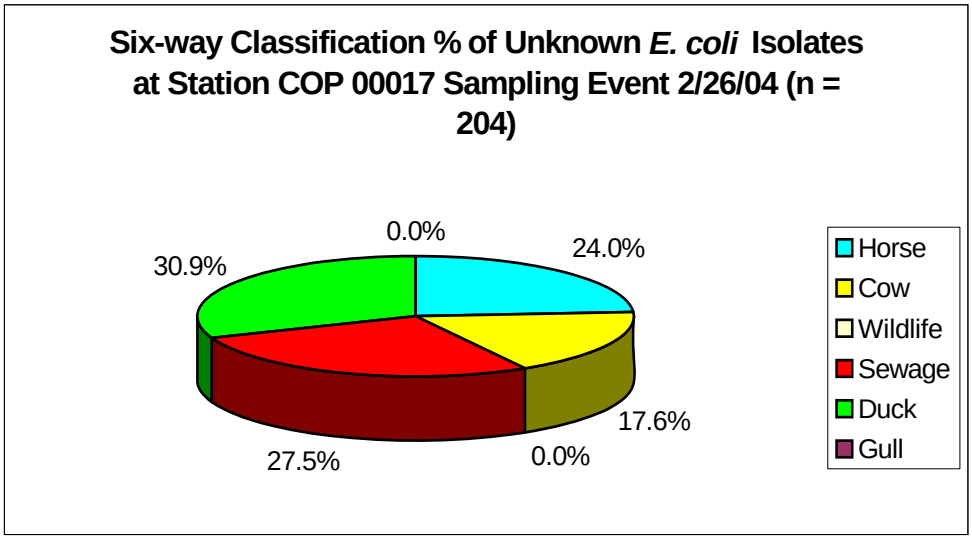


Figure 54b. Proportion of unknown isolates classified as each source for station COP 00017 for sampling event 02/26/04.

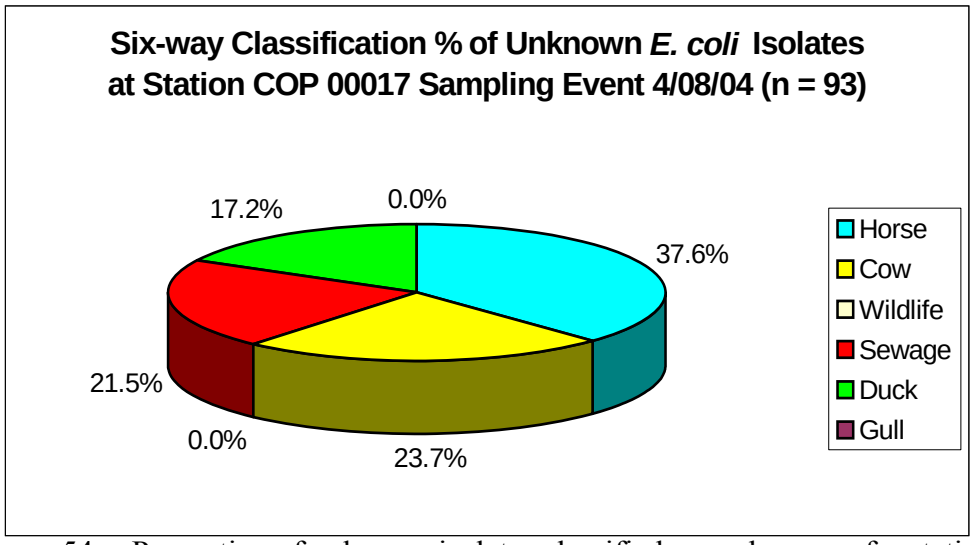


Figure 54c. Proportion of unknown isolates classified as each source for station COP 00017 for sampling event 04/08/04.

Table 74. Percent classification of *E. coli* isolates for each sampling event from station COP 00019 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	100.0	0.0	0.0	0.0	0.0	0.0	1
11/17/2003	15.2	2.2	0.0	71.7	10.9	0.0	46
12/17/2003	42.9	0.0	0.0	14.3	42.9	0.0	7
01/08/2004	35.8	35.2	0.0	15.7	8.8	4.4	159
02/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
02/26/2004	20.0	46.7	0.0	13.3	6.7	13.3	15
03/02/2004	0.0	100.0	0.0	0.0	0.0	0.0	9
04/08/2004	32.1	21.4	0.0	28.6	17.9	0.0	28
Total							265

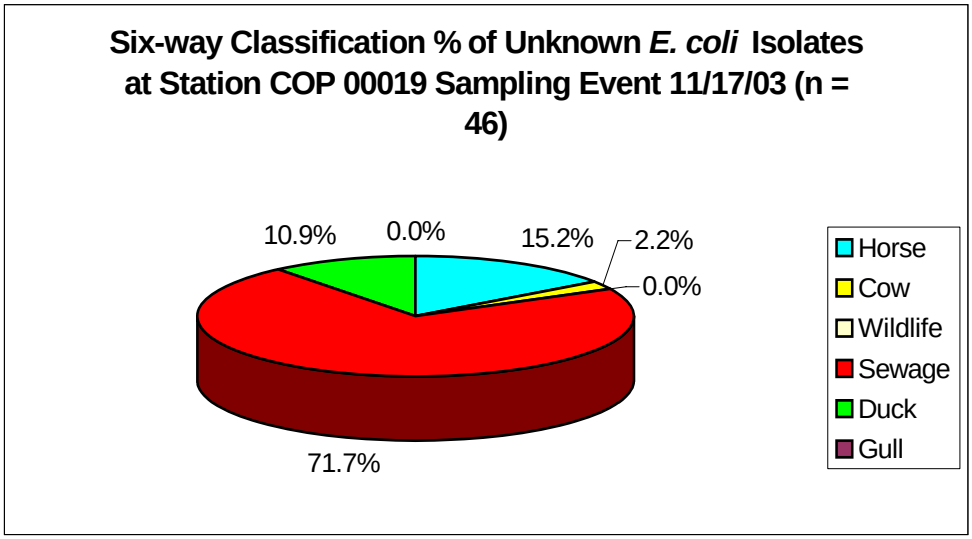


Figure 55a. Proportion of unknown isolates classified as each source for station COP 00019 for sampling event 11/17/03.

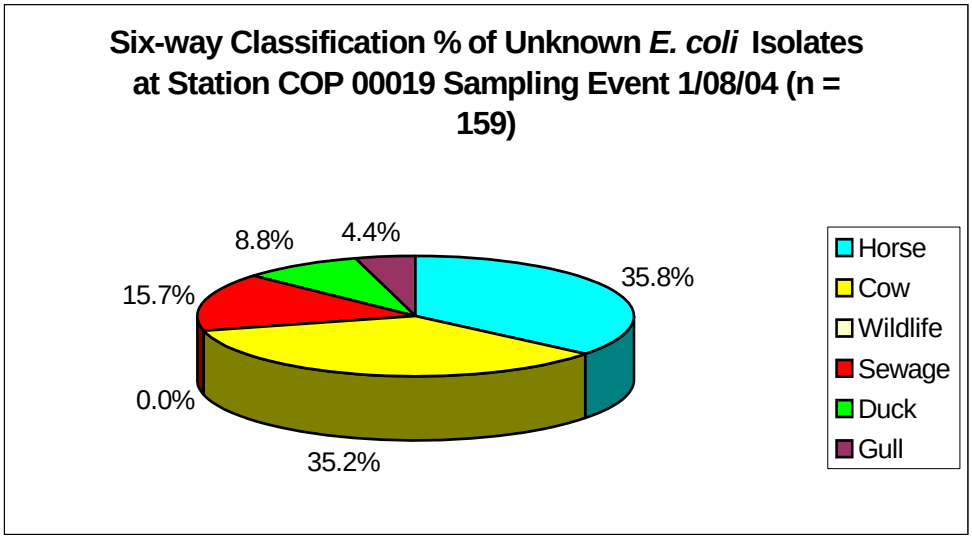


Figure 55b. Proportion of unknown isolates classified as each source for station COP 00019 for sampling event 01/08/04.

