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Catchment-scale export of antibiotic resistance genes and bacteria from an agricultural watershed in central Iowa.

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Catchment-scale export of antibiotic resistance genes and bacteria from an agricultural watershed in central Iowa.

by

Timothy P. Neher

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Agricultural and Biosystems Engineering

Program of Study Committee:
Michelle Soupir, Major Professor
Adina Howe
Thomas Moorman

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2019

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ABSTRACT

Manure produced by animal feeding operations (AFOs) is commonly applied to surrounding agriculture fields as an agroeconomic practice to improve soil fertility and properly dispose of manure on-site. Antibiotics are utilized by AFOs to control, prevent, and treat disease in livestock animals. Manure from antibiotic treated livestock contains undigested active antibiotic metabolites that provides selective pressure and facilitates the spread of antibiotic resistance to the environment. This two year study monitors a wide range of antibiotic resistance genes *ermB, ermF* (macrolides), *tetA, tetM, tetO, tetW* (tetracyclines), *sul1, sul2* (sulfonamides), *aadA2* (aminoglycosides), *vgaA*, and *vgaB* (pleuroutilins) and total fecal indicator bacteria (FIB) (*E. coli* and enterococci) and tylosin and tetracycline resistant enterococci in catchment outlet waters from a highly agriculturalized watershed. Samples were collected at catchment outlets with areas ranging from 221 to 804 hectares, a scale in which comparisons can be made between areas with and without manure application. Results of the total watershed analysis shows higher concentrations of FIB resistance indicators in the growing season (June-August) and higher ARG indicators in the pre-planting (March-May) and post-harvest (September-December) seasons. The manured catchment had significantly higher (p<0.05) percent tetracycline and tylosin resistant bacteria than the non manured catchment in 2017. Average *ermB* and *tetM* concentrations above limit of quantification (LOQ) from catchment outlet waters ranged from <LOQ to 4,462 copies 100 mL\(^{-1}\) and <LOQ to 39,223 copies 100 mL\(^{-1}\). Two larger catchments of comparable size both showed higher antibiotic resistance indicators compared to a control site outside of the watershed. This study provides evidence that manure application on row crops significantly increases antibiotic resistance genes and bacteria in catchment waters. The early spring and late fall time-periods are determined to be associated with the highest risk of transporting ARGs through catchment outlet water.
CHAPTER 1. INTRODUCTION

The animal agricultural industry is the largest consumer of antibiotics worldwide, used for the prevention and treatment of diseases, and as growth supplements in livestock animals. In the period between 2010 and 2030, global antibiotic usage rates in agriculture are predicted to increase by at least 67% and nearly double in developing countries where legislative enforcement on antibiotic use is lacking (Van Boeckel et al., 2015). The United States in 2017 added new regulations to the Veterinary Feed Directive, changing medically important antibiotics from over the counter to requiring a veterinary prescription (FDA 2016). Manure from antibiotic treated livestock is known to contain low levels of undigested antibiotic metabolites that provides selective pressure on bacteria and facilitates the spread of antibiotic resistance to the environment (Mira, Meza, Nandipati, & Barlow, 2015; Schwaiger et al., 2009). Opportunities for interaction between antibiotics and bacteria occur twofold; in the guts of treated animals and in manure storage facilities. Antibiotic resistance is a natural mechanism in bacteria (S. Y. Kim et al., 2016; Sanderson, Fricker, Brown, Majury, & Liss, 2016), though is accelerated when opportunities of antibiotic and bacteria interaction are increased. Antibiotic resistance is a global public health concern, as antibiotic resistance can pass to disease causing bacteria and cause an incurable infection. It is estimated that 700,000 deaths are attributed to antibiotic resistance per year and could cost the global economy a massive 100 trillion USD between now and 2050 if action is not taken (O’Neill 2016).

Manure application on row crops as fertilizer is commonly practiced in global agriculture (Bowen et al., 2017) to improve soil fertility and properly dispose of animal waste. Manure is rich in essential nutrients necessary for plant growth as well as provides physical improvements to soils such as increases in water holding capacity, regulation of soil
temperature, and improved aggregate stability. However, manure is known to contain excessive amounts of bacteria, and manure application on row crops is the primary source of fecal contamination to receiving downstream waters in the agricultural landscape (Unc & Goss, 2004). Trends in animal production show livestock owners shifting to concentrated animal feeding operations (CAFOs) to maximize production efficiency, leading to the accumulation of excess manure. Fields amended by manure application typically occur in early spring before planting and in late fall, ideally once soil temperatures reach below 10 degrees Celsius to limit nutrient leaching potential. The American Upper Midwest consists of areas with high amounts of CAFOs that can be breeding grounds for antibiotic resistance where bacteria and antibiotics heavily interact (Mira et al., 2015).

Bacteria transport in the environment is closely associated with water flow. In the Great Lakes and Corn Belt states, 37% of cropland is artificially drained to promote soil infiltration in otherwise poorly drained soils (Fausey, Brown, Belcher, & Kanwar, 1995). Although this has led to some of the most productive cropland in the world, tile-drainage has changed the natural hydrology of the region by increasing water flow to downstream catchments, consequently increasing erosion and expediting the transport of nutrients and bacteria to receiving waterbodies (Jamieson, Gordon, Sharples, Stratton, & Madani, 2002). Because water transports bacteria containing the potential for antibiotic resistance from manure amended lands, the importance of monitoring is crucial to understand the factors that most closely influence the potency and export of bacteria and antibiotic resistance.

Several studies have monitored fecal indicator bacteria and a handful of antibiotic resistance genes (ARGs) at plot and larger scale watershed views, but few have monitored at the catchment scale to observe differences in bacterial export from areas with differences in
manure application. The goal of this study is to characterize the extent of antibiotic resistance in a small-scale watershed. Objectives are to monitor tile and surface water for total fecal indicator bacteria, antibiotic specific resistant fecal indicator bacteria, and ARGs at the catchment scale in an agricultural watershed in central Iowa. We hypothesize that 1.) The antibiotic resistance indicators will be highest during the pre-planting (April – May) and post-harvest (September – December), and lowest during the growing season (Jun-August) 2.) The catchment known to receive manure will have higher antibiotic resistance indicators in runoff than the similarly sized catchment known to not receive manure. 3.) Two comparable catchments within the watershed will have greater antibiotic resistance indicators than an outside of the watershed control catchment.
CHAPTER 2. LITERATURE REVIEW

2.1 Antibiotics in Agriculture

The use of antibiotics in agriculture is universal and continues to remain effective at
curing disease and supplementing the health and growth of food-producing animals
(Khachatourians, 1998). Therapeutic uses of antibiotics are intended to treat disease in
individual animals when showing signs of illness. Sub-therapeutic uses of antibiotics are
administered via drinking water or feedstuff to control the spread of disease and prevent
disease outbreak. Sub-therapeutic doses also provide favorable effects in the health of food
producing animals such as improved animal growth and feed efficiency (Lee, Carmosini,
Sassman, Dion, & Sepúlveda, 2007). Both therapeutic and sub-therapeutic practices are
intended to improve animal well-being and increase animal production to meet consumer
demand.

In January 2017, the Veterinary Feed Directive was amended by the United States
Food and Drug Administration requiring livestock owners to obtain a veterinary prescription
before administering medically important antibiotics to livestock animals. The amendment is
intended to eliminate the use of medically important antibiotics as growth-promoters and as
an initiative to protect human and animal health. Although the use of antibiotics is expected
to decrease in feedlots across the United States, the presence of antibiotic residues may
persist in the environment over time (Kolpin, Skopec, Meyer, Furlong, & Zaugg, 2004;
Washington, Moorman, Soupir, Shelley, & Morrow, 2018). A study in Denmark where
similar legislation was passed led to significant declines in antibiotic use, however
therapeutic usage doubled over a ten year period (Aarestrup, 2012).
Medically important classes of antimicrobials are those that are used in human medicine. The overuse of medically important antibiotics in food producing animals could pose potential risks for the acceleration of antibiotic resistance in the environment. Medically important antibiotic classes include aminoglycosides, cephalosporins, fluoroquinolones, lincosamides, tetracyclines, macrolides, penicillins, sulfas, amphenicols, diaminopyrimidines, polymyxins, and streptogramins. Non-medically important antimicrobials used in livestock production include ionophores, aminocoumarins, glycolipids, orthosomycins, pleuromutilins, polypeptides, and quinoxalines. Medically important tetracyclines and non-medically important ionophores are the most widely produced and sold drug classes in America in 2016 (FDA, 2016). Tetracyclines and macrolides are both strongly associated with swine and cattle production.

2.1.1 Antibiotics in Swine Production

The United States Food and Drug Administration’s 2016 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals, estimates that 37% of all medically important and 8% of all non-medically important antimicrobials distributed domestically were intended for use in swine. Estimated annual totals for antimicrobials used in swine production is 3,133,262 kg of medically important and 425,568 kg of non-medically important antimicrobials. Out of all meat and meat products, pigmeat is listed as having the highest predicted increase in global demand from 2017 to 2018 with an increase of 2%, furthering the need for antimicrobials to keep up with demand (FAO, 2018). This increase is set to be the fastest pace since 2012 (FAO, 2018). An estimated 118.7 million tons of pigmeat was produced in 2017 and forecasted to be 121.1 million tons in 2018 (FAO, 2018). Distribution of major classes of medically important antimicrobials used in swine production in 2016 were aminoglycosides, fluoroquinolones, lincosamides, macrolides, penicillins,
sults, and tetracyclines (FDA, 2016). Non-medically important classes of antimicrobials used in swine production include ionophores (FDA, 2016).

2.1.2 Antibiotics in Beef Production

Antibiotics are regularly used in beef production with 43% of antibiotics administered classified as medically important and 55% classified as non-medically important (FDA, 2016). Bovine meat production is expected to increase 1.8% from 2017 to 2018 with an estimated 70.8 million tons in 2017 produced worldwide, and global production of bovine meat is set to increase for the third consecutive year (FAO, 2018). Medically important beef antibiotic classes are aminoglycosides, cephalosporins, fluoroquinolones, macrolides, penicillins, sulfas, and tetracyclines. Non-medically important antibiotics used in beef production are classified as ionophores.

2.2 Antibiotic Characteristics and Function

Antibiotics are classes of drugs used to disrupt microorganism viability and or inhibit vital metabolic functions needed for survival. They are used in agriculture to treat, prevent, and control the spread of disease. In sub-therapeutic doses, antibiotics act as growth promoters in feed animals. Possible mechanisms for increases in animal growth are less intestinal absorption competition within intestinal microbe population (McAllister & Topp, 2012), fewer subclinical infections, and decreased growth-depressing microbial metabolites (Kumar, C. Gupta, Chander, & Singh, 2005). Tetracycline and tylosin are two antibiotics commonly used in feedlots in the U.S. and remain used as growth promoting supplements in parts of the world.

2.2.1 Tetracycline

Tetracycline antibiotics are used widely to treat various types of bacterial infections in humans and livestock due to a broad effectiveness against gram-negative and gram-
positive bacteria (Chopra, Hawkey, & Hinton, 1992). The basic mechanism of tetracycline is the blocking of aminoacyl-tRNA from attaching to the ribosomal acceptor site, causing the inhibition of protein synthesis used for the production of vital proteins for survival. It is estimated in 2016 that 43% and 49% of all domestic sales and distribution of tetracycline in the United States were intended for the use in swine and cattle respectively (FDA, 2016).

2.2.2 Tylosin

Tylosin is a broad range antibiotic in the macrolide antibiotic class used in livestock health that is effective at inhibiting gram-positive bacteria (McGuire et al., 1961). It is often used as a substitute for patients allergic to penicillin (Sande, 1985). The macrolide mode of action is to bind to the 50S ribosomal subunit of susceptible organisms inhibiting bacterial ribosomal action (Noli & Boothe, 1999). The FDA 2016 Report estimates that 61% of domestic sales and distribution of macrolides were intended for use in swine and none intended for cattle.

2.3 Antibiotic Detection in the Environment

The presence of antibiotics in natural ecosystems is well known (Durso & Cook, 2014). Antibiotics are produced naturally by microorganisms to inhibit competition and may play a role in the structure of microbial communities (Broszat & Grohmann, 2014). Shortly after the discovery of penicillin in 1928, natural and synthetic antibiotics have been exploited by humans to combat disease (Gaynes, 2017).

Humans manufacture vast quantities of antibiotics for use in human healthcare and animal agriculture. The animal agricultural industry has caused a surge of antibiotic metabolites to enter into the soil and water environment due to excessive treatment of livestock. Many studies have detected antibiotics in agricultural runoff. Washington et al. (2018) detected tylosin and tetracycline in all tile effluent sites monitored in an Iowa
watershed dominated by swine confinements, Sura et al. (2015) detected concentrations of antimicrobial metabolites in feedlot pen runoff, and Kolpin et al. (2004) detected various antibiotics in several surface water streams in Iowa at low flow conditions.

2.3.1 Tetracycline

Tetracycline is frequently detected in soil and water in agriculture. The main route of export to the environment is as undigested metabolites in animal manure that is then applied to row crops as fertilizer. Tetracycline is generally associated with the solid fractions of manure and soil (Frey et al., 2015). These compounds are able to form chelated compounds with protons and become bound to soil particles (Yeom, Yoon, & Kim, 2017).

2.3.2 Tylosin

Tylosin is used in lower quantities than tetracycline, and is mainly used in swine and less so in cattle production. Tylosin persists in the environment by the attachment of soils due to its weak base nature, it is able to bond to negatively charged soils and sediments (Q. Zhang et al., 2016). A study by Topp, Renaud, Sumarah, and Sabourin (2016) show that soils with a history of manure application retained macrolides such as tylosin for shorter time than soils without a history of manure application (Topp et al., 2016).

2.4 Antibiotic Resistance

Antibiotic resistance has been around as long as naturally occurring antibiotics have (Sanderson et al., 2016) and is known to occur in pristine environments (S. Y. Kim et al., 2016). The most common modes of resistance encoded by antibiotic resistance genes (ARGs) are to create proteins that 1. Physically pump the antibiotic out of the cell, known as efflux 2. Modification of an enzyme that interacts with the drug to neutralize it, known as enzymatic modification 3. The modification of the antibiotic’s intended target area, known as altered target (Dever & Dermody, 1991). Antibiotic resistance is transferred from one organisms to
another through mobile genetic elements such as plasmids that can be incorporated into foreign genomes to confer resistance capabilities (Barlow, 2009). Resistance is selected for, meaning a positive pressure by an antibiotic must be present for strains of bacteria to become resistant and pass along the antibiotic resistance gene for the survival of future generations (Kolář, Urbánek, & Látal, 2001). Sub-lethal concentrations of antibiotics are concentrations below the minimum inhibitory concentration (MIC), the lowest concentration of a specific antibiotic that will prevent the growth of bacteria (Andrews, 2001). Multiple studies have shown that sub-lethal concentrations of antibiotics lead to populations with higher instances of antibiotic resistance (Mira et al., 2015; Schwaiger et al., 2009).

Although the presence of antibiotics facilitate the selection of antibiotic resistance, other factors such as temperature and nutrients have been determined to have an effect on antibiotic resistance (Fahrenfeld et al., 2014). Muurinen et al. (2017) studied the effects of cold stress on bacteria communities, showing increases in ARG relative abundances with colder temperatures. An elevated concentration of nutrients have been shown to increase antibiotic resistance transfer efficiency (West, Liggit, Clemans, & Francoeur, 2011).

ARGs are the basic foundation of antibiotic resistance expression of a microorganism. The gene particles are able to survive extracellularly and may even accumulate in the soil environment over time (Carini et al., 2016). These extracellular genes are not completely stable, and can degrade in the environment if not bound to a stable favorable material such as organic matter or clay particles (Fahrenfeld et al., 2014). Extracellular ARGs is a potential route for microbial organisms to transform antibiotic resistance genes into existing DNA through horizontal gene transfer (von Wintersdorff et al., 2016).
2.4.1 Macrolide

Macrolide resistant bacteria share resistance to tylosin, erythromycin, clindamycin, lincomycin, streptogramin, and azithromycin (Davies & Davies, 2010) through the functional familial relationship of macrolide-lincosamide-streptogramin B (MLS$_B$). The known modes of resistance are hydrolysis, glycosylation, phosphorylation, efflux, and altered target (Davies & Davies, 2010). There are more than 20 known *erm* genes (Roberts et al., 1999) which encode the ribosomal binding site modification (Ghanbari et al., 2016). The *erm* genes work by methylating the 23S rRNA by encoding 23S rRNA adenine-specific N$_6$-methyltransferases (J. Chen, Yu, Michel, Wittum, & Morrison, 2007). This prevents the binding of any MLS$_B$ drug.

2.4.2 Tetracycline

Tetracycline resistant bacteria common modes of resistance are efflux, ribosomal protection, and enzymatic modification (Grossman, 2016). There are 46 known genes that encode resistance to tetracycline (Roberts & Schwarz, 2016). Three of the most commonly studied are *tetA*, *tetM*, and *tetO*, all of which encode for ribosome protection mechanisms to remove tetracycline from the ribosomal binding site, much like the translational GTPase EF-G (Li et al., 2013).

2.4.3 Sulfonamide

Sulfonamide resistant bacteria common modes of resistance are efflux and altered target (Davies & Davies, 2010). The *sul1* and *sul2* genes are associated with sulfonamide resistance and perform by creating an altered target mode of resistance. Both are plasmid-borne, meaning they are linked to free floating plasmids that can be integrated into a bacterial genome. Either *sul1* or *sul2* can initiate resistance by enabling the production of sulfonamide resistant dihydropteroate synthase (DHPS) (Sköld, 2000).
2.4.4 Pleuromutilin

Pleuromutilin is a naturally occurring antimicrobial leading to the development of semi-synthetic antibiotics tiamulin and valnemulin (Paukner & Riedl, 2017). The antimicrobial mode of action is the inhibition of protein synthesis in gram-positive bacteria. Resistance to pleuromutilin is relatively slow to develop and occurs in sequential fashion (Paukner & Riedl, 2017). The plasmid-borne vgaA and vgaB genes are suspected to cause pleuromutilin resistance in staphylococci (van Duijkeren et al., 2014).

2.4.5 Aminoglycoside

Aminoglycoside resistant bacteria common modes of resistance are efflux, altered target, and enzymatic modification; phosphorylation, acetylation, methylation, and adenylation (Davies & Davies, 2010). The gene aadA2 is an ARG that grants resistance to the aminoglycoside streptomycin and the aminocyclitol spectinomycin by enzymatic modification of the adenyltransferase (Michael, Cardoso, & Schwarz, 2005).