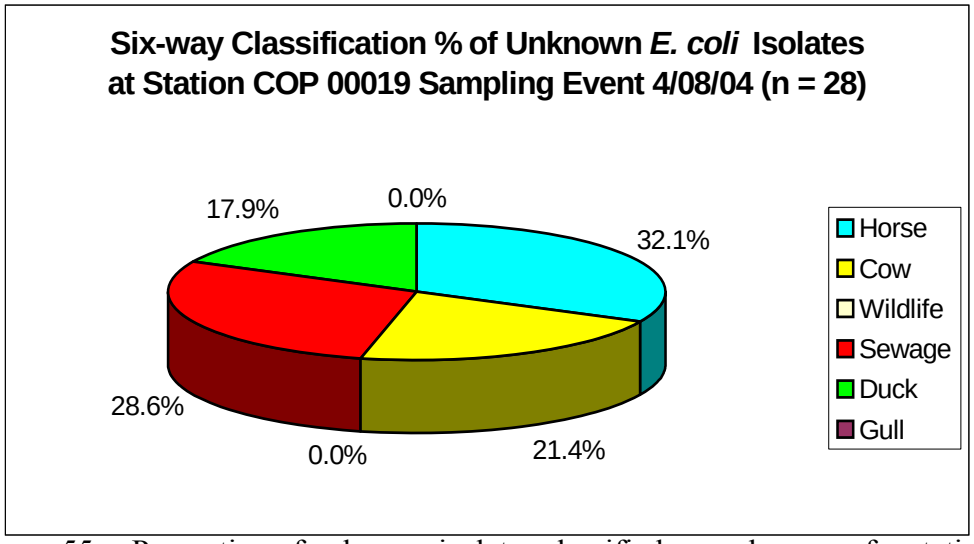


Figure 55c. Proportion of unknown isolates classified as each source for station COP 00019 for sampling event 04/08/04.

Table 75. Percent classification of *E. coli* isolates for each sampling event from station MBY 00002 using SPSS Discriminant analysis six-way classification (all groups equal).

Sampling Event	Horse	Cow	Wildlife	Sewage	Duck	Gull	Total Isolates
10/15/2003	16.7	0.0	0.0	83.3	0.0	0.0	6
11/17/2003	0.0	0.0	0.0	83.3	16.7	0.0	6
12/17/2003	58.3	16.7	0.0	8.3	16.7	0.0	12
01/08/2004	0.0	100.0	0.0	0.0	0.0	0.0	3
2/17/2004	0.0	0.0	0.0	0.0	0.0	0.0	0
2/26/2004	0.0	0.0	0.0	100.0	0.0	0.0	2
03/02/2004	23.1	38.5	0.0	23.1	15.4	0.0	13
04/08/2004	46.2	22.6	0.5	9.7	21.0	0.0	186
Total							228

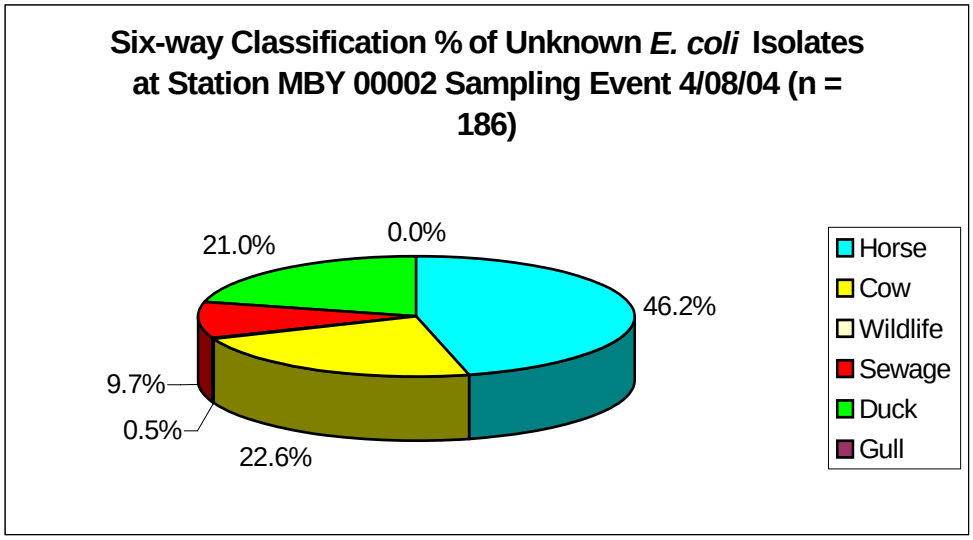


Figure 56. Proportion of unknown isolates classified as each source for station MBY 00002 for sampling event 04/08/04.

in which they are located around Copano Bay, so that adjacent stations can be better compared.

STATION COP 00001

Over the eight sampling events conducted in this study only 36 presumptive *E. coli* colonies were isolated on mTEC agar plates from water samples collected at Copano Bay station COP 00001. This was the lowest number of colonies obtained from a single station in Copano Bay (Table 24). Most probable number of fecal coliforms (MPN) for a single station (2.2/100 ml) as determined by TDH were also very low (Table 22). The highest fecal coliform MPN (4.5/100 ml) for station COP 00001 occurred during sampling event 02/26/04. The majority of the isolates (31/100 ml) were obtained from water collected during sampling events 01/08/04 and 04/08/04 (Table 25).

Twenty-five of the colonies were verified as *E. coli* and were analyzed for antibiotic resistance (Table 25). For sampling event 01/08/04 45.5% *E. coli* isolates were classified as horse and 36.4 % human (Table 62). The majority of the *E. coli* isolates from the sampling event of 4/8/04 classified as cow (45.5%) while other *E. coli* isolates were evenly identified at 18.2% as duck, human, and horse. However, the low number of *E. coli* isolates collected from this station does not allow any conclusions regarding source classification to be made. Based on this study and on historical fecal coliform data, bacteriological loading of station COP 00001 is not significant (TDH, 2003a).

Other stations that are located near the Copano and Aransas Bay interface include stations COP 00003, COP 00004, and COP 00011. These three stations along with station

COP 00001, were the stations from which the fewest colonies were isolated (Table 24).**STATION COP 00003**

A total of 76 presumptive *E. coli* colonies were isolated from station COP 00003 water samples during sampling events 01/08/04, 02/26/04, and 03/02/04 with the majority (40) isolated during sampling event 03/02/04 (Table 24.). The higher number of isolates from sampling event 03/02/04 corresponded with the highest fecal coliform MPN (7.8/100 ml) and lowest salinity (9.6 psu) for station COP 00003 (Tables 22, 13). Of the 76 colonies, 70 were verified as *E. coli* isolates using the Biolog™ MicroLog System. Sixty-five of the verified *E. coli* isolates were analyzed for antibiotic resistance (Table 25). Six-way (all groups equal) discriminant analysis classified 39.1 and 34.8% of the 23 *E. coli* isolates from sampling event 1/8/04 as cow and horse *E. coli* isolates, respectively (Table 63). During sampling event 2/26/04 three of the nine *E. coli* isolates were classified as cow, three as duck isolates. During sampling event 3/2/04 45.5% and 24.2% of the 37 *E. coli* isolates were classified as duck and horse *E. coli* isolates, respectively. The small number of *E. coli* isolates collected from sampling event 2/26/04 makes it difficult to assess possible pollution sources. The majority of the 33 *E. coli* isolates for event 3/02/04 were classified as ducks (45.5%) (Table 63, Fig. 45). Copano Bay is a common migratory habitat for ducks from fall through early spring (Stunz, personal communication). Of the *E. coli* isolates from sampling event 3/02/04 18.5% classified as sewage *E. coli* isolates.

STATION COP 00004

A total of 48 presumptive *E. coli* colonies were obtained from Copano Bay station COP 00004 (Table 24). However, the majority (28) of these isolates occurred during

sampling event 4/08/04. High numbers of isolates during this event also correlated with the highest fecal coliform MPN (13.0/100 ml) and lowest salinity (8.5 psu) values observed over the study period (Tables 22, 13). Low salinity values were presumably due to a 7-day average rainfall of 5.99 inches, which resulted in increased freshwater inflows into Copano Bay from the Mission and Aransas Rivers, as well as Copano Creek. The rainfall and flow rates from Copano Creek (1020 cfs), Mission River (9340 cfs) and Aransas River (190 cfs) were the highest observed for a single sampling event during the study (Fig. 21, Table 19).

Of the 48 colonies, 38 were verified as *E. coli* isolates using Biolog™ MicroLog System. Antibiotic resistance profiles were developed for 37 of the verified *E. coli* isolates (Table 25). All four of the *E. coli* isolates from sampling event 10/15/03 were classified as human sewage *E. coli* isolates, while five of the six *E. coli* isolates from sampling event 2/26/04 were classified as cow (83.3%) (Table 50). The 25 *E. coli* isolates analyzed for sampling event 4/08/04 were classified as 40.0, 36.0, and 16.0% for cow, horse, and human *E. coli* isolates, respectively (Table 64, Fig. 46). During higher levels of rainfall and when flow increased from Copano Creek, Mission and Aransas Rivers, as occurred in sampling events 2/26/04 and 4/08/04, the majority of *E. coli* isolates were classified as cow and horse *E. coli* isolates.

STATION COP 00011

A total of 38 presumptive *E. coli* colonies were isolated from Copano Bay station COP 00011. (Table 24). The majority of these were isolated during sampling events 10/15/03, 01/08/04, and 04/08/04. The highest number of colonies (16) occurred during sampling event 04/08/04 when the 7 day rainfall average equaled 5.99 inches (Table 19).

The fecal coliform level for all sampling events of station COP 00011 was 1.8/100 ml (Table 22). The lowest salinity level (9.3 ppt) occurred during sampling event 2/26/04, however only one colony was obtained from this sampling event (Table 13).

Thirty-three of the CFU's were verified as *E. coli* isolates using Biolog™ MicroLog System. ARP was conducted on 31 of the *E. coli* isolates (Table 25). For the first sampling event (10/15/03) seven of the nine *E. coli* isolates classified as human/sewage (Table 54). The six *E. coli* isolates from sampling event 1/08/04 classified as 50% cow and 50% horse *E. coli* isolates.

E. coli isolates from sampling event 4/08/04 classified as 35.7, 28.6, and 28.6% for horse, cow, and sewage *E. coli* isolates, respectively (Table 68). This event was associated with northeast winds and high flow rates from Copano Creek and the Mission and Aransas rivers (Table 16, Fig. 21).

As mentioned earlier the low numbers of isolates from these four stations (00001, 00003, 00004 and 00011), located close to the Copano Bay interface with Aransas Bay, support the MPN data for fecal coliforms that fecal bacteria loadings at these stations is minimal. Source identification information is limited due to the paucity in *E. coli* isolates obtained.

STATION COP 00019

Over 340 presumptive *E. coli* colonies were obtained for station COP 00019 throughout the course of the study (Table 24). The majority of the *E. coli* were isolated during sampling events 11/17/03, 12/17/03 01/08/04, 02/26/04 and 04/08/04, when 61, 26, 192, 24, and 31 colonies were obtained, respectively. Elevated levels of fecal coliforms

corresponded with the *E. coli* isolations for each sampling event with values of 13, 13, 49, 12, and 4.5, respectively ((Table 22). Although the highest fecal coliform and colony isolations occurred during sampling events 11/17/03 and 01/08/04, the corresponding salinity values of 11.3 and 11.4 psu were higher than the salinity values for sampling events 10/15/03 (7.8 psu) 12/17/03 (9.8 psu), and 2/26/04 (8.5 psu), when lower fecal bacteria levels were found (Table 13). The salinity levels correlate with freshwater inflow from Copano Creek prior to sampling events 10/15/03, 12/17/03, and 02/26/04 (Tables 14-21). However, salinity remained high (12.7 psu) regardless of high flow rates from Copano Creek, and the Mission and Aransas Rivers during sampling event 4/08/04.

The high fecal bacteria levels found when flow rates and rainfall were low imply that the bacteriological water quality for station COP 00019 is not an effect of rainfall (and runoff) events. This compares to previous reports by the TDH in 2000 where 9.1% of the samples had greater fecal coliform MPN's than the recommended 43 for shellfish harvesting areas at this station (TDH, 2003a). Antibiotic resistance analysis was conducted on 265 of the 270 verified *E. coli* isolates (Table 25). Classification results for sampling event 11/17/03 indicated that 71.7% of the 46 *E. coli* isolates were human/sewage source isolates (Table 74, Fig. 55a).

Classification results for sampling event 01/08/04 identified 35.8, 35.2, and 15.7% of the 159 *E. coli* isolates as horse, cow, and sewage *E. coli* isolates, respectively (Table 74, Fig. 55b). In two-way analysis almost 30% were identified as human/sewage. Other conditions for this event were northwest winds and a 24 hr rainfall event of 0.5 inches; however flow rates for Copano Creek remained low throughout January (Tables 16, 19,

Fig.18). Low numbers of *E. coli* isolates were collected at all other stations for this event (Tables 24, 43).

The 28 *E. coli* isolates for sampling event 04/08/04 resulted in 32.1, 21.4, and 28.6% of the *E. coli* isolates classifying as horse, cow, and human sewage *E. coli* isolates. There was significant rainfall during this sampling event (7 day 5.99 inches) and high flow rates prior to and during the sampling event from Copano Creek, and the Mission and Aransas Rivers (Table 19, Fig.21). Northeast winds may have minimized flow toward station COP 00019, as indicated by the high salinity of this station (12.7 ppt), compared with other stations in Copano Bay (Tables 16, 13).

STATION COP 00017

More than 1,500 presumptive *E. coli* colonies were isolated from water samples collected at station COP 00017 over the sampling events during the study (Table 24). The majority were obtained during sampling events 12/17/03, 02/26/04, and 04/08/04, where 174, 1,193, and 150 colonies were isolated, respectively. The fecal coliform numbers were also high in water samples from this station with values of 33, 1600, and 110 for the December, February 26, and April sampling events (Table 22). Water salinity for sampling event 02/26/04 was low (7.7 psu) compared to that of sampling events 12/17/03 (10.0 psu) and 04/08/04 (11.8 psu) (Table 13).

There were very low inflow rates at Copano Creek, during sampling event 12/17/03 (Fig. 17), correlating with the higher salinity measured. However, high flow rates were recorded from 11/21/03-11/27/03, prior to the sampling date (Fig. 16).

A 4-day rainfall average of 0.42 inches (Table 19) increased flow rates recorded from Copano Creek during sampling event 02/26/04 and may have resulted in the lower salinity values observed. Flow rates from Copano Creek, and the Mission and Aransas Rivers during sampling event 04/08/04 were very high due to the 7 day rainfall average of 5.99 inches and bacteria levels were high (Fig. 21, Tables 19, 22). The elevated flow rates would be expected to decrease salinity levels; however, this was not the case.

Biolog™ MicroLog System was used to confirm 712 of the colonies at station COP 00017 as *E. coli* isolates. ARPs were developed for 428 of the verified *E. coli* isolates (Table 25). Discriminant analysis classified 89 *E. coli* isolates for sampling event 12/17/03 as 37.1% and 53.9% for horse and duck *E. coli* isolates, respectively (Table 73, Fig. 54a). Large migratory duck and geese populations inhabit the Copano Creek area (TDH, 2003a) from late fall through early spring (Stunz, personal correspondence). Station COP 00017 is located at the mouth of Copano Creek. Flow gauges of Copano Creek indicate low flow rates for this sampling event, however there were higher flow rates prior to the sampling event from 11/21/03 to 11/27/03 (Figs. 16, 17). Southwest winds may have also affected water movement (Table 16).

For sampling event 02/26/04, 24.0%, 17.6%, 27.5%, and 30.9% of the 204 *E. coli* isolates classified as horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 73, Fig. 54b). There was high inflow from the Mission River and Copano Creek during this event, while the wind direction was northwest (Fig 19, Table 16).

For sampling event 04/08/04, 37.6%, 23.7%, 21.5% and 17.2 % of the 93 *E. coli* isolates for this event were horse, cow, sewage, and duck *E. coli* isolates, respectively (Table

73 Fig. 54c). High flow rates from Copano Creek and the Mission and Aransas Rivers with rainfall characterized this event (Fig. 21, Table 19).

STATION MBY 00002

There were almost 700 presumptive *E. coli* colonies isolated from station MBY 00002 during the course of the study (Table 24). The majority (approx. 640) were obtained during sampling event 4/08/04, when fecal coliform levels were 240/100 ml (MPN) (Table 22). The salinity for sampling event 4/08/04 at station MBY 00002 (0.5 psu) was the lowest recorded during the course of the study for all sampling events and stations (Table 13). The average salinity for MBY 00002 for all sampling events was also low (7.4 psu) with salinities never exceeding 10.5 psu. This is presumably due to the location of station MBY 00002 in Mission Bay, which receives freshwater inflow from the Mission River. The freshwater inflow from the Mission River never dropped below 14 cfs during the course of the study, and had the highest flow rates at sampling event 4/08/04 (9,430 cfs) (Figs. 15-21). A 7 day average rainfall of 5.99 inches and low salinities for sampling event 04/08/04 correlate with the high flow rates recorded during this event (Tables 19, 13, Fig. 21).