2.5 Antibiotic Resistance in Agriculture

Agricultural contribution to the enrichment of antibiotic resistance in the environment cannot be overlooked (Givens et al., 2016; Mallin, McIver, Robuck, & Dickens, 2015; West et al., 2011). Antibiotics are commonly used by concentrated animal feeding operations (CAFOs) to prevent and treat disease in livestock animals. Manure produced by CAFOs is commonly applied to surrounding agricultural fields as an agroeconomic practice to improve soil fertility and properly dispose of manure on-site. This manure from antibiotic treated livestock contains low levels of undigested antibiotics that provide selective pressure on and facilitate the spread of antibiotic resistance to the soil and water environment (Baguer, Jensen, & Krogh, 2000; Mira et al., 2015; Schwaiger et al., 2009). Opportunities for
interaction between antibiotics and bacteria occur in the gut of treated animals, in manure storage facilities, and in soil treated with manure (Kumar et al., 2005).

2.5.1 Manure as a source of antimicrobial resistance

Animal manure is not naturally a source of antibiotic resistance, however when livestock animals are treated with antibiotics, up to 75% of the chemical remains undigested in the animal gut and excreted largely unchanged or as broken up active metabolites (Massé, Saady, & Gilbert, 2014). Manure is known to contain high concentrations of bacteria ranging from $10^3$ to $10^6$ CFU/ml fecal coliforms in liquid swine manure and $10^5$ to $10^7$ CFU/ml fecal coliforms in solid beef manure (Unc & Goss, 2004). Therefore, resistance in manure is commonly observed. Cotta, Whitehead, and Zeltwanger (2003) reported 4 to 32% of bacteria in swine manure resistant to tylosin while another study observed high abundances of sul1 and sul2 ARGs in manure from animals treated with antibiotics and lower abundances in similar animals treated with little antibiotics (Binh, Heuer, Gomes, Kaupenjohann, & Smalla, 2010). Another study monitored eight swine slurry lagoons in the Midwestern United States with varying levels of tetracycline use reported that the high-use lagoon had significantly higher detection of tetracycline resistant gene markers (Peak et al., 2007).

The application of manure on row crops is a major source of antibiotic resistance entering into the environment (Marti et al., 2014). Many studies have documented the spread of antibiotic resistance markers from manure to the soil and water environment. In a long-term study testing the effects of straw, cow, and swine manure application on the abundance of tetL, tetB, tetO, tetW, sul1, ermB, and ermF in soil, the researchers found that straw had no detectable effect, cow manure increased tetM and tetW to detectable levels, and swine manure increased the abundances of all ARGs studied (Peng et al., 2017). Another study compared swine manured soils with non-manured soils and found a substantially higher
antibiotic resistant bacteria abundance in the manured soils as well as observed the integration of the genus *Sphingobacterium* into the soil microbial community, suggesting the persistence of antibiotic resistant bacteria from manure to the soil environment (Yang, Wang, Ren, Szoboszlay, & Moe, 2016).

Several studies have documented the amount of time it takes for ARGs to return to background levels after manure application (Fahrenfeld et al., 2014; Marti et al., 2014; Muurinen et al., 2017). X. Zhou, Qiao, Wang, and Zhu (2017) conducted a soil microcosm experiment by applying swine manure based commercial organic fertilizer on soil determining that two months is not an acceptable time for ARGs to reach background levels. Another study measured the amount of time for ARGs to return to background levels in swine and dairy manure applied soils finding that swine applied soils took 6 weeks while cattle manure amended soils took within 6 months to return to background levels (Sandberg & LaPara, 2016). Furthermore, concentrations of ARGs *erm* B and *erm* F in soil from a plot-scale study by Garder, Moorman, and Soupir (2014) found ARG concentrations from manure applied plots to take six months to a year to reach concentrations observed in the no-manure control plots.

The method of applying manure on row crops may indicate the degree of antibiotic resistance attenuated in soil. Swine manure is generally applied in liquid slurry form and injected into soils while cattle manure is generally solid and broadcast across the surface of the soil followed by tillage. The broadcasting of cattle manure can leave associated organisms and gene fragments exposed to UV penetration and harsh temperatures (McAllister & Topp, 2012). A study by Joy et al. (2013) observed higher *erm* genes in
surface water from manure applied plots by the broadcast method as opposed to the injection method, but found no differences of *erm* genes in the soil profile.

### 2.6 Tile-drainage

In the Great Lakes and Corn Belt states, 37% of cropland is artificially drained to promote soil infiltration in otherwise poorly drained soils (Fausey et al., 1995). Although this has led to some of the most productive cropland in the world, tile-drainage has changed the natural hydrology of the region by increasing water flow to downstream catchments, consequently increasing erosion and expediting the transport of nutrients and bacteria to receiving waterbodies (Jamieson et al., 2002). Studies have observed elevated antibiotic resistant bacteria and genes in tile-water from fields applied with manure. A study by Garder et al. (2014) reported in a plot-scale study that tylosin treated swine manure led to a short-term increase in antibiotic resistant bacteria and *erm* genes in soil, but did not have an impact on tile-drainage water during a year of below-average precipitation. A similar study at the same study location observed statistically higher *erm* genes in tile-drained water from swine manure applied plots, with the study taking place during a more normal and above-average precipitation regime (Luby, Moorman, & Soupir, 2016). Furthermore, a monitoring study in a large watershed with several CAFOs reported tile-drainage consisted of higher concentrations of *ermB* and *ermF* ARGs than in surface water (Rieke, Moorman, Douglass, & Soupir, 2018).

### 2.7 Best Management Practices

Best Management Practices (BMPs) are in-field and edge-of-field practices known to reduce sediment and nutrient pollution to the environment (J. M. Kim et al., 2006; P. Inamdar, Mostaghimi, W. McClellan, & M. Brannan, 2001). However, the impact of BMPs on bacteria and antibiotic resistance remains unclear. Some examples of BMPs implemented
in agriculture are no-till, cover crops, constructed wetlands, riparian buffers, nutrient management plans, grassed waterways, and buffer strips, among others.

Studies have offered insight into whether BMPs can reduce bacteria and antibiotic resistance in agriculture. A study by Richkus, Wainger, and Barber (2016) evaluated the co-benefits of BMP implementation in the Chesapeake Bay on reducing bacteria inputs, estimating that there is the potential for a 27% reduction of bacteria export if practices were implemented. Another study identified constructed wetlands as an effective practice with 80-87% reduction of *E. coli* and 88-97% reduction of enterococci (Diaz, O’Geen, & Dahlgren, 2010). Moreover, narrow grass hedges in swine manure applied fields were tested in a rainfall simulation reducing average tylosin concentrations from 1.47 µg L\(^{-1}\) to 0.12 µg L\(^{-1}\) and average *ermB* concentrations from 2.43 x 10\(^5\) copies mL\(^{-1}\) to 1.09 x 10\(^4\) copies mL\(^{-1}\) in runoff water (Soni et al., 2015).
CHAPTER 3. MATERIALS AND METHODS

3.1 Site Description

The Black Hawk Lake (BHL) watershed is located in western Iowa on the border of Sac and Carroll Counties and along the western edge of the Des Moines Lobe, the furthest extent of glacial formation from around 12,000 years ago. The area is characterized by poorly drained soils and consisting of glacial till derivatives. The 5,324 hectare watershed drains into Black Hawk Lake, a class A1 primary contact recreational lake. The lake is 387 hectares and is the southernmost glacially formed lake in Iowa with a maximum depth of only 4.6 meters.

The watershed is dominated by loams and clay loams formed from glacial till. Land use in the watershed includes 74.6% row crops, 6.7% grass/hay, 5.8% wetlands, 1.9% timber, and 11% other. Two municipalities lie in the watershed: Lake View, population 1,142, lies on the northwest corner of the lake and includes residential housing outlining the north and east perimeter of the lakeshore. Breda, population 409, is situated at the southernmost end of the watershed at the headwaters.

Much of the cropland in the BHL watershed is tile-drained due to the naturally hydric and poorly drained soils native to the Des Moines Lobe. Based on land use, slopes, and soil types, it is estimated that 68% of the land is tile-drained, and it is assumed that all surface flow channels in the watershed are influenced by tile-drainage discharge.

Swine and cattle animal feeding operations (AFOs) are scattered throughout the watershed (Figure 2) documented in a windshield survey. There are ten swine confinements and six cattle lots within and just outside of the watershed border. Manure application on row crops as fertilizer is commonly practiced in agriculture as a way to recycle accumulating
animal waste in AFOs and apply nutrients to crop fields. Manure generated by AFOs in the watershed are applied to approximately 20% of the cropland with the bulk of manure applied in the west-central region as estimated in Figure 1.

Figure 1. Black Hawk Lake watershed manure extent map. Provided by T.J. Lynn, BHL Watershed Coordinator.
Furthermore, Best Management Practices (BMPs) are implemented within the watershed by land-owner discretion, intended to reduce nutrient and sediment losses to downstream waters. Consequently, the distribution of BMPs are uneven throughout the watershed. The most common BMPs in the BHL watershed are terraces, grass waterways, cover crops, no-till, strip-till, perennial native grass plantings (CRP), and nutrient management plans.

3.2 Study Design

Monitoring of fecal indicator bacteria and antibiotic resistance in the BHL watershed occurred from May 2017 through early December 2018. The primary watershed was delineated into fifteen catchments with independent drainage areas for study. The locations monitored within the BHL watershed includes four catchment outlets, S15, ST12, S11, and T8, two control sites 490 and Marina (Figure 2). The “S” and “T” denote surface flow and tile-drain discharge, respectively. The number refers to the catchment from which the discharge originated. Each of the four catchments range in manure application extent and levels of best management practice implementation. The two drainage tiles at the north end of the lake, 490 and Marina, were planned to be used as control sites draining areas without manure application. However preliminary data collected at these sites suggest there is bacterial contamination from septic discharge from the nearby municipality, Lake View, and data from these sites are only used for the total watershed analysis. A surface runoff location outside of the watershed and in the Des Moines Lobe was added to the study in 2018 to serve as a new no-manure control (East Otter Creek, 42°00'00.9"N 94°27'16.7"W).
Figure 2. Black Hawk Lake watershed monitoring locations marked by yellow stars. The shaded regions indicate monitored catchments known to receive manure. Triangles indicate beef/dairy feedlots and squares indicate swine confinements. The East Otter Creek monitoring location is not shown.
Table 1. Area and extent of each site in the study. Sites 490 and Marina do not have corresponding delineated catchments.

| Site          | S11  | ST12 | T8   | S15   | East Otter Creek | 490 | Marina |
|---------------|------|------|------|-------|------------------|-----|--------|
| Area (ha)     | 229.46 | 221.36 | 804.51 | 727.87 | 727.11           | X   | X      |
| Manure Application | Y   | N    | Y    | N     | N                | N   | N      |

Catchments T8 and S11 are known to receive manure (Personal communication, T.J. Lynn Dec. 14th 2017) and have low mixed BMP coverage (<30%) by at least one type of BMP and consisting of at least two AFOs within the drainage area. Catchment ST12 has high mixed BMP coverage (87.5%) by at least one type of BMP and no manure application. This catchment has two monitoring locations at the outlet; a surface outlet and a tile discharge outlet. These flow pathways were analyzed separately and results were combined flow-weighted by dividing the sum of concentrations from the two sites by the combined flow to evaluate total export from catchment ST12. Catchment S15 has low mixed BMP coverage (21.4%) by at least one type of BMP and does not receive manure application, but drains effluent from the town of Breda (Pop=409) along with two AFOs that may contribute to bacteria inputs measured at monitoring site S15. A summary can be found in Table 1.
3.3 Sample Collection

Grab samples were collected at each site in 1 L high-density polyethylene (HDPE) bottles every two weeks in sampling year 2017 and every week in sampling year 2018. Surface water samples were collected at the mid-point of each stream channel, typically in the highest velocity flow. Tile-drainage samples were taken directly from the tile discharge. Each sampling location was sampled on the same day to minimize influence of outside factors to reduce variability between sites such as changes in temperature, precipitation, or sunlight exposure. All grab samples were preserved on ice while transferred to the Water Quality Research Lab at Iowa State University and stored at 20°C for no longer than 24 hours before filtering and storage, however bacterial enumerations determined to be too numerous to count (TNTC) halfway through incubation were re-analyzed no more than 48 hours after collection.

Flow was measured at each location and on each sampling date. Manual measurements were taken using the float method as described in Dobriyal, Badola, Tuboi, and Hussain (2017). Discharge measurements were taken at tile-drained sites by filling a known volume and dividing by time. Beginning in March 2018, a Model 2100 Current Velocity Meter (Swoffer Instruments, Inc.) was used to measure velocity for the manual flow measurements at each site location. In addition, select catchment sites were equipped with ISCO model 750 Area Velocity Module (sites T8, S11, and T12) or an ISCO Model 720 Submerged Probe Module (site S12) throughout the entirety of the study and this flow data is used preferentially to the manual flow measurements at respective sites.

3.4 Phenotypic Plate Count Analysis

Grab samples from the field were analyzed for total enterococci, total E. coli, tylosin resistant and tetracycline resistant enterococci by the membrane filtration technique as
described by APHA (1998). Each sample was filtered individually through a 0.45um sterile disc filter wetted with phosphate-buffered saline (PBS) buffer. Selective Agar used for the analysis was mEnterococcus agar (Difco, Detroit Michigan), mEnterococcus agar spiked with 16 mg/L tetracycline (Sigma-Aldrich, St. Louis, MO), mEnterococcus agar spiked with 35 mg/L tylosin (Sigma-Aldrich, St. Louis, MO), and Modified mTEC agar (Difco, Detroit Michigan). Each antibiotic infused agar was made so that the concentration of active antibiotic in each plate was at the resistance breakpoint concentration to select for antibiotic resistance bacteria growth (Clinical and Laboratory Standards Institute, 2011). The mEnterococcus plates were incubated at 35°C for 48 hours and the Modified mTEC plates were incubated at 35°C for two hours and 44.5°C for 22 hours in a hot water bath. Plates were counted for CFU’s (Colony Forming Units). Samples were diluted and re-plated if resulted in above 250 CFU, indicating TNTC. Final counts were reported as CFU/100mL. If plates were deemed TNTC, but not re-plated in time, then they were treated as 250 CFU/plate and reported as CFU/100mL based on the dilution factor. These results are included in the statistical analysis of the Wilcoxon Rank-Sum Test described subsequently. Because of the ranking system of the test, the measurement represents a sample consisting of high bacterial concentration that should not be disregarded.

3.5 DNA Extraction

A portion of each water sample (250 mL) was filtered through a 0.25µm sterile filter within 24 hours of sampling and stored at -80°C until DNA extraction. Subsequent DNA extraction from the filters were done following the Mo Bio Qiagen MagAttract PowerWater DNA/RNA Kit protocol and Eppendorf epMotion 5075 automated robot. DNA concentrations were measured by the ThermoFisher Scientific Quant-iT TM dsDNA Assay Kit, high sensitivity. The DNA was then stored at -80°C until further analysis.
3.6 Genotypic qPCR Analysis

All environmental water samples were analyzed for the 16S rRNA bacterial gene abundance and a range of genes coding for antibiotic resistance for common antibiotics used in swine and cattle production. These include *ermB, ermF* (macrolides), *tetA, tetM, tetO, tetW* (tetracyclines), *sul1, sul2* (sulfonamides), *aadA2* (aminoglycosides), *vgaA,* and *vgaB* (pleuropneumonia) (Table 2). The qPCR analysis was performed in four runs on the Wafergen SmartChip Realtime PCR System (Wafergen Inc. USA) in the 144 sample x 36 target format. The threshold cycle value (Ct) of 28 was used as the limit of quantification (LOQ) for ARGs without an associated standard curve, and any sample with a Ct-value above the threshold was not used for analysis. Gene targets *16S rRNA, ermB, ermF, and tetM* were run with associated standard curves for gene quantification (copies 100 mL⁻¹) and have unique separate LOQs per qPCR run (Table 4). All other gene targets are reported as relative abundance respective to the 16S rRNA Ct-value using the relationship:

$$\text{Relative abundance} = 2^{-\Delta Ct} \text{, where } \Delta Ct = Ct_{\text{sample}} - Ct_{16S \text{ rRNA}}$$

(Schmittgen & Livak, 2008)

All primer sets and samples were run in triplicate. The gene copy and relative abundance analyses are separate and all genes for the relative abundance analysis including 16S rRNA, *ermB, erF,* and *tetM* follow the same LOQ (Ct<28) requirement instead of calculated standard curve LOQ from the gene copy analysis for consistency between methods. Quantified gene copy ARGs were normalized to the bacteria conserved gene 16S rRNA and represented as a percentage to evaluate proportion of total bacteria with resistance genes in each sample.
Table 2. Table of primers used in the study (Williams et al., 2018). Gene target with associated forward and reverse primers, antibiotic resistance mechanisms, and bacterial targets.

| Gene | Forward Primer (5'-3’) | Reverse Primer (5'-3’) | Mechanism | Bacterial Targets |
|------|------------------------|------------------------|-----------|------------------|
| 16S  | CCTACGGGAGGCAGCAG       | ATTACCGCGGCTGCTGGC     | N/A       | All bacteria     |
| ermB | GAAACACTAGGGTTGTCTGGCA  | CTGAACATCTGTGGATGGC    | protection| Streptococcus,  |
|      |                        |                        |           | Escherichia,    |
|      |                        |                        |           | Staphylococcus, |
|      |                        |                        |           | Enterococcus    |
| ermF | TCTGATGCCCGAAATGGTCAAG | TGAAGGACAATTTAACCTC   | protection| Bacteroides      |
| tetM | GGAGCGATTACAGAATTAGGAAGC| TCCATATGTCCTGGCGTGTCC | protection| Streptococcus,  |
|      |                        |                        |           | Enterococcus,   |
|      |                        |                        |           | Escherichia,    |
|      |                        |                        |           | Listeria        |
| tetA | CTCACCAGCCTGACCTCGAT    | CACGTTGTTATAGAAGCGCATAG| efflux    | Escherichia,    |
|      |                        |                        |           | Actinobacillus, |
|      |                        |                        |           | Citrobacter,    |
|      |                        |                        |           | Pseudomonas,    |
|      |                        |                        |           | Aeromonal,      |
|      |                        |                        |           | Klebsiella,     |
|      |                        |                        |           | Proteus,        |
|      |                        |                        |           | Vibrio,         |
|      |                        |                        |           | Salmonella      |
| tetO | CAACATTTACGGAAATTGTATGATACCA | TTGACGCTCCAATTTACCTGATC | protection| Bifidobacterium |
|      |                        |                        |           | Campylobacter,  |
|      |                        |                        |           | Enterococcus,   |
|      |                        |                        |           | Streptococcus,  |
|      |                        |                        |           | Megasphaera     |
| tetW | ATGAAACATTTCCACCGTATCTTT | ATATCGGCGGAGACCTTATCC | protection| Bifidobacterium |
| sul1 | GCGGATGAGATCAGACGTATTG  | CGCATAGCGGCTGGTTTCT    | protection| Escherichia,    |
|      |                        |                        |           | Actinobacillus, |
|      |                        |                        |           | Citrobacter,    |
|      |                        |                        |           | Pseudomonas,    |
|      |                        |                        |           | Aeromonal,      |
|      |                        |                        |           | Klebsiella,     |
|      |                        |                        |           | Proteus,        |
|      |                        |                        |           | Vibrio,         |
|      |                        |                        |           | Salmonella      |
| sul2 | TCATCTGCCAAACTCGTGTTA   | GTCAAAGAACGCGCAATGT    | protection| Escherichia,    |
|      |                        |                        |           | Actinobacillus, |
|      |                        |                        |           | Citrobacter,    |
|      |                        |                        |           | Pseudomonas,    |
|      |                        |                        |           | Aeromonal,      |
|      |                        |                        |           | Klebsiella,     |
|      |                        |                        |           | Proteus,        |
|      |                        |                        |           | Vibrio,         |
|      |                        |                        |           | Salmonella      |
| aadA2| ACGGCTCCGCAGGATG        | GGCCACAGTAAACACAAAATCA | deactivate| Escherichia,    |
|      |                        |                        |           | Actinobacillus, |
|      |                        |                        |           | Citrobacter,    |
|      |                        |                        |           | Pseudomonas,    |
|      |                        |                        |           | Aeromonal,      |
|      |                        |                        |           | Klebsiella,     |
|      |                        |                        |           | Proteus,        |
|      |                        |                        |           | Vibrio,         |
|      |                        |                        |           | Salmonella      |
| vgaA | GGAAGCTATAGAGGCCTTGAATC| CGGAAGGTTCAATCTCAATCGAC| efflux    | Staphylococcus  |
| vgaB | CAGCCGAGTCTGGCTTCA      | TACGATCTCCATTGAGGGTA   | efflux    | Staphylococcus  |
|      |                        |                        |           | aureus          |
3.7 Statistical Analysis