Antibiotic resistance analysis was conducted on 228 of the 338 confirmed *E. coli* isolates (Table 25). Discriminant analysis of 186 of the *E. coli* isolates from MBY 00002 during sampling event 04/08/04 classified the isolates as 46.2%, 22.6%, 9.7%, 0.5% and 21.0% for horse, cow, sewage, wildlife and duck *E. coli* isolates, respectively (Table 75, Fig. 56). Misclassification of isolates from other sources may be resulting in an increased

classification of *E. coli* isolates as horse. Only 18 of the 186 *E. coli* isolates classified as human sewage *E. coli* isolates.

Low numbers of *E. coli* were verified and analyzed from all other sampling events for station MBY 00002 (Tables 26-33). However, it can be noted that for sampling event 10/15/03 and also for 11/17/03 five of six isolates were classified as sewage source (Table 61). Higher flow rates of Mission River and southeast winds in November were recorded for these sampling events (Fig. 16, Table 16).

STATION COP 00009

There were 222 presumptive *E. coli* colonies isolated from station COP 00009 of Copano Bay during the course of the study (Table 24). The majority (188) occurred during sampling event 04/08/04, when fecal coliform levels were also elevated (33.0/100 ml) (Table 22). Flow from the Mission River (9340 cfs) was elevated and the 7 day rainfall average was 5.99 inches for this sampling event compared with other sampling events of station COP 00009 (Fig. 21, Table 19). Salinity values were also low (5.7 psu); however sampling event 10/15/04 had the lowest salinity (5.3 psu) recorded for station COP 00009 (Table 13). This may be attributed to the increased freshwater inflow rates from the Mission River prior to sampling.

The Mission River probably impacts station COP 00009 more than other inflow sources due to reefs that channel the flow from Mission Bay towards station COP 00009, as explained in the study site description (Fig. 3). The two reefs that are responsible for channeling the freshwater inflow from the Mission river to station COP 00009 are the Copano Bay and Shell Bank Reefs. They protrude from the eastern and western portion of

Mission Bay, respectively and extend into Copano Bay (TDH, 1994). Northern winds may also contribute to the channeling effect out of Mission Bay towards the middle of Copano Bay and impact station COP 00009 (TDH, 1994).

Antibiotic resistance analysis was conducted on 162 of the 172 confirmed *E. coli* isolates (Table 25). Discriminant analysis on 138 of the *E. coli* isolates from station COP 00009 during sampling event 04/08/04 classified as 46.4%, 18.1%, 19.65% 15.2% and 0.7% for horse, cow, sewage, duck, and gull *E. coli* isolates, respectively (Table 67, Fig. 49). The similar *E. coli* isolate source classifications for nearby station MBY 00002 (Fig 56) during sampling event 04/08/04 may reflect similar sources of fecal contamination. More *E. coli* were classified as sewage at station COP 00009 (27) compared to station MBY 00002 (18) (Tables 53, 61). The northeast winds may have contributed to water movement (Table 16).

A high proportion of *E. coli* isolates from sampling events 10/15/03 and 11/17/03 also classified as sewage. Although, the number of *E. coli* isolates for these two sampling events was low, at 3 and 12 respectively, they classified as 100% and 50% sewage *E. coli* isolates, respectively (Table 53). There were high flow rates for each of these sampling events for station COP 00009 from the Mission River (Figs. 15, 16) with southeast winds for sampling event 11/17/03 (Table 16).

STATION COP 00008

There were over 1,600 presumptive *E. coli* colonies isolated from station COP 00008 of Copano Bay during the course of the study (Table 24). Antibiotic resistance analysis was conducted on 282 of the verified *E. coli* isolates (Table 25). Almost all the isolates were obtained during sampling event 04/08/04 (approx. 1,635). Fecal coliform levels were also

extremely high at this station for the sampling event (1600.0/100 ml) (Table 22). High flow rates were recorded from the Aransas River a day prior to sampling (1090 cfs), and the 7 day rainfall average was 5.99 inches, winds were northeast (Fig. 21, Tables 19, 16). Salinity values were very low (0.6 psu) (Table 13), the lowest of all of the sampling events at station COP 00008, corresponding to the freshwater inflow of the Aransas River and high rainfall event. A storm drain is also located near the Bayside community close to this station. Discriminant analysis on 252 of the *E. coli* isolates from station COP 00008 during sampling event 4/08/04 classified as 39.7%, 18.3%, 0.8%, 16.7%, and 0.4% for horse, cow, wildlife, duck, and gull *E. coli* isolates, respectively (Table 66, Fig. 48). Almost 25% of the isolates were classified as human/ sewage. As with some of the previous stations the number of *E. coli* classifying as horse is high. Two-way analysis classified some of these isolates as sewage, which may indicate underestimation of the human/sewage signature contributing to the bacteriological loading of Copano Bay. The classification of duck *E. coli* isolates corresponds to duck populations that migrate to marsh areas at the mouth of the Aransas River.

STATION COP 00013

There were over 650 presumptive *E. coli* colonies isolated from station COP 00013 of Copano Bay during the course of the study (Table 24). The majority occurred from water collected during sampling events 02/26/04, 03/02/04, and 04/08/04, from which approximately 230, 54, and 296 colonies were isolated, respectively. The high MPN of fecal coliforms (130/100 ml) for sampling event 04/08/04 (130.0) correlated with the number of

colonies isolated (Table 22). However, the MPN's for sampling events 02/26/04 (33.0) and 3/02/04 (1.8) did not reflect the high numbers of presumptive *E. coli* colonies.

Station COP 00013 is located just east of Egery Island, where the Aransas River and Chiltipin Creek flow into Copano Bay. The Aransas River experienced low flow rates during sampling events 2/26/04 and 3/02/04 (Figs. 19, 20). There were extremely high flow rates from the Aransas River (1090 cfs) a day prior to sampling event 04/08/04 due to an average 7 day rainfall event of 5.99 inches (Fig. 21, Table 19). The salinity values for sampling events 02/26/04 (7.3), 03/02/04 (10.7), and 04/08/04 (3.5) correlate with the flow rates from the Aransas River (Table 13, Figs.19, 20, 21). Northwest winds may have contributed to the low salinity of sampling event 2/26/04 by moving freshwater inflow from the Mission River to station COP 00013 (Table 16).

Antibiotic resistance analysis was conducted on 408 of the 471 confirmed *E. coli* isolates (Table 25). Discriminant analysis on 26 of the *E. coli* isolates from station COP 00013 during sampling event 01/08/04 classified as 38.5%, 42.3%, 11.5%, and 7.7 % for horse, cow, sewage, and duck *E. coli* isolates, respectively, after a 24 hr 0.5 inch rainfall event and northwest winds (Table 70, Fig. 51a; Tables 16, 19). While percentages of horse isolates appear high they represent only 10 isolates (Table 56). The classification of 2 duck *E. coli* isolates for this event is consistent with the migratory duck populations that inhabit marshes surrounding the mouth of the Aransas River.

Discriminant analysis on 160 of the *E. coli* isolates from station COP 00013 during sampling event 02/26/04 classified as 50.0%, 18.8%, 10.6% and 19.4% for horse, cow, sewage, and duck *E. coli* isolates, respectively, following an average 4 day rainfall of 0.42

inches with northwest winds (Table 70, Fig. 51b; Tables 16, 19). There was a higher number of isolates classified as duck (31) for this event compared with event 01/08/04 (Table 56).

Discriminant analysis on 27 of the *E. coli* isolates from station COP 00013 from sampling event 03/02/04 resulted in classifications of 33.3%, 11.1%, 33.3%, and 22.2% for horse (9 isolates), cow (3), sewage (9), and duck (6) *E. coli* isolates, respectively (Table 70, Fig. 51c; Table 56). Rainfall was minimal, and flow rates from the Aransas River were low, and winds were southeast (Table 19, Fig. 20, Table 16). The classification of duck *E. coli* isolates from station COP 00013 during sampling event 03/02/04 correlates with the migratory duck populations that inhabit marshes in Port Bay (TDH, 2003a).

Discriminant analysis on the 165 *E. coli* isolates from station COP 00013 from sampling event 04/08/04 resulted in classifications of 30.9%, 19.4%, 1.2%, 19.4% and 29.1% for horse, cow, wildlife, sewage, and duck *E. coli* isolates, respectively (Table 70, Fig. 51d). The event was characterized by northeast winds, high rainfall (7 day average 5.99 inches), and high inflow rates a day prior to sampling (1090 cfs) from the Aransas River (Table 16, 19, Fig. 21). Large migratory duck populations are known to reside in marsh areas from Aransas River and Port Bay, while domesticated and resident duck populations have been reported east of Port Bay in an area known as Salt Lake (TDH, 2003a; Stunz, personal correspondence).

STATION COP 00014

There were over 1,150 presumptive *E. coli* colonies isolated from station COP 00014 of Copano Bay during the course of the study (Table 24). The majority were obtained during sampling events 12/17/03, 02/26/04, and 04/08/04, which generated approximately 42, 212,

and 879 colonies, respectively. The fecal coliform levels for sampling event 12/17/03 (7.8/100 ml), 2/26/04 (33.0), and 4/08/04 (540.0) were also the highest of those found at station COP 00014 (Table 22).

Station COP 00014 had the lowest average salinity (7.1 psu) out of the 14 stations sampled in this study (Table 13). This reflects the location of station COP 00014 in the southeast corner of Copano Bay where the Aransas River and Chiltipin Creek flow into Copano Bay.

Sampling event 12/17/03 was characterized by low flow rates from the Aransas River (7.7 cfs), a low average 7 day rainfall of 0.87 inches and west/northwest winds (Fig. 17, Tables 19, 16). Sampling event 02/26/04 was also characterized by low inflow rates from the Aransas River (21.0 cfs), and had a low 7 day rainfall average of 0.42 inches (Fig. 19, Table 19). Flow rates were high for the Aransas (88.0 cfs) and the Mission River (107 cfs) a day prior to sampling with northwest winds; however the salinity (8.3 psu) remained higher than the salinity for sampling event 2/17/04 (Tables 16, 13). Sampling event 04/08/04 had the lowest salinity (1.2 psu) recorded of the eight sampling events conducted at station COP 00014, with an average 7 day rainfall of 5.99 inches and increased flow rates from the Aransas River (1090 cfs) a day prior to sampling (Table 13, 19; Fig. 21).

Antibiotic resistance analysis was conducted on 308 of the 348 confirmed *E. coli* isolates (Table 25). Discriminant analysis on 20 of the *E. coli* isolates from station COP 00014 during sampling event 12/17/03 classified 30% as horse (6 isolates) and 70% as duck (14 isolates) source (Tables 71, 57). High migratory duck populations occur in the marshes surrounding the Aransas and Mission Rivers (TDH, 2003a).

Discriminant analysis on 112 of the *E. coli* isolates from station COP 00014 during sampling event 2/26/04 classified as 37.5%, 37.5%, 17.0%, and 8.0% for horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 71, Fig. 52a). Scattered rainfall with high flow rates from the Mission and Aransas Rivers and northwest winds characterized the event. (Table 19, 16; Fig. 19).

Discriminant analysis on 156 of the *E. coli* isolates from station COP 00014 during sampling event 04/08/04 classified as 32.1%, 9.0%, 0.6%, 9.6%, 47.4% and 1.3% for horse, cow, wildlife, sewage, duck, and gull *E. coli* isolates, respectively (Table 71, Fig. 52b). The elevated inflow rates from the Aransas and Mission Rivers and the high amounts of rainfall that occurred during this event distinguish it from the other events (Fig. 21, Table 19). This was one of the few sampling events where isolates were identified as wildlife or gull *E. coli* isolates. However, the levels of these *E. coli* isolates were very low compared to numbers of horse, cow, sewage, and duck *E. coli* isolates (Table 57).

STATION COP 00012

A total of 357 presumptive *E. coli* colonies were isolated from station COP 00012 of Copano Bay during the course of the study (Table 24). The majority occurred during sampling events 02/26/04, 03/02/04, and 04/08/04, where 42, 108, and 154 colonies were obtained. The fecal coliform levels for sampling event 02/26/04 (13.0), and 04/08/04 (13.0) were the highest of those analyzed for station COP 00012 (Table 22). However, the MPN of fecal coliforms for sampling event 03/2/04 was low.

Sampling event 02/26/04 was characterized by a low 7 day rainfall average (0.42 inches), but high flow rates from the Aransas (88 cfs) and Mission River (108 cfs) a day prior

to sampling (Table 19, Fig. 19). The salinity of water at the station for this event was 8.6 ppt and winds were northwesterly (Tables 13, 16). Sampling event 03/02/04 had a 7 day rainfall average of only 0.36 inches, which corresponded to low flow rates from the Aransas (9 cfs) and Mission (26 cfs) Rivers (Table 19, Fig. 20). The low rainfall and freshwater inflow from the Aransas and Mission Rivers are reflected in the higher salinity (11.2 psu) observed during the sampling event (Table 13). The low salinity (6.3 psu) observed during sampling event 04/08/04 corresponded with a high 7 day average rainfall of 5.99 inches and elevated flow rates from Copano Creek (1020 cfs) and Mission River (9340 cfs) (Tables 13, 19; Fig. 21). The Aransas River also had high flow rates of 1090 cfs a day prior to sampling (Fig. 21).

Antibiotic resistance analysis was conducted on 280 of the 292 confirmed *E. coli* isolates (Table 25). Discriminant analysis on 25 of the *E. coli* isolates from station COP 00012 during sampling event 02/26/04 classified them as mainly cow (40.0%) and horse (36.0%), with lesser proportions of sewage (4.0%), duck (12.0%) and gull (8.0%) (Table 69, Fig. 50a). This proportion of gull isolates was the highest found for a location and sampling event during the study, but in terms of actual numbers consisted of 2 isolates (Table 55).

Discriminant analysis of 88 of the *E. coli* isolates from station COP 00012 during sampling event 03/02/04, with little rainfall and southeast winds, classified as 18.2%, 22.7%, 48.9%, and 10.2% for horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 69, Fig. 50b). Wind direction differed from the February sampling event and the proportion and number of human/sewage isolates was much higher than the previous event (48.9% or 43 isolates, compared with 44% or 1 isolate) (Tables 16, 69, 55, Fig. 50b). Duck *E. coli* isolates may have originated from the marshes of the Aransas River and in the Port Bay area, where

large migratory duck populations reside (TDH, 2003a), or southeast winds may have contributed in water circulation from Salt Lake, where large populations of domesticated and whistling ducks have been reported to reside year round (Stunz, personal correspondence).

Discriminant analysis of 131 of the *E. coli* isolates from station COP 00012 during sampling event 04/08/04 classified as 45.8%, 11.5%, 25.2%, and 17.6% for horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 69, Fig. 50c). This event was characterized by northeast winds, high rainfall and Aransas River flow rates (Tables 16, 19; Fig. 21). Duck *E. coli* isolates may have originated from the marshes of the Aransas River and Port Bay areas, where large migratory duck populations reside (TDH, 2003a) or from domesticated and whistling ducks in Salt Lake, as described previously.

STATION COP 00016

A total of 272 presumptive *E. coli* colonies were isolated from station COP 00016 of Copano Bay during the course of the study (Table 24). The majority of the colonies were obtained during sampling events 02/26/04, 03/02/04, and 04/08/04, with approximately 67, 78, and 85 colonies, respectively. The fecal coliform levels for sampling event 3/2/04 (13.0), and 4/08/04 (46.0) were the highest found at station COP 00016 (Table 22). However, the level for sampling event 02/26/04 (1.8) was low.

Sampling event 02/26/04 was characterized by a low 7 day rainfall average (0.42 inches), but high flow rates from the Aransas (88 cfs) and Mission River (108 cfs) a day prior to sampling (Table 19, Fig. 19). The water salinity of this event was 8.6 psu and may have been influenced from the northwest winds enhancing freshwater inflow from the Aransas and Mission Rivers (Tables 13, 16).

Sampling event 03/02/04 had a 7 day rainfall average of only 0.36 inches, which corresponded to low flow rates from the Aransas (9 cfs) and Mission (26 cfs) Rivers (Table 19, Fig. 20). The low rainfall and freshwater inflow from the Aransas and Mission Rivers reflect the higher salinity (12.2 psu) observed during the sampling event (Table 13).

The lowest salinity (6.1 psu) observed during all sampling events at station COP 00016 occurred during sampling event 04/08/04 (Table 13). This was probably due to a high 7 day average rainfall of 5.99 inches and high flow rates from Copano Creek (1020 cfs) and Mission River (9340 cfs) (Table 19, Fig. 21). The Aransas River also had a high flow rate of 1090 cfs the day prior to sampling event 4/08/04 (Fig. 21). Copano Creek and Mission River may not have affected the salinity values of station COP 00016 due to northeast winds that could have impeded freshwater inflow from reaching station COP 00016 (Table 16). Nevertheless, the extreme freshwater flow rates may have been sufficient to decrease the salinity at station COP 00016.