All statistical analyses were completed using JMP Pro 14. The non-parametric Wilcoxon Rank-Sum Test was used to test for significant differences among growing seasons, between catchments with differing manure management, and among catchments with human influence and manure application. Resulting p-values < 0.05 between two groups were classified as significantly different. The total watershed analysis separately combines plate count data and qPCR data from all BHL sites (S11, T8, ST12, S15, 490, and Marina) per each sampling year and is grouped by seasonality based on sampling date. The three seasonality groups are pre-planting, growing, and post-harvest. The selection of seasonality is to ensure the pre-planting and post-harvest seasons would include manure application practices and the growing season would not. The pre-planting season encompasses all samples collected between March-May, the growing season June-August, and the post-harvest season September-December. Sampling years 2017 and 2018 are analyzed separately. Comparisons between sites of similar size were made using the Wilcoxon Rank-Sum Test. Site comparisons include manured site S11 versus non-manure site ST12, and manured T8 versus human-influenced S15. The latter comparison includes a comparison to a non-manured, non-human influenced control site EOC that is of equal area and similar land use. All data below limit of quantification were assigned a value of zero and included in statistical analyses. Flow relationships were measured by simple linear regression analysis. Relationships with $r^2 > 0.80$ were considered significant.
CHAPTER 4. RESULTS

4.1 BHL Watershed Assessment

4.1.1 Total and Resistant Fecal Indicator Bacteria

Total enterococci concentrations in catchment outlet waters of the BHL watershed (T8, ST12, S11, S15, 490, and marina) were not significantly different between 2017 and 2018. Concentrations ranged from 1 CFU 100 mL\(^{-1}\) to greater than 10^4 CFU 100 mL\(^{-1}\) (Table 1). The median enterococci at the manured catchments S11 and T8 in 2017 were 246 and 58 CFU 100mL\(^{-1}\) respectively, and were 160 and 40 CFU 100 mL\(^{-1}\) in 2018. Total concentrations of \textit{E. coli} in the BHL watershed were significantly greater (p=0.0395) in 2017 than in 2018. The concentrations in 2017 ranged from 1 CFU 100 mL\(^{-1}\) to 10^4 CFU 100 mL\(^{-1}\) while concentrations in 2018 ranged up to 10^3 CFU 100 mL\(^{-1}\) (Table 3). Concentrations of \textit{E. coli} were typically lower than enterococci concentrations.

There was no significant difference between total \textit{E. coli} and total enterococci in the watershed in 2017, but 2018 data shows significantly higher (p=0.0075) enterococci in the BHL watershed than \textit{E. coli}. There was no flow correlation (r^2<0.5) with total enterococci or total \textit{E. coli}.

Seasonal enterococci and \textit{E. coli} concentration trends were observed in the watershed. Total enterococci concentrations in growing and post-harvest seasons were significantly higher (p=0.0248 and p=0.0251) than the pre-planting season in 2017. Total enterococci concentrations in sampling season 2018 were significantly different among all three groups (P<0.0001) with the growing season observed as the highest, followed by post-harvest and pre-planting. Total \textit{E. coli} concentrations in the growing (p=0.0100) and post-harvest (p=0.0019) seasons were both significantly greater than the pre-planting season in 2017 and
all three groups were significantly different (p=0.0074) to each other in 2018 with the
growing season greatest.

Table 3. Median values of *E. coli* and enterococci at each monitoring location and from each
eyear. Average percent tetracycline and tylosin are reported as tetracycline or tylosin resistant
bacteria plate counts divided by the total. Monitoring location names denoted by and “S” and
“T” indicate surface and tile-drainage, respectively. Marina and 490 are tile-outlets and EOC
is a surface water site outside of the watershed used as a control.

| Parameter                        | Year | Monitoring Location |
|----------------------------------|------|---------------------|
| Median E.coli (CFU/100mL)        |      | S11     | ST12 | T8  | S15 | 490  | Marina | EOC |
|                                  | 2017 | 109     | 38   | 37  | 174 | 3150 | 4750   | N/A |
|                                  | 2018 | 57      | 15   | 19  | 144 | 82   | 400    | 156 |
| Median Enterococci (CFU/100mL)   |      | 246     | 80   | 58  | 315 | 3700 | 820    | N/A |
|                                  | 2017 | 246     | 80   | 58  | 315 | 3700 | 820    | N/A |
|                                  | 2018 | 160     | 62   | 40  | 224 | 119  | 382    | 790 |
| Average Percent Tetracycline Resistance (%) |      | 2017 | 16.25 | 5.52 | 18.89 | 4.83 | 0.79 | 1.61 | N/A |
|                                  | 2018 | 3.26   | 2.65 | 21.54 | 11.16 | 4.56 | 2.37 | 1.49 |
| Average Percent Tylosin Resistance (%) | 2017 | 4.49 | 0.64 | 12.04 | 1.98 | 0.12 | 0.16 | N/A |
|                                  | 2018 | 0.98   | 0.64 | 6.77 | 0.94 | 0.15 | 0.04 | 0.04 |

Tetracycline and tylosin resistant enterococci were detected at all sites in the
watershed. Overall, the growing (p=0.0352) and the post-harvest (p=0.0424) seasons had
significantly higher percentage of tetracycline-resistant enterococci than in the pre-planting
season in 2017 while there was no significant difference between the three seasons in 2018.
Tylosin resistant enterococci were detected at all sites in the BHL watershed, but not as
frequently as tetracycline resistant enterococci. No differences in seasonal groups were found
in 2017, but the growing season in 2018 was significantly greater than both pre-planting
(p=0.0003) and post-harvest (p<0.0001) groups. The growing season was observed to have
higher percent resistant tetracycline and tylosin enterococci than the pre-planting and post-
harvest seasons in both years. There was no correlation with flow ($r^2<0.5$) with percent resistant tylosin and tetracycline enterococci.

### 4.1.2 16S rRNA, \textit{ermB}, \textit{ermF}, and \textit{tetM} Copies 100mL$^{-1}$

Table 4. Limits of quantification (LOQ) and limits of detection (LOD) for the four selected genes with associated standard curves 16S rRNA, \textit{ermB}, \textit{ermF}, and \textit{tetM}. Each LOQ is run specific and samples below LOQ are reported as zero for analysis.

| Gene | Run | Date Range | LOQ (copies/100mL) | Total samples | % samples > LOQ | LOQ > % samples > LOD | % sample < LOD |
|------|-----|------------|-------------------|---------------|-----------------|-----------------------|----------------|
| 16S  | 2017| 4/20 - 12/19 | 330,142           | 125           | 98.4            | 0.0                   | 1.6            |
|      | 2018-1 | 3/27 - 7/11 | 49,577            | 117           | 94.0            | 4.3                   | 1.7            |
|      | 2018-2 | 7/18 - 10/2  | 1,598,059         | 117           | 37.6            | 41.9                  | 20.5           |
|      | 2018-3 | 10/11 - 11/27 | 248,521         | 91            | 71.4            | 16.5                  | 12.1           |
| \textit{ermB} | 2017 | 4/20 - 12/19 | 1,774            | 125           | 7.2             | 24.8                  | 68.0           |
|      | 2018-1 | 3/27 - 7/11 | 1,466            | 117           | 9.4             | 13.7                  | 76.9           |
|      | 2018-2 | 7/18 - 10/2  | 18,482           | 117           | 0.0             | 7.7                   | 92.3           |
|      | 2018-3 | 10/11 - 11/27 | 298             | 91            | 14.3            | 0.0                   | 85.7           |
| \textit{ermF} | 2017 | 4/20 - 12/19 | 2,744            | 125           | 0.0             | 24.0                  | 76.0           |
|      | 2018-1 | 3/27 - 7/11 | 2,727            | 117           | 4.3             | 17.1                  | 78.6           |
|      | 2018-2 | 7/18 - 10/2  | 2,211            | 117           | 0.9             | 6.8                   | 92.3           |
|      | 2018-3 | 10/11 - 11/27 | 4,949          | 91            | 0.0             | 14.3                  | 85.7           |
| \textit{tetM} | 2017 | 4/20 - 12/19 | 3,258            | 125           | 14.4            | 36.0                  | 49.6           |
|      | 2018-1 | 3/27 - 7/11 | 2,858            | 117           | 11.1            | 28.2                  | 60.7           |
|      | 2018-2 | 7/18 - 10/2  | 2,734            | 117           | 6.9             | 14.5                  | 78.6           |
|      | 2018-3 | 10/11 - 11/27 | 20,065         | 91            | 2.2             | 28.6                  | 69.2           |

The general bacteria gene indicator 16S rRNA was above the limit of quantification (LOQ) in 76.0% of the total water samples collected (Table 4). Detection of ARGs with an associated standard curve (\textit{ermB}, \textit{ermF}, and \textit{tetM}) are quantified if values fell within the range acceptable of each relative standard curve. Otherwise, data was not used for analysis (Table 4). The total 16S rRNA in the watershed in 2017 was not different from 2018. There was no significant seasonal differences in 2017, but in 2018 significant differences were found among each seasonal group where pre-planting was observed as the highest (p=0.0064), followed by growing (p<0.0001) and post-harvest (p<0.0001).
Figure 3. Time-series plots of ARGs Erm(B), Erm(F), and Tet(M) from 2017 and 2018. Seasonal groups are marked by the vertical dashed lines. Values below LOD were assigned a value of zero.

Antibiotic resistance gene markers for ermB, ermF, and tetM were detected at catchment outlets within the watershed but at low concentrations (Table 5). The ARGs ermB and tetM were detected more frequently than ermF which was below limit of quantification (LOQ) in all 125 samples collected in 2017 and seven out of 325 samples in 2018 (2.2%).
Table 5. The frequency of qPCR detection and median concentration of selected antibiotic resistance genes in stream water of the Blackhawk Lake Watershed above LOQ for each selected gene and corresponding mean copies 100 mL\(^{-1}\) of reads above LOQ at each site location.

| Year | Gene | Parameter | Site Location |
|------|------|-----------|---------------|
|      |      |           | S11 | ST12 | T8  | S15 | 490 | Marina | EOC |
| 2017 | 16S  | % > LOQ   | 100 | 94.4 | 100 | 100 | 100 | 100    | -    |
|      |      | Mean of % > LOQ (copies/100mL) | 5.5E+06 | 2.2E+06 | 1.6E+06 | 9.8E+06 | 1.1E+07 | 4.2E+06 | -    |
|      | ermB | % > LOQ   | 6   | <LOQ | 25.0 | 36.8 | 35.7 | <LOQ   | -    |
|      |      | Mean of % > LOQ (copies/100mL) | 648 | <LOQ | 945 | 1,724 | 17,742 | <LOQ   | -    |
|      | ermF | % > LOQ   | <LOQ | <LOQ | <LOQ | 5.3 | <LOQ | <LOQ   | -    |
|      |      | Median of % > LOQ (copies/100mL) | <LOQ | <LOQ | <LOQ | 61  | <LOQ   | <LOQ   | -    |
|      | tetM | % > LOQ   | 12.5 | 6.7  | 20.0 | 26.3 | 50.0 | <LOQ   | -    |
|      |      | Mean of % > LOQ (copies/100mL) | 4,678 | 1,490 | 6,754 | 4,045 | 6,981 | <LOQ | -    |

| Year | Gene | Parameter | Site Location |
|------|------|-----------|---------------|
|      |      |           | S11 | ST12 | T8  | S15 | 490 | Marina | EOC |
| 2018 | 16S  | % > LOQ   | 54.5 | 75.8 | 63.6 | 75.8 | 71.9 | 62.1    | 65.2 |
|      |      | Mean of % > LOQ (copies/100mL) | 3.3E+06 | 1.3E+06 | 2.7E+06 | 5.0E+06 | 7.0E+06 | 1.8E+06 | 4.2E+06 |
|      | ermB | % > LOQ   | 9.1  | 12.1 | 9.1  | 33.3 | <LOQ | 3.4    | <LOQ |
|      |      | Mean of % > LOQ (copies/100mL) | 4,086 | 937  | 2,240 | 4,462 | <LOQ   | 6,011 | <LOQ |
|      | ermF | % > LOQ   | 6.1  | 3.0  | 3.0  | 9.1  | <LOQ | <LOQ   | <LOQ |
|      |      | Mean of % > LOQ (copies/100mL) | 5,317 | 2,110 | 3,194 | 5,755 | <LOQ   | <LOQ   | <LOQ |
|      | tetM | % > LOQ   | 18.2 | <LOQ | 30.3 | 24.2 | 12.5 | <LOQ   | <LOQ |
|      |      | Mean of % > LOQ (copies/100mL) | 12,880 | <LOQ | 39,223 | 8,328 | 15,863 | <LOQ | <LOQ |

Temporal differences were observed in ARG concentration data between seasonal groups in the watershed (Figure 3). In 2017, ARG \textit{ermB} was significantly higher (p=0.0231) in the post-harvest season than in the growing season, but not different than the pre-planting season. No significant difference was found in seasonality for \textit{ermF}, and \textit{tetM}. Sampling year 2018 does not follow the same trend as 2017, as higher ARG gene copies were observed.
earlier in the year in the pre-planting season. Here, the pre-planting \textit{ermB} was significantly greater than the growing (p=0.0132) and the post-harvest (p=0.0214) seasons. There were no other seasonal trends in quantified ARG copies in 2018. No flow correlations were observed ($r^2<0.5$) with ARG/100 ml$^{-1}$.

\textbf{4.1.3 Relative Abundance}

Catchments S15 and T8 had the highest detection of ARGs in this study (Table 6). The ARG \textit{vgaB} was below LOQ in both sampling years. The ARG \textit{vgaA} was only detected at T8 and S15 in 2018 but rarely. The relative abundance of ARGs (\textit{ermB}, \textit{ermF}, \textit{tetM}, \textit{tetA}, \textit{tetO}, \textit{tetW}, \textit{sul1}, \textit{sul2}, \textit{aadA2}, \textit{vgaA}, \textit{vgaB}) for each sampling year in the BHL watershed were grouped to create a dataset of total relative abundance in order to compare catchments and overall resistance in the BHL watershed. The comparison of relative abundance grouped by seasonality shows significantly higher total relative abundance occurring during the pre-planting (0.0099) and post-harvest (p=0.0198) seasons than the growing season in 2017. In 2018, we observed a similar trend with the pre-harvest season significantly greater (p<0.0001) than the post, but not significantly different to the growing season. The growing season was significantly greater (p=0.0028) than the post-harvest season. This agrees well with the observed ARG gene copy data. There was no correlation with flow ($r^2<0.5$) with any ARG relative abundance.
Table 6. The average relative abundance from each selected ARG at each site in 2017 and 2018. The total number of samples analyzed for each site is shown to the right of every site name as n # of samples analyzed. The amount of detections above LOD (Ct<28) is reported to the right of each corresponding mean relative abundance and is out of the total number of samples analyzed. For example, site S11 tetW was detected in 6 out of 16 samples during sampling year 2017. The Mean relative abundance only includes values above the LOD.

### 4.2 Manure vs. Non-manure Assessment

Total enterococci concentrations compared at manured catchment S11 versus non-manured catchment ST12 revealed that S11 had significantly higher (p=0.0291) total enterococci concentrations in catchment outlet water during 2017 but no significant difference in 2018. Total *E. coli* concentrations between sites were not different in 2017, but S11 contained significantly higher (p=0.0360) concentrations in 2018. Likewise, the catchment comparison of the resistant enterococci showed significantly higher percent
tetracycline (p=0.0060) and tylosin (p=0.0033) resistance at S11 in 2017, but no significant differences in 2018 (Figure 4).

Figure 4. Box-plots of percent tetracycline and tylosin resistant enterococci at manured catchment S11 and non-manured catchment ST12. The middle line on the box-plot represents the median while the upper quartile of the box represents the 75th-percentile and the lower quartile the 25th-percentile. The whiskers denote the range of the quartiles and outliers are marked as dots. The letters above each plot represent significant differences based on the Wilcoxon Rank-Sum Test. Capital letters are associated with 2017 and lower-case letters are associated with 2018. Differing letters mean significant difference between groups (p<0.05).

In 2017, we observed significantly higher (p=0.0003) 16S rRNA in catchment outlet water of S11 than at ST12, and no significant difference between the two catchments in 2018. The ARGs between catchments showed no difference in ARGs with standard curves or total relative abundance between sites in 2017, however, in 2018 there was significantly higher tetM (p=0.0113) copies 100 mL⁻¹ in outlet waters from manured catchment 11 than from non-manured catchment 12.

The catchment outlet water at each site through both years consisted of ARGs determined through the relative abundance analysis. Site S11 contained a larger diversity of ARGs than ST12 (Table 6), more than likely due to manure application practices and the varying antibiotics administered to livestock. The total relative abundance comparison
between sites showed no difference in 2017, however, 2018 data showed S11 contained significantly greater (p=0.0302) total relative abundance than ST12 in catchment effluent.

4.3 Manured vs. Urban Influenced with Control Site

The site comparison of urban influenced catchment S15 versus manured catchment T8 showed that S15 had significantly higher (p=0.0136) enterococci concentrations in outlet waters in 2017 and in 2018. The control site outside of the BHL watershed, East Otter Creek (EOC), had significantly higher enterococci concentrations than both T8 (p<0.0001) and S15 (p=0.0049) in 2018. Catchment discharge water at EOC was not collected in 2017. The urban influenced catchment S15 had significantly higher concentrations of *E. coli* than the manured catchment T8 in both 2017 (p=0.0300) and 2018 (p<0.0015). The control catchment had significantly higher (p=0.0077)) concentrations of total *E. coli* than T8 and no significant difference between EOC and S15 in 2018. The control catchment’s high concentrations of FIB may be due to other sources of FIB such as geese or grazing cows in pastureland. The observation of higher enterococci and *E. coli* in catchment discharge without manure application follow similar trends already observed in this study that manure may not contribute to a prolonged elevation of FIB bacteria in runoff waters.

The comparison of percent resistant enterococci between the manured catchment T8 and the urban influenced catchment S15 showed that T8 had significantly higher percent tetracycline in outlet waters in both 2017 (p=0.0050) and 2018 (p=0.0454) (Figure 5). The site comparison showed that catchments T8 and S15 both had significantly higher (p<0.0001) percent resistance to tetracycline than the control catchment outside of the watershed. The comparison of percent resistant tylosin enterococci between the two BHL sites showed no significant difference in 2017, however, T8 had significantly higher (p=0.0133) percent tylosin resistance in 2018. These two catchments both showed significantly higher
(p=0.0017) percent tylosin resistant enterococci when compared to the similarly sized control site EOC.

Figure 5. Box-plots of percent tetracycline and tylosin resistant enterococci at urban influenced S15, animal manured T8, and control EOC. The middle line on the plot represents the median while the upper quartile of the box represents the 75th-percentile and the lower quartile the 25th-percentile. The whiskers denote the range of the quartiles and outliers are marked as dots. The letters above each plot represent significant differences based on the Wilcoxon Rank-Sum Test. Capital letters are associated with 2017 and lower-case letters are associated with 2018. Differing letters mean significant difference between groups.

Catchment S15 effluent contained significantly higher 16S rRNA than catchment T8 in both 2017 (p<0.0001) and 2018 (p=0.0130). There was no difference in gene copies, except for the \textit{ermB} concentration at S15 was significantly higher in 2017 (p=0.0236) and 2018 (p=0.0162) than at T8. Both T8 and S15 contained the highest observed \textit{ermB} in drainage waters. Additionally, catchment T8 contained spikes of \textit{tetM} in catchment waters in 2018 with a mean of above LOQ at 32,223 copies 100 mL$^{-1}$ compared to mean \textit{tetM} copies of 8,328 copies 100 mL$^{-1}$ at S15 (Table 5 and Figure 3).

The manured catchment T8 consistently contained significantly greater total relative abundance in drainage effluent than the urban influenced S15 in both 2017 (p<0.0001) and 2018 (p=0.0355). The control comparison showed catchments T8 and S15 had significantly higher (p<0.0001) total relative abundances than EOC.
CHAPTER 5. DISCUSSION

The FIB and ARG results in this study show opposing seasonal trends determined by the whole watershed analysis. The FIB concentrations of both total enterococci, total *E. coli*, tetracycline and tylosin resistant enterococci were all greater during the growing season (June-August), while ARG indicators were greater in the pre-planting (March-May) and post-harvest (September-December) seasons. Higher FIB indicators in the growing season may be attributed to the plate count method bias towards warmer months when bacteria cells are more viable and readily grown in the lab (Haack et al., 2016). This aligns with the work by Tomer, Moorman, and Rossi (2008) who measured *E. coli* in the South Fork Watershed in central Iowa, an area consisting of a high density of CAFOs, and observed that the summer season in each of the three tributaries monitored contributed the highest geometric means to the watershed outlet.

The concentrations of ARGs in catchment waters observed in this study follows similar temporal trends reported by Rieke et al. (2018) in the South Fork of the Iowa River Watershed of central Iowa that reported significantly higher *ermB* copies 100 mL⁻¹ in surface water during the drainage (April-July) and the post-fall manure periods (Oct-Dec) than during the pre-fall manure period (July-Sep). Additionally, the concentrations of *ermF* in the South Fork study were observed highest in the drainage period (April-July) in both surface and tile outlets. The observed *ermF* in this study had too few quantifiable results to show any trends among seasons. Additionally, Keen, Knapp, Hall, and Graham (2018) reported higher concentrations of *tetO, tetM, tetQ, and tetW* during the wet season (November-March) than the dry season (April-October) in stream waters in Canada, attributing manure application as the likely contributor. The similar temporal trends observed in ARG concentration export in
agricultural runoff water suggests that early-spring and post-fall manure application practices may contribute to the accumulation of ARGs in soil that are reflected in water monitoring (Han et al., 2018; Nõlvak et al., 2016). Moreover, FIB indicators may be suitable for comparing fecal contamination among locations with little variance of outside factors such as in plot-scale studies, and ARGs should be used preferentially as indicators for antibiotic resistance in watershed-scale studies.

Similarities were found between the antibiotic resistance indicator results of tetracycline and tylosin by the genotypic and phenotypic analyses. We observed higher percent tetracycline resistant enterococci associated with higher tetM resistance genes in comparison to percent tylosin resistant enterococci and associated ermB and ermF resistance genes. This is expected since the corresponding resistance genes are the primary mechanisms for enterococci growth on antibiotic infused agar. The reasons for observing higher tetracycline than tylosin resistance indicators in the BHL watershed may be tied to the types of AFOs within the watershed (ten swine confinements and six cattle lots) and what antibiotics are administered to livestock (Peak et al., 2007; West et al., 2011). Tetracycline is popularly used in both swine and cattle, however tylosin is used vastly more in swine production.

The scale of the current study is unique to studies on antibiotic resistance in agriculture that have been conducted mostly at a plot scale. Median total enterococci concentrations in outlet waters were close to the ranges reported by Luby et al. (2016) who studied enterococci concentrations in tile-drainage from 1-acre plots treated with swine manure. The drainage area of the plot scale study was 0.17% the size of catchment S11 and 0.05% of T8, but remain to observe similar enterococci concentrations in outlet waters.
Average \textit{ermB} concentrations of samples above LOQ at manure applied catchments S11 and T8 were 648 and 945 copies mL$^{-1}$ in 2017 and 4,086 and 2,240 copies 100 mL$^{-1}$, respectively. The \textit{ermF} concentrations were not detected at the two sites in 2017, but in 2018 averages were 5,317 at S11 and 3,194 copies 100 mL$^{-1}$ at T8. The plot scale \textit{ermB} and \textit{ermF} 2-yr mean of drainage water samples above LOQ were reported by Garder et al. (2014) as \(9 \times 10^3\) and \(2.4 \times 10^5\) copies 100 mL$^{-1}$, respectively and Luby et al. (2016) as 4,670 and 1,810 copies 100 mL$^{-1}$, respectively. The differences between magnitudes of ARG concentrations in the two plot scale studies may be attributed to differing precipitation regimes, as the Garder et al. (2014) study was during below average precipitation and the Luby et al. (2016) study was during above average precipitation that perhaps created a dilution effect. The current study precipitation was average in 2017 and above average in 2018. Higher concentrations of \textit{erm} genes are predictable in the two plot scale experiments because the manure applied was known to originate from animal livestock treated with the macrolide antibiotic tylosin and the manure was verified to contain high concentrations of \textit{ermB} and \textit{ermF} genes. At the watershed scale, there is higher variability in antibiotics usage where adjacent fields may receive manure composed of a mix of antibiotic residues and associated ARGs (Cheng, Feng, Liu, Xue, & Li, 2019; Spielmeyer, 2018). Other factors at the watershed scale that can reduce the impact of ARGs in outlet waters are uncertainty in manure application extent, a lack of documented history of manure application (Rahman et al., 2018; Xie et al., 2018) and a knowledge gap of the extent of ARG mobility in soil to connecting waterways (Huang et al., 2019). The observed ARG concentrations of this study compared to other watershed scale studies (Rieke et al., 2018; Topp et al., 2016) are lower. The BHL watershed is 7% the size of the watershed in the study by Rieke et al. (2018) and roughly the same size as in the study
by Keen et al. (2018). The breadth in variations of influencing factors innate in environmental monitoring is reflected in watershed scale studies such as these. Unlike plot scale, watershed wide studies reflect real world conditions to better understand the actual risk of antibiotic resistance in agriculture and subsequently to public health (Thanner, Drissner, & Walsh, 2016).

Manure applied catchment T8 contained the highest mean relative abundance and most frequently detected ARGs in drainage water of all sites in the two year study. The most commonly detected ARG was tetW. The ARG vgaA was only detected at T8 and S15 in 2018 but rarely and vgaB was not detected at all. The vga genes confer resistance to tiamulin, an antibiotic not regulated by the VFD and known to persist in animal manure (Berendsen et al., 2018). Low detection of tiamulin resistance genes are expected, as previous studies on in vitro resistance development have shown low potential for success for resistance to fully develop (Miller, Dunsmore, Fishwick, & Chopra, 2008; Pringle, Poehlsgaard, Vester, & Long, 2004), as resistance develops in a slow step-wise fashion (Paukner & Riedl, 2017). Alternatively, failure to detect tiamulin resistance could result from either poor amplification by our primers or the antibiotic is not used in sufficient quantity to generate detectable resistance genes. The total relative abundance comparison between the two larger catchments S15 and T8 shows that T8 had significantly greater total relative abundance in both sampling years. Additionally, This follows similar observances from Uyaguari-Díaz et al. (2018) who conducted a study that compared ARGs from different watersheds each dominated separately by agriculture, human, or the natural environment and found the agricultural watershed comprised of the highest diversity of ARGs, followed closely by urban and natural containing the lowest. They concluded that sul1 and sul2 were more prevalent in agriculture
dominated watersheds while *tetW* was equally prevalent in agricultural and human dominated areas (Lin et al., 2019). These findings were reflected in this study as the manured catchment T8 had higher detections and average relative abundance of *sul1* and *sul2* than the urban influenced catchment S15. S15 and T8 shared almost equal detection frequency and average relative abundance of *tetW*. The ARG copy 100mL\(^{-1}\) comparison between manure applied T8 and urban influenced S15 showed differences in *ermB* concentration at S15 were significantly higher (p=0.0063) than at T8. It is possible that this detection of *ermB* could originate from human influence (Z.-C. Zhou et al., 2018) such as leaky wastewater infrastructure, as *ermB* is a class of macrolide that shares cross resistance with other MLS\(_B\) drugs used in human medicine (Ghanbari et al., 2016). However, the waste water treatment facility for the municipality is downstream of the monitoring location. It is also possible that some crop fields in catchment S15 are unknowingly applied with manure, as two swine confinements straddle the catchment border. Additionally, catchment T8 contains spikes of *tetM* in catchment waters in 2018. These spikes occurred during low to normal flow rates in the growing season, suggesting other unknown drivers may have influenced the increases in export of ARGs, for example the lag-time between ARG source deposition and subsequent mobility within the soil/water matrix (Heuer, Schmitt, & Smalla, 2011). Another possibility is a sudden release of active antibiotics previously held inert by soil may create a localized rapid production of ARGs in bacteria that are then transported to catchment outlets through normal flow conditions (Jechalke, Heuer, Siemens, Amelung, & Smalla, 2014). Regardless, both catchments contributed to antibiotic resistance in comparison to the control site outside of the watershed.
The catchment comparison between the two similarly sized catchments with and without manure application practices suggests that manure application influences bacteria and antibiotic resistance indicators in catchment outlet waters (Q.-L. Chen et al., 2017). Total enterococci was significantly higher at the manured catchment in 2017 and *E. coli* was significantly higher in 2018. This result contradicts the findings of A. Pappas, S. Kanwar, L. Baker, C. Lorimor, and Mickelson (2008) who observed similar concentrations of enterococci in drainage waters from plots treated with swine manure and plots free of manure. The catchment with manure application consistently had significantly greater or no difference between measured antibiotic resistance indicators measured in outlet waters than the catchment without. However, notable differences between these two catchments are levels of best management practices (BMPs) implemented by land owners, and the extent of tile-drainage, which may or may not influence the presence of antibiotic resistance indicators in outlet waters. Little research has gone into the effects of BMPs on bacteria export (Díaz et al., 2010; Richkus et al., 2016), and even fewer on BMPs to reduce antibiotic resistance in agriculture (Soni et al., 2015). Tile-water on the other hand has been observed to contain higher concentrations of resistance constituents than in surface water and could also provide a reason for observing higher total relative abundance at tile-drainage driven T8 than at mainly surface drained S15 (Rieke et al., 2018). Reasons for this may be the persistence of ARGs in the soil matrix (Y.-J. Zhang et al., 2017), favorability of cell growth and decay in tile-lines, or the rapid transport to drainage discharge with increased opportunity for horizontal gene transfer in the presence of low levels of antibiotics (Fahrenfeld et al., 2014). Surface water samples from all catchment outlets are assumed to have inputs from upstream tile-discharge, but the extent is unknown.
CHAPTER 6. CONCLUSIONS

In this study, we observed significant differences in antibiotic resistance indicators among different crop seasons from catchment outlet waters in an agriculturally dominated small-scale watershed. Plate count FIB and antibiotic resistant enterococci detection in catchment outlet waters were greatest in the growing season. Conversely, the ARG detection in catchment outlet waters was significantly greater during the pre-planting months suggesting that spring manure application may contribute the most to the facilitation of antibiotic resistance to agricultural soils as reflected in our water monitoring. Moreover, FIB indicators may be suitable for comparing fecal contamination among locations, however ARGs should be used preferentially as indicators for antibiotic resistance.

The scale of this study allowed for better confidence in comparisons of monitored antibiotic resistance indicators due to differences in manure application practices or land use factors. At the catchment scale, we observed differences between catchments within the watershed that differed in manure application practices and urban influence. The comparison between manured and non-manured catchments suggests that manure application significantly increases resistance indicators in catchment outlet waters. The catchment with manure application was always either significantly greater or had no difference in bacteria and antibiotic resistance factors than the non-manured catchment. The comparison between the urban influenced and manured catchments with control suggests that human-influences may contribute more to FIB in outlet waters, and manured fields may contribute slightly more ARG to the environment. However, both catchments contributed to antibiotic resistance in comparison to the control site.
6.1 Implications and Recommendations for Future Work

This study provides strong evidence that manure application on row crops will significantly increase antibiotic resistance genes and bacteria in catchment waters. Seasonal time-periods associated with the highest risk of transporting ARGs through catchment outlet waters is in early spring and late fall. Plate count methods were limited by growth factors and genetic analyses should be used preferentially. Antibiotic usage in livestock production is necessary for animal well-being, however new regulations with the VFD and a greater awareness of antibiotic usage in agriculture may help reduce the input drivers of antibiotic resistance, yet the persistence of antibiotics and antibiotic resistance in soils will remain a risk to public health.

Future studies should use metagenomic analyses to focus on identifying classifications of bacteria that are most likely to confer antibiotic resistance capabilities and that also persist in the soil and water environment. Experiments that characterize the mobility of ARGs in soil will also help improve our understanding of public health risk. The detection of ARGs in water is of the greatest concern to human health because of the potential for the spread of resistance to recreational and urban areas. Studies should focus on in-field mitigation strategies for ways to improve ARG attenuation in soils after manure application to limit the degree of ARG pollution in waterways. Although the scale of this study is relatively small, confounding factors between catchments are possible such as the amount and types of BMPs implemented, the extent of tile-drainage contributions, and human influences. Further research should investigate the potential for specific BMPs to reduce ARG export from manure applied fields. Additionally, future studies should add human specific fecal indicator gene targets to better assess human contributions to antibiotic resistance in agriculture.
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### APPENDIX A. DATA 2017 & 2018