Antibiotic resistance analysis was conducted on 220 of the 232 confirmed *E. coli* isolates (Table 25). Discriminant analysis on 55 of the *E. coli* isolates from station COP 00016 during sampling event 02/26/04 classified as 47.3%, 16.4%, 9.1%, and 27.3% for horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 72, Fig. 53a). High flow rates of the Aransas River were measured a day prior to sampling in combination with northwest winds (Fig. 19, Table 16). Only 9.1% of *E. coli* isolates from sampling event 02/26/04 were classified as sewage *E. coli* isolates. The largest per cent classification of duck *E. coli* isolates for sampling events with over 25 *E. coli* isolates at station COP 00016, occurred during sampling event 02/26/04. The most likely sources of duck *E. coli* isolates are the marsh areas at and surrounding Salt Lake, where very large populations of

domesticated and whistling ducks are found year round (Stunz, personal correspondence) which are located just south of station COP 00016. Migratory duck populations could also have contributed to the fecal loading.

Discriminant analysis on 66 of the *E. coli* isolates from station COP 00016 during sampling event 03/02/04 (with southeast winds and low flow rates from the Aransas River, Table 16, Fig. 20) classified as 48.5%, 7.6%, 24.2%, and 19.7% for horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 72, Fig. 53b). There was a higher proportion of sewage *E. coli* isolates from station COP 00016 during sampling event 03/02/04 compared with sampling event 02/26/04, while numbers of duck isolates were similar (Table 58).

Discriminant analysis of 65 of the *E. coli* from station COP 00016 during sampling event 04/08/04 classified as 29.2%, 44.6%, 13.8%, and 12.3% for horse, cow, sewage, and duck *E. coli* isolates, respectively (Table 72, Fig. 53c). Numbers of isolates classified for February, March and April were similar (55, 66, 65) (Table 58). The biggest difference between these events was the increase in cow isolates to 44% for the April event, compared with 16% and 7.6% for the earlier events, corresponding with high rainfall and flow rates from the Aransas River and northeast winds (Tables 16,19; Fig. 21).

STATION COP 00007

A total of 117 presumptive *E. coli* colonies were isolated from station COP 00007 of Copano Bay during the course of the study (Table 24). The majority (90) were obtained from water samples collected 04/08/04. The fecal coliform levels for this sampling event (64.0/100 ml) were also elevated (Table 22). A high 7 day average rainfall event of 5.99 inches and high flow rates were recorded at the Aransas River (1090.0 cfs) and Mission

River (9230.0 cfs) a day prior to sampling event 04/08/04, with low salinity (6.5 psu) (Table 19, 13; Fig. 21).

Antibiotic resistance analysis was conducted on 72 of the 76 confirmed *E. coli* isolates (Table 25). Discriminant analysis of 54 of the *E. coli* isolates from station COP 00007 during sampling event 04/08/04 classified as 31.5% horse, 18.5% cow, 33.3% sewage, 14.8% duck and 2.0% gull, respectively (Table 65, Fig. 47). As for other stations in this area it is likely that most of the duck *E. coli* isolates originated from Salt Lake, or from the marshes of Port Bay and the Aransas River, where migratory ducks are known to reside.

SUMMARY OF RESULTS

Over the period of the study the proportions of *E. coli* isolates classifying as each source varied considerably, both by date and location. Wind and rainfall are probably primary environmental factors affecting impacts of fecal contamination at different stations. Wind is considered to be the primary factor in controlling water movement within the Bay (TDH, 2003a). Rainfall prior to each sampling event had a significant impact on Copano Bay, affecting runoff and freshwater inflow from Copano Creek, Aransas River, and Mission River.

Copano Bay stations 00001, 00003, 00004, 00007 and 00011 had low numbers of *E. coli* isolates compared to other sampling stations in Copano Bay. These stations are in close proximity to the Copano and Aransas Bay interface with station COP 00007 being the farthest away, 800 yards east of Salt Lake. These stations have historically exhibited excellent bacteriological water quality under various conditions due to the water exchange with Aransas Bay (TDH, 2003a). Station COP 00007 did have a high proportion of *E. coli* isolates classified as human/sewage for the April sampling event 4/08/04, following high rainfall and northeast winds.

Copano Bay stations 00008, 00013, 00014 were probably affected by freshwater inflow that originated from the Aransas River and Chiltipin Creek. Most of the *E. coli* isolates classified as cow and horse. A proportion of *E. coli* isolates from stations COP 00013 (29.1%) and 00014 (47.4%) classified as duck isolates during sampling event 04/08/04, correlating with the large populations of migratory ducks that inhabit the marsh areas surrounding stations COP 00013 and 00014. The highest classification of human *E. coli*

isolates from this area occurred at stations COP 00013 and 00008 during sampling events 03/02/04 and 04/08/04, respectively.

Stations MBY 00002 and COP 00009 are probably both impacted by the Mission River. The highest numbers of presumptive *E. coli* colonies isolated for both stations occurred during sampling event 04/08/04 following a period of heavy rainfall. These two stations had very similar proportions of isolate classifications during this sampling event, with high proportions classifying as cow and horse. There was also a relatively high classification of duck *E. coli* isolates at both stations, corresponding with the migratory duck habitat of Mission Bay area (TDH, 2003a).

More than 25 *E. coli* isolates were analyzed for three events at station COP 00019. For sampling event 11/15/03 71.7% of the isolates classified as human/sewage, which was the highest proportion of isolates classified as human/sewage for any station with over 25 *E. coli* isolates for a single sampling event during the course of this study. Sampling events 01/08/04 and 04/08/04 had a lower proportion of isolates classified as sewage and a higher combined proportion classified as horse and cow *E. coli* isolates.

Station COP 00017 had the highest proportion (54.0 %) of duck *E. coli* isolates during a single sampling event compared with all other stations through the course of the study. This occurred during sampling event 12/17/03 when large migratory populations of ducks would be expected in the marshes near Copano Creek near station COP 00017. A high proportion of isolates from sampling events 02/26/04 (105 isolates) and 04/08/04 (251) were classified as human/sewage source (Tables 45, 47).

The second highest proportion of sewage *E. coli* isolates for stations with over 25 *E. coli* isolates occurred at station 00012 during sampling event 03/02/04. High numbers of cow and horse *E. coli* isolates were found at Station 00012 and Station 00016.

The highest proportions of isolates classifying as human/sewage were found for sampling events 10/15/03 (77.3%) and 11/17/03 (61.8%). December through February proportions were below 20%, while 30.7% of isolates for the 03/02/04 sampling event were grouped as human/sewage. Isolates identified as horse *E. coli* were scarce for events 10/15/03 and 11/17/03 but remained relatively consistent from 12/17/03 through 4/08/04 at over 30% isolates. Duck *E. coli* isolates classification was low until sampling event 12/17/03 which had the highest percentage classification of ducks (42.1%). The duck classification declined for sampling event 1/08/04, but afterward remained close to 20%. *E. coli* isolates classifying as cow were low for the first two sampling events, but for sampling event 01/08/04 peaked at 35.4%, remaining near 20% thereafter. Few isolates classified as wildlife, excluding duck (6) or gull (24) *E. coli* for any station or sampling event.

The highest proportion of duck *E. coli* isolates occurred at stations COP 00017 (31.1%), 00014 (32.1%), 00013 (23.3 %) and 00016 (20.0%) and COP 00003 (29.2%). The position of each station, excluding COP 00003 where low numbers of *E. coli* isolates analyzed, and COP 00016, correlated with areas where large populations of migratory ducks reside. COP 00016 is located near a large population of residential Black-Bellied Whistling Ducks.

SUMMARY

- Both antibiotic resistance profiling and PFGE results suggest a human/sewage contribution to fecal contamination of Copano Bay.
- Wildlife and gulls, as assessed by antibiotic resistance profiling, were found to contribute relatively little contamination (in terms of *E. coli*) compared with human/sewage, cow and horse.
- Livestock (cow, horse) appear to contribute to fecal contamination at many stations under certain environmental conditions, such as rainfall and high river water flow.
- Isolates identified as duck were found in areas known to be colonized by either migratory or resident ducks suggesting these birds contribute to fecal contamination of the Bay.
- Additional studies are needed to examine loadings and sources in the contributing rivers –Mission and Aransas, and Copano Creek.
- Other questions such as potential contribution of fecal bacteria from sediments still need to be addressed.

The strategy of using a screening phenotypic technique in conjunction with a genotypic technique to analyze a subset of the data and provide a level of confirmation shows promise; however, library sizes were a constraint for both techniques. A high proportion of human/sewage isolates analyzed by PFGE confirmed antibiotic resistance results. For horses and ducks, the PFGE library was not only much smaller but contained a different, or restricted species group of isolates. This was due to the timing of library development.

PFGE library was developed using isolates from the first group of collections only. The ARP library was expanded with isolates from a second period of collection and augmented with isolates from an existing TAMU-CC library. Specifically, for horse, the ARP library did not contain the horse isolates from fairground samples; whereas these were the only isolates in the PFGE library. For ducks a second set of isolates were obtained at a later collection date from different duck species, not included in the PFGE library. These differences probably accounted for the lower levels of confirmation for these two groups. The PFGE library did not contain wildlife and gulls, thus these groups were not included in the isolate identification comparisons. The library issues were primarily related to funding constraints. Ideally the ARA and PFGE library would include the same isolates, with a subset of unknown isolates analyzed to support the ARA results. Our evaluation of isolates from other libraries at TAMU-CC suggest that some source isolates from other watersheds may sometimes be used; however, each case needs to be considered individually as there are many factors to be evaluated e.g. land use, urban vs. rural areas (for example, dogs from urban households are more likely to have been exposed to antibiotics), types of feed used for cattle and other livestock, etc. In order to maximize confidence in the results, libraries should ideally be developed for the watershed being studied, over the same time period of the water (unknown source) sample collections.

Since the inception of this project the science of bacteria source tracking and the techniques available have developed significantly. For future studies enterococci should be considered for study instead of *E. coli*. *E. coli* was used for this study as Copano Bay water quality is evaluated using fecal coliforms (due to its shellfish classification) a group of which *E. coli* is a member. An additional factor in the decision was the existence of libraries which

could be expanded with Copano Bay watershed isolates, thus minimizing costs associated with library development. Carbon source utilization data, obtained when confirming *E. coli* colonies, is showing promise in another study (Mott and Lehman, unpublished). For this study, the early isolates were analyzed using a manual plate reader and not stored electronically; requiring manual input to use the results for source tracking.

REFERENCES

American Public Health Association. 1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, Washington D.C.

Biolog. 1994. Rainbow Agar O157. Fast and easy isolation of *E. coli* O157:H7 and other *E. coli* strains. Technical Document, Biolog, Inc.

Biolog. 1999. MicroLog™ System Release 4.0 User Guide.

Bio-Rad Laboratories. 1995. CHEF-DR III Pulsed Field Electrophoresis Systems: Instruction Manual and Applications Guide. Hercules, California. 40 pp.

Bio-Rad Laboratories. 1999. Quality One Quantitation Software: Instruction Manual and Applications Guide. Hercules, California.

Bio-Rad Laboratories. 1999. Diversity Database: Instruction Manual and Applications Guide. Hercules, California.

Cameron, D.N., F. M. Khambathy, I. K. Wachsmuth, R.V. Tauxe, and T.J. Barrett. 1994. Molecular characterization of *Vibrio cholerae* O1 strains by Pulsed Field Gel Electrophoresis. J. Clin. Microbiol. 32:1685-1690.

Dombek, P. E., L. K. Johnson, S. T. Zimmerley, and M. J. Sadowsky. 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. 66:2572-2577.

Duck, W.M., C.D. Steward, S.N. Banerjee, J.E. McGowan Jr., and F. C. Tenover. 2003. Optimization of computer software settings improves accuracy of Pulsed Field Gel Electrophoresis macrorestriction fragment pattern analysis. J. Clin. Microbiol. 41:3035-3042.

Duffy, G., S.B. O'Brien, E. Carney, J.J. Sheridan, D.A. McDowell, and I.S. Blair. 2005. Characterisation of *E. coli* O157 isolates from bovine hide and beef trimming in Irish abattoirs by pulsed field gel electrophoresis. J. Microbiol. Meth. 60: 375- 382.

Dufour, A.P., E.R. Strickland and V.J. Cabelli. 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. 41: 1152-1158.

Ferris, M.M., X. Yan, R.C. Habbersett, Y. Shou, C. L. Lemanski, J.H. Jett, T.M. Yoshida, and B. L. Marrone. 2004. Performance assessment of DNA fragment sizing by high-sensitivity flow cytometry and pulsed-field gel electrophoresis. J. Clin. Microbiol. 42:1965-1976.

Field, A. 2000. Discovering statistics using SPSS for Windows. Sage Publications Ltd., London. 496 pp.

Hagedorn, C. 2004. Development of known source libraries. American Society for Microbiology. Workshop 104-20. Microbial Source Tracking using Indicator Organisms. May 23, 2004.

Hagedorn, C., S.L. Robinson, J.R. Filtz, S.M. Grubbs, T.A. Angier, and R.B. Reneau, Jr. 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. Appl. Environ. Microbiol. 65: 5522-5531.

Hagedorn, C., J. B. Crozier, K. A. Mentz, A. M. Booth, A. K. Graves, N. J. Nelson, and R. B. Reneau, Jr. 2003. Carbon source utilization profiles as a method to identify sources of faecal pollution in water. J. Appl. Microbiol. 94:792-799.

Hahm, B.-K., Y. Maldonado, E. Schreiber, A.K. Bhunia, and C.H. Nakatsu. 2003. Subtyping of foodbourne and environmental isolates of Escherichia coli by multiplex-PCR, rep-PCR, PFGE, ribotyping and AFLP. J. Microbiol. Meth. 53:387-399.

Harwood, V.J., J. Whitlock, and V. Withington. 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. Appl. Environ. Microbiol. 66:3698-3704.

Huberty, C.J. 1994. Applied discriminant analysis. John Wiley and Sons, Inc., New York, N.Y.

IQA/WP. 2000. DNA fingerprinting to identify sources of bacteria in coastal waters of Texas. GLO.

Lu, L., M.E. Hume, K.L. Sternes, and S.D. Pillai. 2004. Genetic diversity of *Escherichia* coli isolates in irrigation water and associated sediments: implications for source tracking. 2004. Wat. Res. 38:3899-3908.

McLellan, S.L., A.D. Daniels, and A.K. Salmore. 2003. Genetic characterization of *Escherichia coli* populations from host sources of fecal pollution by using DNA fingerprinting. Appl. Environ. Microbiol. 69:2587-2594.

Meays, C.L., K. Broersma, R. Nordin, and A. Mazumder. 2004. Source tracking fecal bacteria in water: a critical review of current methods. J. Environ. Manag. 73:71-79.

Mott, J.B. 2000. Application of antibiotic resistance patterns to differentiate sources of E. *coli* in coastal waters of Texas. TNRCC study work plan approved by TNRCC (2000).

NCCLS. 2000. Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard-Seventh Edition. NCCLS document M2-A7.

NCCLS. 2002a. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Second Edition. NCCLS document M31-A2.

NCCLS. 2002b. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement. NCCLS document M100-S12

Okwumabua, O., M. O'Connor, E. Shull, K. Strelow, M. Hamacher, T. Kurzynski, and D. Warshauer. 2005. Characterization of *Listeria monocytogenes* isolates from food animal clinical cases: PFGE pattern similarity to strains from human listeriosis cases. FEMS Microbiol. Letters. In Press.

QAPP. 2003. Development of an *E. coli* bacterial source tracking library and assessment of bacterial sources impacting Lake Waco and Lake Belton. Prepared for the Texas State Soil and Water Conservation Board by Parsons, Texas A&M El Paso Agricultural Research and Extension Center, TAMU and TAMU-CC.

Parveen, S., N.C. Hodge, R.E. Stall, S.R. Farrah, and M.L. Tamplin. 2001. Phenotypic and genotypic characterization of human and nonhuman *Escherichia coli*. Wat. Res. 35: 379-386.

Scott, T.M., J.B. Rose, T.M. Jenkins, S.R. Farrah, and J. Lukasik. 2002. Microbial source tracking: current methodology and future directions. Appl. Environ. Microbiol. 68: 5796-5803.

Simmons, G. M., D. F. Waye, S. Herbein, S. Myers, and E. Walker. 2000. Estimating nonpoint fecal coliform sources in Northern Virginia's Four Mile Run watershed, p. 248-267. In: T. Younos and J. Poff (ed.), Abstracts of the Virginia Water Research Symposium, Blacksburg, Virginia.

Simpson, J.M., Santo Domingo, J.W., and D.J. Reasoner. 2002. Microbial source tracking: state of the science. Environ. Sci. Technol. 36: 5279-5288.

Singer, R.S., W.M. Sischo, and T.E. Carpenter. 2004. Exploration of biases that affect the interpretation of restriction fragment patterns produced by pulsed-field gel electrophoresis. J. Clin. Microbiol. 42:5502-5511.