**FIB Plate Counts (CFU 100 mL\(^{-1}\)) and Discharge (m\(^3\)/s)**

| Site | Date       | FIB Plate Count (ent) | FIB Plate Count (ent+TYL) | FIB Plate Count (ent+TET) | E. coli | Discharge (m\(^3\)/s) |
|------|------------|-----------------------|---------------------------|---------------------------|--------|-----------------------|
| T12  | 5/17/17    | 80                    | 3                         | n/a                       | n/a    | 0.10                  |
| S12  | 5/17/17    | 80                    | 2                         | n/a                       | n/a    | 0.08                  |
| S15  | 5/17/17    | 80                    | 25                        | n/a                       | n/a    | 0.30                  |
| T8   | 5/17/17    | 80                    | 28                        | n/a                       | n/a    | 0.07                  |
| S11  | 5/17/17    | 80                    | 39                        | n/a                       | n/a    | n/a                   |
| 490  | 5/17/17    | 80                    | 0                         | n/a                       | n/a    | n/a                   |
| Marina | 5/17/17   | 80                    | 1                         | n/a                       | n/a    | n/a                   |
| ST12 | 5/17/17    | 80                    | 3                         | 0                         | 0      | 0.18                  |
| T12  | 5/31/17    | 10                    | 0                         | 0                         | 4      | 0.01                  |
| S12  | 5/31/17    | 16                    | 0                         | 0                         | 5      | 0.01                  |
| S15  | 5/31/17    | 206                   | 8                         | 2                         | 12     | n/a                   |
| T8   | 5/31/17    | 6                     | 0                         | 0                         | 5      | 0.09                  |
| S11  | 5/31/17    | 37                    | 0                         | 9                         | 48     | 0.05                  |
| 490  | 5/31/17    | 10                    | 0                         | 0                         | 3      | n/a                   |
| Marina | 5/31/17   | 356                   | 0                         | 24                        | 320    | n/a                   |
| ST12 | 5/31/17    | 13                    | 0                         | 0                         | 4      | 0.02                  |
| T12  | 6/14/17    | 146                   | 5                         | 10                        | 91     | 0.07                  |
| S12  | 6/14/17    | 310                   | 19                        | 23                        | 200    | 0.00                  |
| S15  | 6/14/17    | 2140                  | 14                        | 135                       | 200    | n/a                   |
| T8   | 6/14/17    | 51                    | 0                         | 26                        | 24     | 0.32                  |
| S11  | 6/14/17    | 1800                  | 68                        | 67                        | 200    | 0.01                  |
| 490  | 6/14/17    | 1330                  | 0                         | 2                         | 273    | n/a                   |
| Marina | 6/14/17   | 5100                  | 12                        | 67                        | 3510   | n/a                   |
| ST12 | 6/14/17    | 147                   | 5                         | 10                        | 92     | 0.07                  |
| T12  | 6/29/17    | 80                    | 0                         | 1                         | 18     | 0.09                  |
| S12  | 6/29/17    | 154                   | 1                         | 8                         | 95     | 0.00                  |
| S15  | 6/29/17    | 244                   | 1                         | 14                        | 82     | n/a                   |
| T8   | 6/29/17    | 78                    | 6                         | 5                         | 91     | 0.05                  |
| S11  | 6/29/17    | 326                   | 8                         | 47                        | 196    | 0.02                  |
| 490  | 6/29/17    | 400                   | 8                         | 15                        | 200    | n/a                   |
| Marina | 6/29/17   | n/a                   | n/a                       | n/a                       | n/a    | n/a                   |
| ST12 | 6/29/17    | 82                    | 0                         | 1                         | 20     | 0.09                  |
| T12  | 7/12/17    | 36                    | 0                         | 1                         | 2      | 0.03                  |
| S12  | 7/12/17    | 204                   | 3                         | 19                        | 82     | 0.01                  |
| S15  | 7/12/17    | 356                   | 1                         | 14                        | 174    | n/a                   |
|   |     |   |   |   |   |   |
|---|-----|---|---|---|---|---|
| T8 | 7/12/17 | 30 | 0 | 6 | 12 | 0.02 |
| S11 | 7/12/17 | 1000 | 67 | 67 | 400 | 0.03 |
| 490 | 7/12/17 | 400 | 85 | 67 | 400 | n/a |
| Marina | 7/12/17 | n/a | n/a | n/a | n/a | n/a |
| ST12 | 7/12/17 | 71 | 1 | 5 | 19 | 0.04 |
| T12 | 7/28/17 | 26 | 0 | 1 | 2 | 0.01 |
| S12 | 7/28/17 | n/a | n/a | n/a | n/a | 0.00 |
| S15 | 7/28/17 | 910 | 3 | 34 | 780 | n/a |
| T8 | 7/28/17 | 278 | 1 | 38 | 220 | 0.01 |
| S11 | 7/28/17 | 1310 | 18 | 92 | 560 | 0.01 |
| 490 | 7/28/17 | n/a | n/a | n/a | n/a | n/a |
| Marina | 7/28/17 | n/a | n/a | n/a | n/a | n/a |
| ST12 | 7/28/17 | 26 | 0 | 1 | 2 | 0.01 |
| T12 | 8/9/17 | 184 | 4 | 15 | 8 | 0.00 |
| S12 | 8/9/17 | n/a | n/a | n/a | n/a | 0.00 |
| S15 | 8/9/17 | 1190 | 122 | 45 | 790 | n/a |
| T8 | 8/9/17 | 32 | 0 | 1 | 36 | 0.01 |
| S11 | 8/9/17 | n/a | n/a | n/a | n/a | 0.00 |
| 490 | 8/9/17 | n/a | n/a | n/a | n/a | n/a |
| Marina | 8/9/17 | n/a | n/a | n/a | n/a | n/a |
| ST12 | 8/9/17 | 184 | 4 | 15 | 8 | 0.00 |
| T12 | 8/25/17 | n/a | n/a | n/a | n/a | 0.00 |
| S12 | 8/25/17 | n/a | n/a | n/a | n/a | 0.00 |
| S15 | 8/25/17 | 12900 | 220 | 995 | 4100 | n/a |
| T8 | 8/25/17 | 278 | 5 | 61 | 236 | 0.00 |
| S11 | 8/25/17 | n/a | n/a | n/a | n/a | 0.00 |
| 490 | 8/25/17 | n/a | n/a | n/a | n/a | n/a |
| Marina | 8/25/17 | n/a | n/a | n/a | n/a | n/a |
| ST12 | 8/25/17 | n/a | n/a | n/a | n/a | 0.00 |
| T12 | 9/7/17 | n/a | n/a | n/a | n/a | 0.00 |
| S12 | 9/7/17 | n/a | n/a | n/a | n/a | 0.00 |
| S15 | 9/7/17 | 800 | 0 | 120 | 100 | n/a |
| T8 | 9/7/17 | 56 | 0 | 1 | 30 | 0.00 |
| S11 | 9/7/17 | n/a | n/a | n/a | n/a | 0.00 |
| 490 | 9/7/17 | n/a | n/a | n/a | n/a | n/a |
| Marina | 9/7/17 | n/a | n/a | n/a | n/a | n/a |
| ST12 | 9/7/17 | n/a | n/a | n/a | n/a | 0.00 |
| T12 | 9/21/17 | n/a | n/a | n/a | n/a | 0.00 |
| S12 | 9/21/17 | n/a | n/a | n/a | n/a | 0.00 |
| S15 | 9/21/17 | 470 | 2 | 13 | 240 | n/a |
| T8 | 9/21/17 | 890 | 6 | 168 | 1890 | 0.01 |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| S11 | 9/21/17 | n/a | n/a | n/a | n/a | 0.00 |
| 490 | 9/21/17 | n/a | n/a | n/a | n/a | n/a |
| Marina | 9/21/17 | n/a | n/a | n/a | n/a | n/a |
| ST12 | 9/21/17 | n/a | n/a | n/a | n/a | 0.00 |
| T12 | 10/9/17 | 161 | 0 | 7 | 126 | 0.01 |
| S12 | 10/9/17 | 740 | 1 | 3 | 360 | 0.07 |
| S15 | 10/9/17 | 550 | 8 | 30 | 430 | n/a |
| T8 | 10/9/17 | 80 | 5 | 24 | 77 | 0.04 |
| S11 | 10/9/17 | 540 | 4 | 23 | 169 | 0.01 |
| 490 | 10/9/17 | 4400 | 2 | 59 | 3400 | n/a |
| Marina | 10/9/17 | 6200 | 1 | 46 | 2400 | n/a |
| ST12 | 10/9/17 | 177 | 0 | 7 | 132 | 0.01 |
| T12 | 10/19/17 | 85 | 0 | 3 | 52 | 0.03 |
| S12 | 10/19/17 | 181 | 1 | 7 | 140 | 0.00 |
| S15 | 10/19/17 | 153 | 1 | 10 | 180 | 0.10 |
| T8 | 10/19/17 | 31 | 9 | 13 | 37 | 0.01 |
| S11 | 10/19/17 | 165 | 6 | 15 | 109 | 0.01 |
| 490 | 10/19/17 | 12600 | 42 | 76 | 23500 | n/a |
| Marina | 10/19/17 | 2600 | 0 | 19 | 6200 | n/a |
| ST12 | 10/19/17 | 86 | 0 | 3 | 53 | 0.03 |
| T12 | 11/6/17 | 63 | 0 | 3 | 169 | 0.00 |
| S12 | 11/6/17 | 85 | 0 | 1 | 940 | 0.00 |
| S15 | 11/6/17 | 274 | 0 | 2 | 570 | n/a |
| T8 | 11/6/17 | 620 | 22 | 19 | 145 | 0.01 |
| S11 | 11/6/17 | 440 | 2 | 7 | 94 | 0.01 |
| 490 | 11/6/17 | 44800 | 229 | 528 | 51000 | n/a |
| Marina | 11/6/17 | 770 | 0 | 5 | 4100 | n/a |
| ST12 | 11/6/17 | 76 | 0 | 2 | 617 | 0.00 |
| T12 | 11/15/17 | 36 | 0 | 1 | 42 | 0.01 |
| S12 | 11/15/17 | 133 | 0 | 0 | 450 | 0.01 |
| S15 | 11/15/17 | 35 | 0 | 1 | 78 | n/a |
| T8 | 11/15/17 | 155 | 8 | 17 | 74 | 0.01 |
| S11 | 11/15/17 | 102 | 13 | 25 | 23 | 0.00 |
| 490 | 11/15/17 | 5000 | 2 | 58 | 11700 | n/a |
| Marina | 11/15/17 | 4600 | 0 | 18 | 22300 | n/a |
| ST12 | 11/15/17 | 86 | 0 | 0 | 251 | 0.01 |
| T12 | 11/28/17 | 24 | 0 | 1 | 40 | 0.00 |
| S12 | 11/28/17 | 34 | 0 | 0 | 93 | 0.00 |
| S15 | 11/28/17 | 33 | 1 | 1 | 127 | 0.01 |
| T8 | 11/28/17 | 60 | 19 | 24 | 72 | 0.02 |
| S11 | 11/28/17 | 100 | 11 | 44 | 12 | 0.02 |
|     | Date     |   |   |   |   |
|-----|----------|---|---|---|---|
| 490 | 11/28/17 | 25700 | 0 | 38 | 16300 | n/a |
| Marina | 11/28/17 | 800 | 4 | 16 | 3800 | n/a |
| T12  | 12/5/17  | 30  | 0 | 0  | 69   | 0.00 |
| S12  | 12/5/17  | 18  | 0 | 0  | 9    | 0.00 |
| S15  | 12/5/17  | 98  | 0 | 0  | 84   | 0.00 |
| T8   | 12/5/17  | 600 | 13 | 32 | 78   | n/a |
| S11  | 12/5/17  | 18  | 2 | 7  | 21   | 0.02 |
| 490  | 12/5/17  | 2900 | 2 | 13 | 2900 | n/a |
| Marina | 12/5/17 | 1300 | 3 | 11 | 8700 | n/a |
| ST12 | 12/5/17  | 49  | 0 | 0  | 38   | 0.00 |
| T12  | 12/12/17 | 9   | 0 | 1  | 5    | 0.00 |
| S12  | 12/12/17 | 8   | 0 | 0  | 11   | 0.00 |
| S15  | 12/12/17 | 20  | 1 | 1  | 17   | 0.01 |
| T8   | 12/12/17 | 10  | 4 | 1  | 14   | 0.01 |
| S11  | 12/12/17 | 6   | 1 | 3  | 3    | 0.02 |
| 490  | 12/12/17 | 3700 | 0 | 60 | 1800 | n/a |
| Marina | 12/12/17 | 490 | 1 | 8  | 5400 | n/a |
| ST12 | 12/12/17 | 9   | 0 | 1  | 6    | 0.00 |
| T12  | 12/19/17 | 240 | 0 | 86 | 290  | 0.00 |
| S12  | 12/19/17 | 18  | 0 | 0  | 16   | 0.00 |
| S15  | 12/19/17 | 32  | 1 | 1  | 14   | 0.02 |
| T8   | 12/19/17 | 36  | 24 | 3  | 26   | 0.01 |
| S11  | 12/19/17 | 337 | 5 | 39 | 1010 | 0.00 |
| 490  | 12/19/17 | 5200 | 9 | 67 | 6500 | n/a |
| Marina | 12/19/17 | 820 | 3 | 6  | 5600 | n/a |
| ST12 | 12/19/17 | 168 | 0 | 58 | 201  | 0.00 |
| 490  | 3/27/2018 | 18 | 0 | 0  | 1    | n/a |
| Marina | 3/27/2018 | 29 | 0 | 0  | 59   | n/a |
| S11  | 3/27/2018 | 9   | 0 | 0  | 8    | 0.06 |
| S15  | 3/27/2018 | 96  | 1 | 8  | 165  | 0.18 |
| Sub 12 | 3/27/2018 | 19 | 0 | 2  | 5    | 0.03 |
| T8   | 3/27/2018 | 61  | 1 | 9  | 11   | 0.07 |
| T12  | 3/27/2018 | 9   | 0 | 2  | 7    | 0.02 |
| S12  | 3/27/2018 | 34  | 0 | 1  | 1    | 0.01 |
| 490  | 4/5/2018  | 3   | 0 | 0  | 0    | n/a |
| Marina | 4/5/2018 | 12 | 0 | 0  | 47   | n/a |
| S11  | 4/5/2018  | 1   | 2 | 1  | 7    | 0.03 |
| S15  | 4/5/2018  | 53  | 0 | 0  | 2    | 0.09 |
| Sub 12 | 4/5/2018 | 5   | 0 | 0  | 2    | 0.01 |
| T8   | 4/5/2018  | 0   | 2 | 0  | 1    | 0.04 |
| Location | Date       | Count | Shape | Size | Thickness |
|----------|------------|-------|-------|------|-----------|
| T12      | 4/5/2018   | 4     | 0     | 0    | 2         | 0.01     |
| S12      | 4/5/2018   | 9     | 0     | 0    | 0         | 0.00     |
| 490      | 4/11/2018  | 119   | 0     | 2    | 316       | n/a      |
| Marina   | 4/11/2018  | 35    | 0     | 3    | 225       | n/a      |
| S11      | 4/11/2018  | 10    | 0     | 1    | 4         | 0.03     |
| S15      | 4/11/2018  | 12    | 0     | 0    | 6         | 0.05     |
| Sub 12   | 4/11/2018  | 15    | 0     | 1    | 0         | 0.01     |
| T8       | 4/11/2018  | 10    | 0     | 1    | 4         | 0.03     |
| T12      | 4/11/2018  | 17    | 0     | 1    | 0         | 0.01     |
| S12      | 4/11/2018  | 9     | 0     | 0    | 0         | 0.00     |
| 490      | 4/24/2018  | 10    | 0     | 0    | 10        | n/a      |
| Marina   | 4/24/2018  | 0     | 0     | 0    | 70        | n/a      |
| S11      | 4/24/2018  | 0     | 0     | 0    | 1         | 0.03     |
| S15      | 4/24/2018  | 4     | 0     | 1    | 1         | 0.07     |
| Sub 12   | 4/24/2018  | 3     | 0     | 1    | 1         | 0.03     |
| T8       | 4/24/2018  | 7     | 0     | 5    | 3         | 0.06     |
| T12      | 4/24/2018  | 8     | 0     | 2    | 4         | 0.01     |
| S12      | 4/24/2018  | 1     | 0     | 0    | 0         | 0.02     |
| 490      | 5/7/2018   | 5     | 0     | 0    | 2         | n/a      |
| Marina   | 5/7/2018   | 162   | 0     | 0    | 284       | n/a      |
| S11      | 5/7/2018   | 11    | 0     | 0    | 2         | 0.03     |
| S15      | 5/7/2018   | 25    | 1     | 1    | 21        | n/a      |
| Sub 12   | 5/7/2018   | 22    | 0     | 0    | 4         | 0.04     |
| T8       | 5/7/2018   | 14    | 1     | 2    | 13        | 0.08     |
| T12      | 5/7/2018   | 31    | 1     | 1    | 5         | 0.01     |
| S12      | 5/7/2018   | 18    | 0     | 0    | 4         | 0.03     |
| 490      | 5/16/2018  | 20    | 0     | 0    | 21        | n/a      |
| Marina   | 5/16/2018  | 382   | 0     | 8    | 411       | n/a      |
| S11      | 5/16/2018  | 11    | 0     | 0    | 0         | 0.03     |
| S15      | 5/16/2018  | 30    | 1     | 3    | 47        | 0.06     |
| Sub 12   | 5/16/2018  | 11    | 1     | 0    | 10        | 0.03     |
| T8       | 5/16/2018  | 4     | 0     | 2    | 5         | 0.06     |
| T12      | 5/16/2018  | 15    | 0     | 0    | 18        | 0.01     |
| S12      | 5/16/2018  | 9     | 1     | 0    | 7         | 0.02     |
| 490      | 5/23/2018  | 111   | 1     | 1    | 222       | n/a      |
| Marina   | 5/23/2018  | 200   | 0     | 54   | 200       | n/a      |
| S11      | 5/23/2018  | 20    | 0     | 1    | 55        | 0.04     |
| S15      | 5/23/2018  | 25    | 1     | 3    | 26        | 0.04     |
| Sub 12   | 5/23/2018  | 47    | 0     | 0    | 21        | 0.03     |
| T8       | 5/23/2018  | 9     | 1     | 2    | 6         | 0.08     |
| T12      | 5/23/2018  | 26    | 0     | 1    | 37        | 0.01     |
|   | Date       | Value | Type | Value | Type | Value | Type |
|---|------------|-------|------|-------|------|-------|------|
| S12| 5/23/2018  | 54    | 0    | 0     | 15   | 0.02  |
| 490| 5/30/2018  | 460   | 0    | 1     | 130  | n/a   |
| Marina| 5/30/2018 | 1,050 | 0    | 28    | 136  | n/a   |
| S11| 5/30/2018  | 94    | 2    | 6     | 171  | 0.02  |
| S15| 5/30/2018  | 91    | 1    | 6     | 80   | 0.04  |
| Sub 12| 5/30/2018 | 147   | 1    | 1     | 19   | 0.02  |
| T8 | 5/30/2018  | 19    | 0    | 1     | 8    | 0.07  |
| EOC| 5/30/2018  | 282   | 0    | 6     | 156  | n/a   |
| T12| 5/30/2018  | 26    | 0    | 1     | 11   | 0.01  |
| S12| 5/30/2018  | 243   | 1    | 1     | 25   | 0.01  |
| 490| 6/6/2018   | 128   | 0    | 1     | 82   | n/a   |
| Marina| 6/6/2018 | 1,790 | 4    | 18    | 2,000| n/a   |
| S11| 6/6/2018   | 180   | 1    | 6     | 76   | 0.02  |
| S15| 6/6/2018   | 235   | 1    | 8     | 162  | 0.04  |
| Sub 12| 6/6/2018 | 98    | 1    | 3     | 76   | 0.02  |
| T8 | 6/6/2018   | 55    | 2    | 3     | 11   | 0.05  |
| T12| 6/6/2018   | 20    | 0    | 0     | 6    | 0.01  |
| S12| 6/6/2018   | 158   | 1    | 5     | 131  | 0.01  |
| 490| 6/13/2018  | 72    | 2    | 3     | 32   | n/a   |
| Marina| 6/13/2018 | 1,840 | 3    | 13    | 2,300| n/a   |
| S11| 6/13/2018  | 374   | 3    | 15    | 116  | 0.00  |
| S15| 6/13/2018  | 400   | 8    | 53    | 598  | 0.24  |
| Sub 12| 6/13/2018 | 155   | 1    | 2     | 81   | 0.05  |
| T8 | 6/13/2018  | 72    | 15   | 20    | 33   | 0.07  |
| T12| 6/13/2018  | 83    | 1    | 2     | 46   | 0.03  |
| S12| 6/13/2018  | 320   | 1    | 1     | 162  | 0.01  |
| 490| 6/20/2018  | 400   | 6    | 131   | 694  | n/a   |
| Marina| 6/20/2018 | 2,600 | 1    | 11    | 700  | n/a   |
| S11| 6/20/2018  | 1,140 | 10   | 44    | 270  | 0.04  |
| S15| 6/20/2018  | 200   | 270  | 200   | 3,600| n/a   |
| Sub 12| 6/20/2018 | 857   | 72   | 83    | 1,337| 0.07  |
| T8 | 6/20/2018  | 200   | 39   | 67    | 200  | 0.14  |
| T12| 6/20/2018  | 400   | 73   | 54    | 400  | 0.05  |
| S12| 6/20/2018  | 2,000 | 70   | 157   | 3,680| 0.02  |
| 490| 6/27/2018  | 1,450 | 1    | 104   | 780  | n/a   |
| S11| 6/27/2018  | 380   | 1    | 16    | 90   | 0.06  |
| S15| 6/27/2018  | 300   | 6    | 114   | 200  | 0.23  |
| Sub 12| 6/27/2018 | 530   | 0    | 12    | 30   | 0.07  |
| T8 | 6/27/2018  | 320   | 0    | 40    | 90   | 0.24  |
| EOC| 6/27/2018  | 4,000 | 0    | 404   | 20   | 0.05  |
| T12| 6/27/2018  | 530   | 0    | 12    | 30   | 0.07  |
| ID    | Date      | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 |
|-------|-----------|---------|---------|---------|---------|---------|
| 490   | 7/2/2018  | 20,000  | 180     | 588     | 2,000   | n/a     |
| S11   | 7/2/2018  | 260     | 3       | 5       | 60      | 0.01    |
| S15   | 7/2/2018  | 320     | 0       | 3       | 90      | 0.19    |
| Sub 12| 7/2/2018  | 162     | 1       | 2       | 27      | 0.04    |
| T8    | 7/2/2018  | 64      | 1       | 10      | 28      | 0.14    |
| EOC   | 7/2/2018  | 520     | 0       | 5       | 40      | 0.12    |
| T12   | 7/2/2018  | 88      | 1       | 1       | 30      | 0.04    |
| S12   | 7/2/2018  | 620     | 0       | 5       | 10      | 0.01    |
| Marina| 7/11/2018 | 500     | 1       | 1       | 1,200   | n/a     |
| S11   | 7/11/2018 | 190     | 1       | 1       | 8       | 0.03    |
| S15   | 7/11/2018 | 200     | 1       | 2       | 26      | 0.09    |
| Sub 12| 7/11/2018 | 63      | 0       | 1       | 6       | 0.02    |
| T8    | 7/11/2018 | 29      | 2       | 0       | 19      | 0.08    |
| EOC   | 7/11/2018 | 760     | 0       | 3       | 154     | 0.12    |
| T12   | 7/11/2018 | 60      | 0       | 1       | 8       | 0.01    |
| S12   | 7/11/2018 | 70      | 0       | 0       | 7       | 0.01    |
| 490   | 7/18/2018 | 206     | 0       | 2       | 46      | n/a     |
| Marina| 7/18/2018 | 460     | 0       | 8       | 330     | n/a     |
| S11   | 7/18/2018 | 212     | 1       | 2       | 46      | 0.00    |
| S15   | 7/18/2018 | 224     | 1       | 12      | 53      | 0.06    |
| Sub 12| 7/18/2018 | 49      | 0       | 1       | 5       | 0.01    |
| T8    | 7/18/2018 | 40      | 2       | 1       | 50      | 0.01    |
| EOC   | 7/18/2018 | 1,500   | 1       | 9       | 188     | 0.05    |
| T12   | 7/18/2018 | 55      | 0       | 2       | 4       | 0.01    |
| S12   | 7/18/2018 | 32      | 0       | 0       | 6       | 0.00    |
| 490   | 7/25/2018 | 14      | 0       | 0       | 66      | n/a     |
| Marina| 7/25/2018 | 1,790   | 1       | 46      | 1,610   | n/a     |
| S11   | 7/25/2018 | 570     | 0       | 6       | 30      | 0.00    |
| S15   | 7/25/2018 | 304     | 0       | 19      | 83      | 0.05    |
| Sub 12| 7/25/2018 | 61      | 0       | 1       | 8       | 0.01    |
| T8    | 7/25/2018 | 115     | 14      | 12      | 94      | 0.04    |
| EOC   | 7/25/2018 | 2,410   | 2       | 24      | 316     | 0.03    |
| T12   | 7/25/2018 | 58      | 0       | 0       | 9       | 0.01    |
| S12   | 7/25/2018 | 65      | 0       | 2       | 50      | 0.01    |
| 490   | 8/1/2018  | 48      | 0       | 1       | 8       | n/a     |
| S11   | 8/1/2018  | 740     | 4       | 10      | 93      | 0.00    |
| S15   | 8/1/2018  | 382     | 2       | 34      | 300     | 0.02    |
| Sub 12| 8/1/2018  | 132     | 1       | 7       | 26      | 0.00    |
| T8    | 8/1/2018  | 98      | 0       | 3       | 12      | 0.03    |
| EOC   | 8/1/2018  | 1,920   | 1       | 15      | 250     | 0.02    |
| T12   | 8/1/2018  | 64      | 0       | 0       | 1       | 0.00    |
|     |     |     |     |      |      |
|-----|-----|-----|-----|------|------|
| S12 | 8/1/2018 | 200 | 1 | 13 | 137 | 0.00 |
| 490 | 8/8/2018 | 400 | 1 | 61 | 266 | n/a  |
| Marina | 8/8/2018 | 560 | 1 | 3 | 480 | n/a  |
| S11 | 8/8/2018 | 816 | 3 | 15 | 169 | 0.00 |
| S15 | 8/8/2018 | 400 | 20 | 73 | 474 | 0.18 |
| Sub 12 | 8/8/2018 | 390 | 1 | 14 | 120 | 0.01 |
| T8 | 8/8/2018 | 400 | 52 | 67 | 200 | 0.03 |
| EOC | 8/8/2018 | 2,180 | 2 | 31 | 300 | 0.02 |
| T12 | 8/8/2018 | 297 | 1 | 15 | 106 | 0.01 |
| S12 | 8/8/2018 | 498 | 1 | 12 | 99 | 0.01 |
| Marina | 8/15/2018 | 550 | 1 | 11 | 236 | n/a  |
| S11 | 8/15/2018 | 1,650 | 26 | 34 | 123 | 0.00 |
| S15 | 8/15/2018 | 2,930 | 11 | 134 | 528 | 0.04 |
| Sub 12 | 8/15/2018 | 369 | 3 | 11 | 63 | 0.00 |
| T8 | 8/15/2018 | 1,180 | 10 | 200 | 996 | 0.02 |
| EOC | 8/15/2018 | 1,790 | 0 | 15 | 240 | 0.01 |
| T12 | 8/15/2018 | 104 | 1 | 2 | 51 | 0.00 |
| S12 | 8/15/2018 | 1,164 | 10 | 39 | 247 | 0.00 |
| Marina | 8/21/2018 | 15,000 | 17 | 310 | 6,400 | n/a  |
| S11 | 8/21/2018 | 3,900 | 3 | 110 | 1,200 | n/a  |
| S15 | 8/21/2018 | 5,760 | 89 | 482 | 2,090 | 0.66 |
| Sub 12 | 8/21/2018 | 1,939 | 3 | 53 | 235 | 0.06 |
| 490 | 8/21/2018 | 1,490 | 16 | 278 | 570 | 0.23 |
| T8 | 8/21/2018 | 790 | 0 | 11 | 240 | 0.07 |
| EOC | 8/21/2018 | 1,910 | 3 | 52 | 229 | 0.04 |
| T12 | 8/21/2018 | 2,000 | 2 | 56 | 2 | 0.02 |
| S12 | 8/21/2018 | 450 | 1 | 5 | 130 | 0.01 |
| S15 | 8/21/2018 | 380 | 0 | 17 | 140 | 0.10 |
| Sub 12 | 8/21/2018 | 193 | 0 | 2 | 15 | 0.01 |
| T8 | 8/21/2018 | 90 | 1 | 18 | 20 | 0.05 |
| EOC | 8/21/2018 | 2,040 | 0 | 21 | 180 | n/a  |
| T12 | 9/6/2018 | 100 | 0 | 5 | 80 | n/a  |
| Marina | 9/6/2018 | 100 | 0 | 7 | 210 | n/a  |
| S11 | 9/6/2018 | 910 | 2 | 16 | 194 | 0.11 |
| S15 | 9/6/2018 | 540 | 6 | 35 | 530 | n/a  |
| Sub 12 | 9/6/2018 | 317 | 0 | 4 | 365 | 0.06 |
| T8 | 9/6/2018 | 135 | 1 | 30 | 102 | 0.14 |
|     |     |     |     |     |
|-----|-----|-----|-----|-----|
|     |     |     |     |     |
| EOC | 9/6/2018 | 3,360 | 1 | 64 | 200 | 0.27 |
| T12 | 9/6/2018 | 296 | 0 | 4 | 500 | 0.04 |
| S12 | 9/6/2018 | 360 | 1 | 5 | 36 | 0.02 |
| 490 | 9/11/2018 | 22 | 0 | 1 | 14 | n/a |
| Marina | 9/11/2018 | 140 | 0 | 13 | 360 | n/a |
| S11 | 9/11/2018 | 160 | 1 | 4 | 108 | 0.02 |
| S15 | 9/11/2018 | 200 | 1 | 18 | 178 | 0.12 |
| Sub 12 | 9/11/2018 | 44 | 0 | 1 | 15 | 0.04 |
| T8 | 9/11/2018 | 6 | 1 | 2 | 9 | 0.06 |
| EOC | 9/11/2018 | 240 | 0 | 4 | 40 | 0.08 |
| T12 | 9/11/2018 | 48 | 0 | 1 | 3 | 0.03 |
| S12 | 9/11/2018 | 36 | 1 | 1 | 20 | 0.02 |
| 490 | 9/26/2018 | 44 | 0 | 5 | 78 | n/a |
| Marina | 9/26/2018 | 60 | 0 | 1 | 0 | n/a |
| S11 | 9/26/2018 | 86 | 0 | 3 | 57 | 0.01 |
| S15 | 9/26/2018 | 390 | 1 | 12 | 172 | 0.16 |
| Sub 12 | 9/26/2018 | 62 | 0 | 1 | 10 | 0.04 |
| T8 | 9/26/2018 | 24 | 1 | 8 | 22 | 0.09 |
| EOC | 9/26/2018 | 2,220 | 1 | 41 | 258 | 0.32 |
| T12 | 9/26/2018 | 52 | 0 | 1 | 5 | 0.03 |
| S12 | 9/26/2018 | 80 | 0 | 0 | 13 | 0.01 |
| 490 | 10/2/2018 | 166 | 0 | 9 | 96 | n/a |
| Marina | 10/2/2018 | 160 | 0 | 1 | 0 | n/a |
| S11 | 10/2/2018 | 618 | 2 | 22 | 138 | 0.11 |
| S15 | 10/2/2018 | 1,630 | 23 | 59 | 538 | 0.38 |
| Sub 12 | 10/2/2018 | 151 | 0 | 1 | 22 | 0.08 |
| T8 | 10/2/2018 | 106 | 1 | 27 | 29 | 0.15 |
| EOC | 10/2/2018 | 2,510 | 3 | 94 | 430 | 0.51 |
| T12 | 10/2/2018 | 158 | 0 | 1 | 26 | 0.05 |
| S12 | 10/2/2018 | 136 | 0 | 1 | 10 | 0.02 |
| 490 | 10/11/2018 | 100 | 0 | 10 | 168 | n/a |
| Marina | 10/11/2018 | 50 | 0 | 0 | 130 | n/a |
| S11 | 10/11/2018 | 280 | 1 | 6 | 70 | 0.17 |
| S15 | 10/11/2018 | 720 | 18 | 51 | 780 | n/a |
| Sub 12 | 10/11/2018 | 124 | 0 | 2 | 11 | 0.09 |
| T8 | 10/11/2018 | 104 | 1 | 18 | 18 | 0.18 |
| EOC | 10/11/2018 | 3,640 | 2 | 44 | 220 | n/a |
| T12 | 10/11/2018 | 132 | 0 | 2 | 11 | 0.06 |
| S12 | 10/11/2018 | 104 | 0 | 1 | 11 | 0.03 |
| 490 | 10/16/2018 | 4 | 0 | 2 | 18 | n/a |
| Marina | 10/16/2018 | 16 | 0 | 0 | 52 | n/a |
|          | Date       | Code | Count | Value | Rate |
|----------|------------|------|-------|-------|------|
| S11      | 10/16/2018 | 32   | 0     | 3     | 68   | 0.01 |
| S15      | 10/16/2018 | 100  | 1     | 13    | 90   | 0.16 |
| Sub 12   | 10/16/2018 | 56   | 0     | 1     | 11   | 0.04 |
| T8       | 10/16/2018 | 16   | 1     | 0     | 8    | 0.12 |
| EOC      | 10/16/2018 | 100  | 0     | 4     | 10   | 0.21 |
| T12      | 10/16/2018 | 80   | 0     | 1     | 11   | 0.03 |
| S12      | 10/16/2018 | 18   | 0     | 0     | 19   | 0.02 |
| 490      | 10/23/2018 | 142  | 0     | 6     | 26   | n/a  |
| Marina   | 10/23/2018 | 400  | 0     | 17    | 400  | n/a  |
| S11      | 10/23/2018 | 20   | 0     | 0     | 36   | 0.01 |
| S15      | 10/23/2018 | 188  | 0     | 87    | 102  | 0.12 |
| Sub 12   | 10/23/2018 | 61   | 0     | 0     | 12   | 0.04 |
| T8       | 10/23/2018 | 7    | 1     | 1     | 3    | 0.08 |
| EOC      | 10/23/2018 | 100  | 0     | 1     | 24   | 0.20 |
| T12      | 10/23/2018 | 10   | 0     | 0     | 6    | 0.02 |
| S12      | 10/23/2018 | 121  | 0     | 0     | 24   | 0.02 |
| 490      | 10/30/2018 | 400  | 0     | 1     | 400  | n/a  |
| Marina   | 10/30/2018 | 1,890| 0     | 4     | 2,000| n/a  |
| S11      | 10/30/2018 | 10   | 0     | 0     | 19   | 0.00 |
| S15      | 10/30/2018 | 92   | 0     | 10    | 144  | 0.10 |
| Sub 12   | 10/30/2018 | 61   | 0     | 0     | 12   | 0.04 |
| T8       | 10/30/2018 | 8    | 6     | 3     | 10   | 0.08 |
| EOC      | 10/30/2018 | 60   | 0     | 1     | 26   | 0.16 |
| T12      | 10/30/2018 | 9    | 0     | 1     | 12   | 0.01 |
| S12      | 10/30/2018 | 68   | 0     | 0     | 79   | 0.01 |
| 490      | 11/6/2018  | 154  | 0     | 1     | 75   | n/a  |
| Marina   | 11/6/2018  | 980  | 0     | 74    | 1,960| n/a  |
| S11      | 11/6/2018  | 147  | 0     | 4     | 11   | 0.02 |
| S15      | 11/6/2018  | 340  | 7     | 92    | 400  | 0.13 |
| Sub 12   | 11/6/2018  | 76   | 0     | 1     | 48   | 0.02 |
| T8       | 11/6/2018  | 95   | 4     | 24    | 46   | 0.07 |
| EOC      | 11/6/2018  | 406  | 1     | 3     | 24   | 0.27 |
| T12      | 11/6/2018  | 114  | 0     | 1     | 28   | 0.01 |
| S12      | 11/6/2018  | 17   | 0     | 1     | 13   | 0.01 |
| 490      | 11/14/2018 | 90   | 0     | 4     | 118  | n/a  |
| Marina   | 11/14/2018 | 550  | 0     | 37    | 3,860| n/a  |
| S11      | 11/14/2018 | 10   | 0     | 1     | 7    | 0.02 |
| S15      | 11/14/2018 | 16   | 0     | 2     | 72   | 0.04 |
| Sub 12   | 11/14/2018 | 10   | 0     | 0     | 5    | 0.02 |
| T8       | 11/14/2018 | 3    | 0     | 1     | 4    | 0.06 |
| EOC      | 11/14/2018 | 40   | 0     | 1     | 44   | n/a  |
| Site  | Date     | 16S rRNA | ermB | ermF | tetM |
|-------|----------|----------|------|------|------|
| T12   | 4/20/17  | 1,622,148| <LOQ | <LOQ | <LOQ |
| S12   | 4/20/17  | 4,737,957| <LOQ | <LOQ | <LOQ |
| S15   | 4/20/17  | 4,430,483| <LOQ | <LOQ | <LOQ |
| T8    | 4/20/17  | 2,177,365| <LOQ | <LOQ | 1,851|
| S11   | 4/20/17  | 5,965,866| <LOQ | <LOQ | <LOQ |
| T12   | 5/4/17   | 1,760,034| <LOQ | <LOQ | <LOQ |
| S12   | 5/4/17   | 1,757,345| <LOQ | <LOQ | <LOQ |
| S15   | 5/4/17   | 4,190,299| <LOQ | <LOQ | <LOQ |
| T8    | 5/4/17   | 275,302  | <LOQ | <LOQ | <LOQ |
| S11   | 5/4/17   | 3,387,290| <LOQ | <LOQ | <LOQ |
| 490   | 5/4/17   | 1,647,552| <LOQ | <LOQ | <LOQ |
| T12   | 5/17/17  | 6,388,844| <LOQ | <LOQ | <LOQ |
| S12   | 5/17/17  | 14,129,606| <LOQ | <LOQ | <LOQ |
| S15   | 5/17/17  | 45,268,028| 1,453| <LOQ | 4,920|
| T8    | 5/17/17  | 11,757,705| 679  | <LOQ | 7,777|
| S11   | 5/17/17  | 32,654,921| <LOQ | <LOQ | <LOQ |
| 490   | 5/17/17  | 11,073,037| <LOQ | <LOQ | <LOQ |
| Marina| 5/17/17  | 7,165,872| <LOQ | <LOQ | <LOQ |