South Texas Regional Water Planning Group. 2001. South Texas Regional Water Plan Volume I. http://www.twdb.state.tx.us/rwp/l/PDFs, March, is also typically the driest season of the west (Table 1-2). There is a general trend of decreasing precipitation from ... reaching the Texas Coast indicate that storm tides

Special Study Plan. 2000. Application of antibiotic resistance patterns to differentiate sources of E. *coli* in coastal waters of Texas. TCEQ.

SPSS. 1999. SPSS Base 9.0 Applications Guide. SPSS Inc. 411 pp.

SPSS. 2003. Online tutorial. Accessed 09/18/03 through SPSS version 11.0 for Windows software.

Stewart, J. R., Ellender, R. D., Gooch, J. A., Jiang, S., Myoda, S. P., and S.B. Weisberg. 2003. Recommendations for microbial source tracking: lessons from a methods comparison study. J. Wat. Health 1: 225-231.

Stoeckel, D. M., Mathes, M. V., Hyer, K. E., Hagedorn, C., Kator, H., Lukasik, T., O'Brien, T. L., Samadpour, M., Strickler, K. M., and B.A. Wiggins. 2004. Comparison of seven protocols to identify fecal contamination sources using *Escherichia coli*. Environ. Sci. Tech. 38: 6109-6117.

Stunz, G. 2005. Personal correspondence. (Familiar with Copano Bay area and duck population distribution). TAMU-CC, Physical and Life Sciences, Assistant Professor, Marine Biology.

TCEQ. 2003. Draft 2002 Texas 303(d) list (October 1, 2002). http://www.tnrcc.state.tx.us/water/quality/02_twqmar/02_305b/02_program_summary/index.html

TDH. 1994. A comprehensive sanitary survey of the shellfish producing waters of Copano Bay. August 1994. Texas Department of Health Division of Shellfish Sanitation.

TDH. 2000. Comprehensive sanitary survey of the shellfish producing waters of Copano Bay, August 2000. Texas Department of Health Seafood Safety Division.

TDH. 2003a. Comprehensive sanitary survey of the shellfish producing waters of Copano Bay, August 2000. Texas Department of Health Seafood Safety Division. Draft.

TDH. 2003b. Bay water sample collection and data management. Standard Operating Procedures. Texas Department of Health Seafood Safety Division.

TDH. 2004. Annual Update of the Shellfish Producing Waters of Aransas and Copano Bays.

United States Census Bureau, 2000 http://factfinder.census.gov

United States Department of Agriculture Soil Conservation Service. 1979. Soil Survey of San Patricio and Aransas Counties, Texas. 64 pp. + appendices and illustrations.

United States Department of Agriculture Soil Conservation Service. 1988. Soil Survey of Refugio County, Texas. 81 pp. + appendices and illustrations.

USEPA. 1986. Bacterial ambient water quality criteria; availability. Federal Register 51(45):8012-8016

USEPA. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *E. coli*. EPA-821-R-97-004.

USEPA. 2001. EPA Requirements for Quality Assurance Project Plans, EPA QA/R-5, EPA/240/B-01/003, Office of Environmental Information, Washington, DC 20460.

United States Geological Survey. 2005. Daily Streamflow for the Nation. http://nwis.waterdata.usgs.gov/nwis/

University of Utah, Department of Meterology. 2005. 30 year monthly averages, 1961-1990. http://www.met.utah.edu/jhorel/html/wx/climate/normrain.html

Webster, L.F., B.C. Thompson, M.H. Fulton, D.E. Chestnut, R.F. Van Dolah, A.K. Leight, and G. I. Scott. 2004. Identification of sources of *Escherichia coli* in South Carolina estuaries using antibiotic resistance analysis. J. Exper.Mar. Biol. Ecol. 298: 179-195.

Whitlock, J.E., D.T. Jones, and V.J., Harwood. 2002. Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. Wat. Res. 36: 4273-4282.

Wiggins, B.A. R.W. Andrews, R.A. Conway, C.L. Corr, E.J. Dobratz, D.P. Dougherty, J.R Eppard, S.R. Knupp, M.C. Limjoco, J.M. Mettenburg, J.M. Rinehardt, J. Sonsino, R.R. Torrijos, and M.E. Zimmerman. 1999. Use of antibiotic resistance analysis to identify non-point sources of fecal pollution. Appl. Environ.Microbiol. 65:3483-3486.

Wiggins, B. A., P. W. Cash, W. S. Creamer, S. E. Dart, P. P. Garcia, T. M. Gerecke, J. Han, B. L. Henry, K. B. Hoover, E. L. Johnson, K. C. Jones, J. G. McCarthy, J. A. McDonough, S. A. Mercer, M. J. Noto, H. Park, M. S. Phillips, S. M. Purner, B. M. Smith, E. N. Stevens, and A. K. Varner. 2003. Use of antibiotic resistance analysis for representativeness testing of multiwatershed libraries. Appl. Environ. Microbiol. 69:3399-3405.

Zhechko, D., M. Michaylova, and S. Mincova. 2005. Characterization of *Lactobacillus helveticus* strains isolated from Bulgarian yoghurt, cheese, plants, and human faecal samples by sodium dodecilsulfate polyacrylamide gel electrophoresis of cell-wall proteins, ribotyping, and pulsed field gel fingerprinting. Inter. Dairy J. 15:998-1005.

APPENDIX

Program	: Biolog MicroLog1 4.20
Unrestricted Access?	: Yes
Read Time	: Feb 21 2004 12:25
Parent File	:
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: H3-B1
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 49 / 9 / 38
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	{/}	<+>	{/}	{/}	{/}	<+>	<+>	{/}	<+>	-+	-
B	-	<+>	<+>	<+>	-	<+>	-	<+>	<+>	<+>	<+>	<+>
C	<+>	<+>	{/}	<+>	<+>	<+>	<+>	<+>	{/}	-	<+>	<+>
D	<+>	-	-	-+	<+>	<+>	<+>	{/}	<+>	<+>	-	-
E	<+>	-	{/}	-	-	<+>	-	<+>	-	<+-	-	<+>
F	<+>	-	<+>	-	<+>	<+>	<+>	<+>	<+>	-	<+>	-
G	-	-	-	-	-	-	-	-+	<+>	-	-	-
H	-	<+>	<+>	<+>	-	-	-	-	<+>	<+>	<+>	<+>

=> Species ID: Escherichia coli <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Escherichia coli	100	0.71	4.41	GN-ENT
2)	Citrobacter freundii	0	0.00	7.62	GN-ENT
3)	Escherichia coli (USP5-7085)	0	0.00	7.64	GN-ENT
4)	Escherichia coli O157:H7	0	0.00	8.52	GN-ENT
5)	Salmonella gp 1 (choleraesuis) ST typhimurium	0	0.00	9.23	GN-ENT
6)	Citrobacter braakii	0	0.00	9.51	GN-ENT
7)	Citrobacter farmeri	0	0.00	9.88	GN-ENT
8)	Citrobacter sedlakii	0	0.00	11.24	GN-ENT
9)	Leclercia adecarboxylata	0	0.00	11.67	GN-ENT
10)	Salmonella gp 4 (houtenae)	0	0.00	11.76	GN-ENT
Other)					

Print Time = Feb 21 2004 12:25

Page 1 of 1 pages

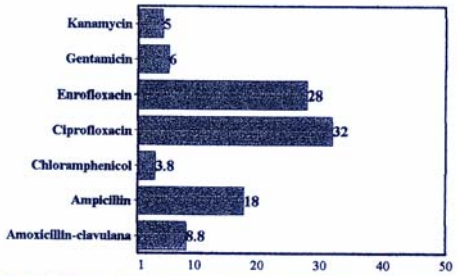
Thursday, March 25, 2004

Specimen Date	Thursday, March 25, 2004	Initialed	E.W /
Specimen Number	H3-A2	Isolate Number	1
		Specimen Type	STOOL
Organism	Escherichia coli		

ANTIBIOTICS	MM	CATEGORY	MIC (mcg/ml)	SERUM IQ *
Amoxicillin-clavulanate	23	S	4	8.8
Ampicillin	21	S	2	18
Chloramphenicol	27	S	4	3.8
Ciprofloxacin	36	S	<0.125	>32
Enrofloxacin	32	S	<0.06	>28
Gentamicin	23	S	1	6
Kanamycin	23	S	4	5
Nalidixic acid	26	S	4	
Neomycin	21	S		
Tetracycline	24	S	4	0.55

RELATIVE ACTIVITY

SERUM IQ *



* IQ, Inhibitory Quotient = Tissue Concentration at lowest usual dosage / MIC.