**16S rRNA, ermB, ermF, tetM Gene Concentrations (Copies 100 mL⁻¹)**
| Sub 12 | 5/17/17 | 9,766,210 | <LOQ | <LOQ | <LOQ |
|-------|---------|-----------|------|------|------|
| T12   | 5/31/17 | 849,670   | <LOQ | <LOQ | <LOQ |
| S12   | 5/31/17 | 2,130,488 | <LOQ | <LOQ | <LOQ |
| S15   | 5/31/17 | 2,372,285 | 775  | <LOQ | <LOQ |
| T8    | 5/31/17 | 676,620   | <LOQ | <LOQ | <LOQ |
| S11   | 5/31/17 | 3,276,045 | <LOQ | <LOQ | <LOQ |
| 490   | 5/31/17 | 756,026   | <LOQ | <LOQ | <LOQ |
| Marina| 5/31/17 | 3,665,846 | <LOQ | <LOQ | <LOQ |
| Sub 12| 5/31/17 | 1,467,759 | <LOQ | <LOQ | <LOQ |
| T12   | 6/14/17 | 984,529   | <LOQ | <LOQ | <LOQ |
| S12   | 6/14/17 | 2,886,589 | <LOQ | <LOQ | <LOQ |
| S15   | 6/14/17 | 7,964,831 | <LOQ | <LOQ | <LOQ |
| T8    | 6/14/17 | 766,171   | <LOQ | <LOQ | <LOQ |
| S11   | 6/14/17 | 4,307,869 | <LOQ | <LOQ | <LOQ |
| 490   | 6/14/17 | 911,869   | <LOQ | <LOQ | <LOQ |
| Marina| 6/14/17 | 7,961,348 | <LOQ | <LOQ | <LOQ |
| Sub 12| 6/14/17 | 1,000,193 | <LOQ | <LOQ | <LOQ |
| T12   | 6/29/17 | 1,099,222 | <LOQ | <LOQ | <LOQ |
| S12   | 6/29/17 | 2,383,392 | <LOQ | <LOQ | <LOQ |
| S15   | 6/29/17 | 441,490   | <LOQ | <LOQ | <LOQ |
| T8    | 6/29/17 | 1,015,244 | <LOQ | <LOQ | <LOQ |
| 490   | 6/29/17 | 47,300,037| <LOQ | <LOQ | 1,032 |
| Sub 12| 6/29/17 | 1,136,358 | <LOQ | <LOQ | <LOQ |
| T12   | 7/12/17 | 891,404   | <LOQ | <LOQ | <LOQ |
| S12   | 7/12/17 | 11,280,218| <LOQ | <LOQ | <LOQ |
| S15   | 7/12/17 | 1,469,185 | <LOQ | <LOQ | <LOQ |
| T8    | 7/12/17 | 536,172   | <LOQ | <LOQ | <LOQ |
| S11   | 7/12/17 | 2,770,801 | <LOQ | <LOQ | <LOQ |
| 490   | 7/12/17 | 1,493,455 | <LOQ | <LOQ | <LOQ |
| Sub 12| 7/12/17 | 3,062,201 | <LOQ | <LOQ | <LOQ |
| T12   | 7/28/17 | 1,173,405 | <LOQ | <LOQ | <LOQ |
| S12   | 7/28/17 | 4,711,471 | <LOQ | <LOQ | <LOQ |
| S15   | 7/28/17 | 846,503   | <LOQ | <LOQ | <LOQ |
| T8    | 7/28/17 | 3,168,598 | <LOQ | <LOQ | <LOQ |
| Sub 12| 7/28/17 | 3,062,201 | <LOQ | <LOQ | <LOQ |
| T12   | 8/9/17  | 1,659,015 | <LOQ | <LOQ | <LOQ |
| S12   | 8/9/17  | 6,320,111 | <LOQ | <LOQ | <LOQ |
| T8    | 8/9/17  | 499,441   | <LOQ | <LOQ | <LOQ |
| Sub 12| 8/9/17  | 1,659,015 | <LOQ | <LOQ | <LOQ |
| S15   | 8/25/17 | 57,635,891| 1,192| <LOQ | 2,534|
| T8    | 8/25/17 | 2,150,404 | <LOQ | <LOQ | 6,974|
| Sub 12 | 8/25/17 |   0  | <LOQ  | <LOQ  | <LOQ  |
|--------|---------|------|-------|-------|-------|
| S15    | 9/7/17  | 16,080,102 | <LOQ  | <LOQ  | <LOQ  |
| T8     | 9/7/17  | 1,507,442   | <LOQ  | <LOQ  | <LOQ  |
| Sub 12 | 9/7/17  |       0     | <LOQ  | <LOQ  | <LOQ  |
| S15    | 9/21/17 | 7,054,387   | <LOQ  | <LOQ  | <LOQ  |
| T8     | 9/21/17 | 3,222,983   | 1,154 | <LOQ  | 10,411 |
| Sub 12 | 9/21/17 |       0     | <LOQ  | <LOQ  | <LOQ  |
| S15    | 9/7/17  | 16,080,102 | <LOQ  | <LOQ  | <LOQ  |
| T8     | 9/7/17  | 1,507,442   | <LOQ  | <LOQ  | <LOQ  |
| Sub 12 | 9/7/17  |       0     | <LOQ  | <LOQ  | <LOQ  |
| S15    | 9/21/17 | 7,054,387   | <LOQ  | <LOQ  | <LOQ  |
| T8     | 9/21/17 | 3,222,983   | 1,154 | <LOQ  | 10,411 |
| Sub 12 | 9/21/17 |       0     | <LOQ  | <LOQ  | <LOQ  |
| T12    | 10/9/17 | 1,028,261   | <LOQ  | <LOQ  | <LOQ  |
| S12    | 10/9/17 | 2,099,789   | <LOQ  | <LOQ  | <LOQ  |
| S15    | 10/9/17 | 7,946,810   | <LOQ  | 61    | 6,113  |
| T8     | 10/9/17 | 900,053     | <LOQ  | <LOQ  | <LOQ  |
| S11    | 10/9/17 | 5,877,466   | <LOQ  | <LOQ  | <LOQ  |
| 490    | 10/9/17 | 52,067,288  | <LOQ  | <LOQ  | <LOQ  |
| Marina | 10/9/17 | 1,135,628   | <LOQ  | <LOQ  | <LOQ  |
| Sub 12 | 10/9/17 | 1,032,532   | <LOQ  | <LOQ  | <LOQ  |
| T12    | 10/19/17| 839,384     | <LOQ  | <LOQ  | <LOQ  |
| S12    | 10/19/17| 2,613,069   | <LOQ  | <LOQ  | <LOQ  |
| S15    | 10/19/17| 2,076,717   | <LOQ  | <LOQ  | <LOQ  |
| T8     | 10/19/17| 767,009     | <LOQ  | <LOQ  | <LOQ  |
| S11    | 10/19/17| 2,273,000   | <LOQ  | <LOQ  | <LOQ  |
| 490    | 10/19/17| 3,086,642   | <LOQ  | <LOQ  | <LOQ  |
| Marina | 10/19/17| 1,075,455   | <LOQ  | <LOQ  | <LOQ  |
| Sub 12 | 10/19/17| 839,510     | <LOQ  | <LOQ  | <LOQ  |
| T12    | 11/6/17 | 1,584,398   | <LOQ  | <LOQ  | <LOQ  |
| S12    | 11/6/17 | 5,537,109   | <LOQ  | <LOQ  | <LOQ  |
| S15    | 11/6/17 | 3,022,655   | <LOQ  | <LOQ  | <LOQ  |
| T8     | 11/6/17 | 1,238,345   | 613   | <LOQ  | <LOQ  |
| S11    | 11/6/17 | 3,306,955   | <LOQ  | <LOQ  | <LOQ  |
| 490    | 11/6/17 | 6,237,852   | 54,992| <LOQ  | 5,920  |
| Marina | 11/6/17 | 2,521,646   | <LOQ  | <LOQ  | <LOQ  |
| Sub 12 | 11/6/17 | 936,049     | <LOQ  | <LOQ  | <LOQ  |
| T12    | 11/15/17| 753,051     | <LOQ  | <LOQ  | <LOQ  |
| S12    | 11/15/17| 1,762,531   | <LOQ  | <LOQ  | <LOQ  |
| S15    | 11/15/17| 2,272,738   | 859   | <LOQ  | <LOQ  |
| T8     | 11/15/17| 484,735     | <LOQ  | <LOQ  | <LOQ  |
| S11    | 11/15/17| 3,082,450   | 648   | <LOQ  | 2,135  |
| 490    | 11/15/17| 3,109,196   | 23,214| <LOQ  | 5,299  |
| Marina | 11/15/17| 3,729,279   | <LOQ  | <LOQ  | <LOQ  |
| Sub 12 | 11/15/17| 381,378     | <LOQ  | <LOQ  | <LOQ  |
| T12    | 11/28/17| 1,352,485   | <LOQ  | <LOQ  | <LOQ  |
| S12    | 11/28/17| 5,021,763   | <LOQ  | <LOQ  | <LOQ  |
|   | Date       | Value        |   |    |    |
|---|------------|--------------|---|----|----|
| T8 | 11/28/17   | 1,262,077    | 1,470 | <LOQ | <LOQ |
| S11| 11/28/17   | 2,623,632    | <LOQ | <LOQ | <LOQ |
| 490| 11/28/17   | 6,616,868    | 6,522 | <LOQ | 8,068 |
| Marina | 11/28/17 | 3,894,628    | <LOQ | <LOQ | <LOQ |
| Sub 12 | 11/28/17 | 1,113,144    | <LOQ | <LOQ | <LOQ |
| T12 | 12/5/17    | 1,726,814    | <LOQ | <LOQ | <LOQ |
| S12 | 12/5/17    | 11,379,362   | <LOQ | <LOQ | <LOQ |
| S15 | 12/5/17    | 4,673,563    | 5,943 | <LOQ | 2,570 |
| T8 | 12/5/17    | 569,972      | <LOQ | <LOQ | <LOQ |
| S11| 12/5/17    | 3,866,974    | <LOQ | <LOQ | <LOQ |
| 490| 12/5/17    | 5,332,685    | <LOQ | <LOQ | 4,312 |
| Marina | 12/5/17 | 7,175,596    | <LOQ | <LOQ | <LOQ |
| Sub 12 | 12/5/17 | 2,486,051    | <LOQ | <LOQ | <LOQ |
| T12 | 12/12/17   | 6,547,661    | <LOQ | <LOQ | <LOQ |
| S12 | 12/12/17   | 3,009,181    | <LOQ | <LOQ | <LOQ |
| S15 | 12/12/17   | 3,581,189    | 1,158 | <LOQ | 4,088 |
| T8  | 12/12/17   | 745,470      | <LOQ | <LOQ | <LOQ |
| S11 | 12/12/17   | 3,839,065    | <LOQ | <LOQ | <LOQ |
| 490 | 12/12/17   | 4,344,255    | 2,205 | <LOQ | 1,649 |
| Marina | 12/12/17 | 3,200,732    | <LOQ | <LOQ | <LOQ |
| Sub 12 | 12/12/17 | 5,626,795    | <LOQ | <LOQ | <LOQ |
| T12 | 12/19/17   | 986,673      | <LOQ | <LOQ | 2,208 |
| S12 | 12/19/17   | 2,549,212    | <LOQ | <LOQ | <LOQ |
| S15 | 12/19/17   | 2,306,936    | <LOQ | <LOQ | <LOQ |
| T8  | 12/19/17   | 1,941,234    | 810  | <LOQ | <LOQ |
| S11 | 12/19/17   | 5,819,677    | <LOQ | <LOQ | 7,221 |
| 490 | 12/19/17   | 9,028,318    | 1,779 | <LOQ | 22,584 |
| Marina | 12/19/17 | 4,918,081    | <LOQ | <LOQ | <LOQ |
| Sub 12 | 12/19/17 | 986,162      | <LOQ | <LOQ | 1,491 |
| T12 | 3/27/2018  | 1,937,458    | <LOQ | 3,843 | <LOQ |
| S12 | 3/27/2018  | 4,491,306    | <LOQ | <LOQ | <LOQ |
| S15 | 3/27/2018  | 16,189,970   | 11,688 | <LOQ | 7,205 |
| T8  | 3/27/2018  | 8,250,592    | 2,233 | <LOQ | 104,568 |
| S11 | 3/27/2018  | 4,446,700    | 3,726 | <LOQ | 5,457 |
| Marina | 3/27/2018 | 3,845,379    | <LOQ | <LOQ | <LOQ |
| 490 | 3/27/2018  | 17,902,423   | <LOQ | <LOQ | <LOQ |
| Sub 12 | 3/27/2018 | 1,197,526    | <LOQ | 2,110 | <LOQ |
| T12 | 4/5/2018   | 1,841,767    | <LOQ | <LOQ | <LOQ |
| S12 | 4/5/2018   | 5,723,089    | <LOQ | <LOQ | <LOQ |
| S15 | 4/5/2018   | 10,427,870   | 1,903 | <LOQ | <LOQ |
|     |       |          |   |   |   |
|-----|-------|----------|---|---|---|
| T8  | 4/5/2018 | 1,099,083 | <LOQ | <LOQ | <LOQ |
| S11 | 4/5/2018 | 6,992,593 | 6,912 | 6,321 | 16,112 |
| Marina | 4/5/2018 | 3,006,293 | <LOQ | <LOQ | <LOQ |
| 490 | 4/5/2018 | 6,663,482 | <LOQ | <LOQ | <LOQ |
| Sub 12 | 4/5/2018 | 1,658,318 | <LOQ | <LOQ | <LOQ |
| T12 | 4/11/2018 | 2,125,992 | 1,527 | <LOQ | <LOQ |
| S12 | 4/11/2018 | 8,122,572 | <LOQ | <LOQ | <LOQ |
| S15 | 4/11/2018 | 13,569,191 | <LOQ | <LOQ | 3,353 |
| T8  | 4/11/2018 | 3,023,427 | <LOQ | <LOQ | <LOQ |
| S11 | 4/11/2018 | 6,158,644 | 4,314 | 9,807 | 490 |
| Marina | 4/11/2018 | 1,944,292 | <LOQ | <LOQ | <LOQ |
| 490 | 4/11/2018 | 11,280,074 | <LOQ | <LOQ | 5,700 |
| Sub 12 | 4/11/2018 | 1,648,697 | 1,130 | <LOQ | <LOQ |
| T12 | 4/24/2018 | 2,247,351 | 1,617 | <LOQ | <LOQ |
| S12 | 4/24/2018 | 5,775,724 | <LOQ | <LOQ | <LOQ |
| S15 | 4/24/2018 | 4,105,185 | <LOQ | <LOQ | 5,667 |
| T8  | 4/24/2018 | 1,622,843 | <LOQ | <LOQ | <LOQ |
| S11 | 4/24/2018 | 2,726,422 | <LOQ | <LOQ | 2,915 |
| Marina | 4/24/2018 | 3,962,368 | 6,011 | <LOQ | <LOQ |
| 490 | 4/24/2018 | 2,772,618 | <LOQ | <LOQ | <LOQ |
| Sub 12 | 4/24/2018 | 770,509 | 539 | <LOQ | <LOQ |
| T12 | 5/7/2018  | 2,132,273 | 3,656 | <LOQ | <LOQ |
| S12 | 5/7/2018  | 3,434,739 | <LOQ | <LOQ | <LOQ |
| S15 | 5/7/2018  | 5,829,276 | 11,845 | <LOQ | 9,109 |
| T8  | 5/7/2018  | 90,317 | <LOQ | <LOQ | <LOQ |
| S11 | 5/7/2018  | 7,378,893 | <LOQ | <LOQ | <LOQ |
| Marina | 5/7/2018  | 1,820,267 | <LOQ | <LOQ | <LOQ |
| 490 | 5/7/2018  | 1,476,286 | <LOQ | <LOQ | <LOQ |
| Sub 12 | 5/7/2018  | 652,628 | 1,105 | <LOQ | <LOQ |
| T12 | 5/16/2018 | 755,778 | <LOQ | <LOQ | <LOQ |
| S12 | 5/16/2018 | 1,606,753 | <LOQ | <LOQ | <LOQ |
| S15 | 5/16/2018 | 8,167,228 | 6,765 | 8,157 | <LOQ |
| T8  | 5/16/2018 | 1,335,821 | <LOQ | <LOQ | <LOQ |
| S11 | 5/16/2018 | 8,105,456 | <LOQ | <LOQ | <LOQ |
| Marina | 5/16/2018 | 2,707,604 | <LOQ | <LOQ | <LOQ |
| 490 | 5/16/2018 | 2,029,153 | <LOQ | <LOQ | <LOQ |
| Sub 12 | 5/16/2018 | 229,885 | <LOQ | <LOQ | <LOQ |
| T12 | 5/23/2018 | 101,696 | <LOQ | <LOQ | <LOQ |
| S12 | 5/23/2018 | 7,691,259 | <LOQ | <LOQ | <LOQ |
| S15 | 5/23/2018 | 5,848,787 | <LOQ | <LOQ | <LOQ |
| T8  | 5/23/2018 | 1,418,880 | <LOQ | <LOQ | <LOQ |
|    | 5/23/2018 | 5/23/2018 | 5/23/2018 | 5/23/2018 |
|----|-----------|-----------|-----------|-----------|
| S11| Marina    | 2,483,737 | <LOQ      | <LOQ      |
|    | 490       |           |           |           |
|    | Sub 12    |           |           |           |
|    | T12       | 859,740   | <LOQ      | <LOQ      |
|    | S12       | 3,818,030 | <LOQ      | <LOQ      |
|    | S15       | 1,440,404 | <LOQ      | <LOQ      |
|    | T8        | <LOQ      | <LOQ      | <LOQ      |
|    | S11       | 1,463,083 | <LOQ      | <LOQ      |
|    | Marina    | 2,069,021 | <LOQ      | <LOQ      |
|    | 490       | 833,524   | <LOQ      | <LOQ      |
|    | EOC       | 1,334,916 | <LOQ      | <LOQ      |
|    | Sub 12    | 403,318   | <LOQ      | <LOQ      |
|    | T12       | 175,936   | <LOQ      | <LOQ      |
|    | S12       | 15,665,035| 1,733     | <LOQ      |
|    | S15       | 2,067,771 | <LOQ      | <LOQ      |
|    | T8        | 523,021   | 2,857     | <LOQ      |
|    | S11       | <LOQ      | <LOQ      | <LOQ      |
|    | Marina    | 485,059   | <LOQ      | <LOQ      |
|    | 490       | 1,297,921 | <LOQ      | <LOQ      |
|    | Sub 12    | 174,878   | 975       | <LOQ      |
|    | T12       | 2,065,899 | <LOQ      | <LOQ      |
|    | S12       | 2,147,815 | <LOQ      | <LOQ      |
|    | S15       | 8,717,552 | 3,278     | <LOQ      |
|    | T8        | 1,534,792 | <LOQ      | <LOQ      |
|    | S11       | 1,522,328 | <LOQ      | <LOQ      |
|    | Marina    | 196,634   | <LOQ      | <LOQ      |
|    | 490       | 2,221,286 | <LOQ      | <LOQ      |
|    | Sub 12    | 1,434,681 | <LOQ      | <LOQ      |
|    | T12       | 2,155,470 | <LOQ      | <LOQ      |
|    | S12       | 1,570,646 | <LOQ      | <LOQ      |
|    | S15       | 588,799   | <LOQ      | <LOQ      |
|    | T8        | 5,686,960 | 1,630     | <LOQ      |
|    | S11       | <LOQ      | <LOQ      | <LOQ      |
|    | Marina    | 173,586   | <LOQ      | <LOQ      |
|    | 490       | 3,340,644 | <LOQ      | <LOQ      |
|    | Sub 12    | 1,541,865 | <LOQ      | <LOQ      |
|    | T12       | 5,115,110 | <LOQ      | <LOQ      |
|    | S15       | 5,680,916 | <LOQ      | <LOQ      |
|    | T8        | 2,225,757 | <LOQ      | <LOQ      |
|    | S11       | 3,158,494 | <LOQ      | <LOQ      |
|        | Date     | Value          | <LOQ | <LOQ | <LOQ |
|--------|----------|----------------|------|------|------|
| 490    | 6/27/2018| 18,926,295     | <LOQ | <LOQ | <LOQ |
| EOC    | 6/27/2018| 5,786,957      | <LOQ | <LOQ | <LOQ |
| Sub 12 | 6/27/2018| 5,115,110      | <LOQ | <LOQ | <LOQ |
| T12    | 7/2/2018 | 3,422,457      | <LOQ | <LOQ | <LOQ |
| S12    | 7/2/2018 | 2,253,841      | <LOQ | <LOQ | <LOQ |
| S15    | 7/2/2018 | 5,404,099      | <LOQ | <LOQ | <LOQ |
| T8     | 7/2/2018 | 563,077        | <LOQ | <LOQ | <LOQ |
| S11    | 7/2/2018 | 1,610,309      | <LOQ | <LOQ | <LOQ |
| 490    | 7/2/2018 | 58,054,501     | <LOQ | <LOQ | <LOQ |
| EOC    | 7/2/2018 | 10,109,152     | <LOQ | <LOQ | <LOQ |
| Sub 12 | 7/2/2018 | 2,950,146      | <LOQ | <LOQ | <LOQ |
| T12    | 7/11/2018| 1,548,335      | <LOQ | <LOQ | <LOQ |
| S12    | 7/11/2018| 133,402        | <LOQ | <LOQ | <LOQ |
| S15    | 7/11/2018| 6,203,659      | 5,836| 3,137| <LOQ |
| T8     | 7/11/2018| 1,273,664      | <LOQ | <LOQ | 2,906|
| S11    | 7/11/2018| 2,770,779      | <LOQ | <LOQ | <LOQ |
| Marina | 7/11/2018| <LOQ           | <LOQ | <LOQ | <LOQ |
| 490    | 7/11/2018| 6,109,904      | <LOQ | <LOQ | <LOQ |
| EOC    | 7/11/2018| 2,052,878      | <LOQ | <LOQ | <LOQ |
| Sub 12 | 7/11/2018| 1,065,314      | <LOQ | <LOQ | <LOQ |
| T12    | 7/18/2018| <LOQ           | <LOQ | <LOQ | <LOQ |
| S12    | 7/18/2018| 3,433,265      | <LOQ | <LOQ | <LOQ |
| S15    | 7/18/2018| 2,662,282      | <LOQ | <LOQ | <LOQ |
| T8     | 7/18/2018| 2,857,812      | <LOQ | <LOQ | 4,315|
| S11    | 7/18/2018| 5,830,594      | <LOQ | <LOQ | <LOQ |
| Marina | 7/18/2018| <LOQ           | <LOQ | <LOQ | <LOQ |
| 490    | 7/18/2018| <LOQ           | <LOQ | <LOQ | <LOQ |
| EOC    | 7/18/2018| 8,044,892      | <LOQ | <LOQ | <LOQ |
| Sub 12 | 7/18/2018| 936,345        | <LOQ | <LOQ | <LOQ |
| T12    | 7/25/2018| 2,024,401      | <LOQ | <LOQ | <LOQ |
| S12    | 7/25/2018| 3,429,847      | <LOQ | <LOQ | <LOQ |
| S15    | 7/25/2018| <LOQ           | <LOQ | <LOQ | <LOQ |
| T8     | 7/25/2018| <LOQ           | <LOQ | <LOQ | <LOQ |
| S11    | 7/25/2018| 2,444,367      | <LOQ | <LOQ | <LOQ |
| Marina | 7/25/2018| 4,477,824      | <LOQ | <LOQ | <LOQ |
| 490    | 7/25/2018| 2,213,958      | <LOQ | <LOQ | <LOQ |
| EOC    | 7/25/2018| 12,532,854     | <LOQ | <LOQ | <LOQ |
| Sub 12 | 7/25/2018| 2,663,240      | <LOQ | <LOQ | <LOQ |
| T12    | 8/1/2018 | <LOQ           | <LOQ | <LOQ | <LOQ |
| S12    | 8/1/2018 | 5,580,408      | <LOQ | <LOQ | <LOQ |
| S15    | 8/1/2018 | 1,759,998      | <LOQ | <LOQ | 11,966|
|   | Date     |   |   |   |   |
|---|----------|---|---|---|---|
| T8 | 8/1/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S11 | 8/1/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| 490 | 8/1/2018 | 8,147,897 | <LOQ | <LOQ | <LOQ |
| EOC | 8/1/2018 | 6,553,931 | <LOQ | <LOQ | <LOQ |
| Sub 12 | 8/1/2018 | 2,790,204 | <LOQ | <LOQ | <LOQ |
| T12 | 8/8/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S12 | 8/8/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S15 | 8/8/2018 | 7,708,093 | <LOQ | <LOQ | 3,966 |
| T8 | 8/8/2018 | 18,245,308 | <LOQ | 3,194 | 123,000 |
| S11 | 8/8/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Marina | 8/8/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| 490 | 8/8/2018 | 4,588,412 | <LOQ | <LOQ | <LOQ |
| EOC | 8/8/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Sub 12 | 8/8/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| T12 | 8/15/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S12 | 8/15/2018 | 2,964,494 | <LOQ | <LOQ | <LOQ |
| S15 | 8/15/2018 | 1,907,396 | <LOQ | <LOQ | <LOQ |
| T8 | 8/15/2018 | <LOQ | <LOQ | <LOQ | 4,414 |
| S11 | 8/15/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Marina | 8/15/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| 490 | 8/15/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| EOC | 8/15/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Sub 12 | 8/15/2018 | 741,124 | <LOQ | <LOQ | <LOQ |
| T12 | 8/21/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S12 | 8/21/2018 | 2,029,749 | <LOQ | <LOQ | <LOQ |
| S15 | 8/21/2018 | 1,907,396 | <LOQ | <LOQ | <LOQ |
| T8 | 8/21/2018 | <LOQ | <LOQ | <LOQ | 11,110 |
| S11 | 8/21/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Marina | 8/21/2018 | 1,656,467 | <LOQ | <LOQ | <LOQ |
| 490 | 8/21/2018 | 7,166,792 | <LOQ | <LOQ | <LOQ |
| EOC | 8/21/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Sub 12 | 8/21/2018 | 664,281 | <LOQ | <LOQ | <LOQ |
| T12 | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S12 | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S15 | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| T8 | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S11 | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| EOC | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| Sub 12 | 8/28/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| T12 | 9/6/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
| S12 | 9/6/2018 | <LOQ | <LOQ | <LOQ | <LOQ |
|     | 9/6/2018  |     |     |     |     |
|-----|-----------|-----|-----|-----|-----|
| Sub 12 |           | T8  | S11 | Marina | 490 |
|     |           |     |     | 9/6/2018 | 9/6/2018 |
|     |           |     |     | <LOQ      | <LOQ      |
|     | 33,991    | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| T12 | 9/11/2018 | S12 | S15 | T8       | S11       |
|     | 9/11/2018 |     |     | 9/11/2018 | 9/11/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Sub 12 | 9/11/2018 | T12 | S12 | S15      | T8        |
|     | 9/11/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Marina | 9/11/2018 | T12 | S12 | S15      | T8        |
|     | 9/11/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| 490 | 9/11/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| EOC | 9/11/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| EOC | 9/11/2018 |     |     |           |           |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Sub 12 | 9/11/2018 | T12 | S12 | S15      | T8        |
|     | 9/26/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Marina | 9/26/2018 | T12 | S12 | S15      | T8        |
|     | 9/26/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| 490 | 9/26/2018 |     |     | 9/26/2018 | 9/26/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| EOC | 9/26/2018 |     |     |           |           |
|     | 8,838,199 | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Sub 12 | 9/26/2018 | T12 | S12 | S15      | T8        |
|     | 10/2/2018 |     |     | 10/2/2018 | 10/2/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Marina | 10/2/2018 | T12 | S12 | S15      | T8        |
|     | 10/2/2018 |     |     | 10/2/2018 | 10/2/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| 490 | 10/2/2018 |     |     | 10/2/2018 | 10/2/2018 |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| EOC | 10/2/2018 |     |     |           |           |
|     | 518,030   | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Sub 12 | 10/2/2018 | T12 | S12 | S15      | T8        |
|     | 10/11/2018|     |     | 10/11/2018| 10/11/2018|
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Marina | 10/11/2018| T12 | S12 | S15      | T8        |
|     | 10/11/2018|     |     | 10/11/2018| 10/11/2018|
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| 490 | 10/11/2018|     |     | 10/11/2018| 10/11/2018|
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| Marina | 10/11/2018|     |     |           |           |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |           |     |     |           |           |
| 490 | 10/11/2018|     |     |           |           |
|     | <LOQ      | <LOQ| <LOQ| <LOQ      | <LOQ      |
|     |          |          |        |        |
|-----|----------|----------|--------|--------|
| EOC | 10/11/2018 | 448,500  | <LOQ   | <LOQ   |
| Sub 12 | 10/11/2018 | <LOQ     | <LOQ   | <LOQ   |
| T12  | 10/16/2018 | 474,549  | <LOQ   | <LOQ   |
| S12  | 10/16/2018 | 361,001  | <LOQ   | <LOQ   |
| S15  | 10/16/2018 | 1,148,633| <LOQ   | <LOQ   |
| T8   | 10/16/2018 | 4,151,352| <LOQ   | <LOQ   |
| S11  | 10/16/2018 | <LOQ     | <LOQ   | <LOQ   |
| Marina | 10/16/2018 | 266,037  | <LOQ   | <LOQ   |
| 490  | 10/16/2018 | 468,167  | <LOQ   | <LOQ   |
| EOC  | 10/16/2018 | 1,039,388| <LOQ   | <LOQ   |
| Sub 12 | 10/16/2018 | 430,237  | <LOQ   | <LOQ   |
| T12  | 10/23/2018 | 964,633  | <LOQ   | <LOQ   |
| S12  | 10/23/2018 | 834,035  | <LOQ   | <LOQ   |
| S15  | 10/23/2018 | 5,944,214| 3,908  | <LOQ   |
| T8   | 10/23/2018 | 269,106  | <LOQ   | <LOQ   |
| S11  | 10/23/2018 | 392,813  | <LOQ   | <LOQ   |
| Marina | 10/23/2018 | 469,408  | <LOQ   | <LOQ   |
| 490  | 10/23/2018 | 263,336  | <LOQ   | <LOQ   |
| EOC  | 10/23/2018 | <LOQ     | <LOQ   | <LOQ   |
| Sub 12 | 10/23/2018 | 904,931  | <LOQ   | <LOQ   |
| T12  | 10/30/2018 | 494,449  | <LOQ   | <LOQ   |
| S12  | 10/30/2018 | 1,712,578| <LOQ   | <LOQ   |
| S15  | 10/30/2018 | 1,968,712| 492    | <LOQ   |
| T8   | 10/30/2018 | 451,266  | <LOQ   | <LOQ   |
| S11  | 10/30/2018 | 620,913  | <LOQ   | <LOQ   |
| Marina | 10/30/2018 | 2,473,462| <LOQ   | <LOQ   |
| 490  | 10/30/2018 | 1,325,142| <LOQ   | <LOQ   |
| EOC  | 10/30/2018 | 1,373,112| <LOQ   | <LOQ   |
| Sub 12 | 10/30/2018 | 1,126,072| <LOQ   | <LOQ   |
| T12  | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| S12  | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| S15  | 11/6/2018  | 478,503  | 535    | <LOQ   |
| T8   | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| S11  | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| Marina | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| 490  | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| EOC  | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| Sub 12 | 11/6/2018  | <LOQ     | <LOQ   | <LOQ   |
| T12  | 11/14/2018 | 313,724  | <LOQ   | <LOQ   |
| S12  | 11/14/2018 | 339,019  | <LOQ   | <LOQ   |
| S15  | 11/14/2018 | 1,034,758| <LOQ   | <LOQ   |
| Site | Date     | aadA2 | ermB | ermF | sul1 | sul2 | tetA | tetM | tetO | tetW | vgaA | vgaB |
|------|----------|-------|------|------|------|------|------|------|------|------|------|------|
| T12  | 4/20/17  | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12  | 4/20/17  | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15  | 4/20/17  | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12  | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12  | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15  | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8   | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11  | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490  | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| ST12 | 5/4/17   | <LOD  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |

Relative Abundance

| Site | Date     | Relative Abundance |
|------|----------|--------------------|
| T8   | 11/14/2018 | 311,283            |
| S11  | 11/14/2018 | 472,106            |
| Marina | 11/14/2018 | 773,018            |
| 490  | 11/14/2018 | <LOQ               |
| EOC  | 11/14/2018 | 1,208,482          |
| Sub 12 | 11/14/2018 | 322,156            |
| T12  | 11/19/2018 | 704,930            |
| S12  | 11/19/2018 | 1,170,736          |
| S15  | 11/19/2018 | 2,436,174          |
| T8   | 11/19/2018 | 619,690            |
| S11  | 11/19/2018 | 1,478,067          |
| Marina | 11/19/2018 | 946,346            |
| 490  | 11/19/2018 | 1,578,229          |
| EOC  | 11/19/2018 | 2,132,561          |
| Sub 12 | 11/19/2018 | 993,898            |
| T12  | 11/27/2018 | 746,438            |
| S12  | 11/27/2018 | 1,426,991          |
| S15  | 11/27/2018 | 3,420,672          |
| T8   | 11/27/2018 | 1,057,506          |
| S11  | 11/27/2018 | 1,044,042          |
| Marina | 11/27/2018 | 306,281            |
| 490  | 11/27/2018 | 438,972            |
| EOC  | 11/27/2018 | 1,348,577          |
| Sub 12 | 11/27/2018 | 1,091,551          |
|     |      |      |      |      |      |      |      |      |      |      |
|-----|------|------|------|------|------|------|------|------|------|------|
| T12 | 5/17/17 | <LOD | <LOD | <LOD | 2.2E-4 | 3.1E-4 | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12 | 5/17/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15 | 5/17/17 | <LOD | <LOD | <LOD | 9.8E-5 | 4.5E-4 | <LOD | <LOD | <LOD | 7.0E-5 | <LOD |
| T8  | 5/17/17 | 1.5E-4 | 8.6E-5 | <LOD | 1.5E-3 | 1.9E-3 | 1.8E-4 | 1.1E-3 | 1.0E-4 | 2.3E-4 | <LOD |
| S11 | 5/17/17 | <LOD | <LOD | <LOD | 1.3E-4 | 9.6E-5 | <LOD | <LOD | 4.6E-5 | 4.5E-5 | <LOD |
| 490 | 5/17/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 5/17/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| ST12 | 5/17/17 | <LOD | <LOD | <LOD | 2.2E-4 | 3.1E-4 | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12 | 5/31/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12 | 5/31/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15 | 5/31/17 | 5.6E-4 | 4.8E-4 | <LOD | 9.1E-4 | 1.4E-3 | <LOD | <LOD | 5.8E-4 | 1.1E-3 | <LOD |
| T8  | 5/31/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11 | 5/31/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490 | 5/31/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 5/31/17 | <LOD | <LOD | <LOD | 4.1E-4 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| ST12 | 5/31/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12 | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12 | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15 | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8  | 6/14/17 | 4.5E-3 | <LOD | <LOD | 2.8E-2 | 2.1E-3 | <LOD | 1.8E-3 | 1.3E-3 | 3.9E-3 | <LOD |
| S11 | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490 | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | 3.5E-4 | <LOD | <LOD | <LOD | <LOD |
| ST12 | 6/14/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12 | 6/29/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12 | 6/29/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8  | 6/29/17 | 9.8E-3 | <LOD | <LOD | 2.2E-2 | 2.7E-3 | <LOD | 1.8E-3 | 9.2E-3 | <LOD | <LOD |
| S11 | 6/29/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490 | 6/29/17 | <LOD | <LOD | <LOD | 2.9E-5 | 3.3E-5 | 7.2E-5 | 8.0E-5 | 2.9E-5 | <LOD | <LOD |
| ST12 | 6/29/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12 | 7/12/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12 | 7/12/17 | <LOD | <LOD | <LOD | 1.2E-4 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15 | 7/12/17 | <LOD | <LOD | <LOD | 6.9E-4 | <LOD | <LOD | <LOD | 1.5E-3 | <LOD | <LOD |
| T8  | 7/12/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 2.1E-3 | <LOD | <LOD |
| S11 | 7/12/17 | <LOD | <LOD | <LOD | 4.0E-4 | <LOD | <LOD | <LOD | 4.3E-4 | <LOD | <LOD |
| 490 | 7/12/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| ST12 | 7/12/17 | <LOD | <LOD | <LOD | 1.2E-4 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12 | 7/28/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15 | 7/28/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8  | 7/28/17 | <LOD | <LOD | <LOD | 1.1E-3 | <LOD | <LOD | 1.1E-3 | <LOD | <LOD | <LOD |
| S11 | 7/28/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
|   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|
| ST12 | 7/28/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
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| ST12 | 8/9/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
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| T8 | 8/25/17 | <LOD | <LOD | <LOD | 8.1E-3 | 3.6E-3 | <LOD | 4.7E-3 | 7.7E-4 | 2.7E-3 | <LOD |
| S15 | 9/7/17 | <LOD | <LOD | <LOD | 1.1E-4 | <LOD | <LOD | 7.3E-5 | <LOD | <LOD |
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| S15 | 9/21/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
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| T12 | 10/9/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
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| T8 | 10/9/17 | <LOD | <LOD | <LOD | 4.7E-4 | 8.6E-4 | 3.1E-4 | 7.1E-4 | 5.7E-4 | 2.7E-3 | <LOD |
| S11 | 10/9/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 2.0E-4 | 4.5E-4 | <LOD |
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| Marina | 10/9/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
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| S15 | 11/6/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8 | 11/6/17 | 2.1E-3 | <LOD | <LOD | 5.3E-3 | 2.3E-3 | <LOD | <LOD | <LOD | 9.5E-4 | <LOD |
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| 490 | 11/6/17 | <LOD | 4.0E-3 | <LOD | <LOD | <LOD | <LOD | 2.2E-3 | 8.1E-4 | 2.7E-4 | <LOD |
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| 490 | 11/15/17 | <LOD | 3.3E-3 | <LOD | <LOD | <LOD | 2.6E-3 | 9.0E-4 | 2.9E-3 | <LOD |

Note: <LOD indicates a value below the limit of detection.
| Marina | Date     | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
|--------|----------|------|------|------|------|------|------|------|------|
| ST12   | 11/15/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12    | 11/28/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12    | 11/28/17 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15    | 11/28/17 | <LOD | 3.9E-4 | <LOD | 7.3E-4 | 1.3E-3 | <LOD | <LOD | <LOD |
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| 490    | 12/5/17  | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
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| S15    | 12/12/17 | 4.2E-4 | 2.6E-4 | 3.6E-4 | 7.1E-4 | <LOD | 1.2E-3 | <LOD | 4.7E-4 | <LOD |
| T8     | 12/12/17 | 1.1E-2 | <LOD | <LOD | 1.7E-2 | 2.2E-3 | 1.8E-3 | <LOD | <LOD |
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| Site   | Date     | aadA2 | erm(B) | erm(F) | sul1 | sul2 | tetA | tetM | tetO | tetW | vgaA | vgaB |
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|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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| T12   | 5/23/18 | <LOD> | <LOD> | <LOD> | <LOD> | <LOD> | <LOD> | <LOD> | <LOD> | <LOD> |
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| Marina | T8 | 10/11/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | S11 | 10/11/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 940 | 10/11/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 10/11/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 10/11/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8    | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11   | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 940 | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 10/16/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 10/23/18 | 8.4E-4 | 5.0E-4 | LOD | 3.9E-4 | 7.8E-4 | <LOD | <LOD | <LOD | <LOD |
| T8    | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11   | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 940 | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 10/23/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8    | 10/30/18 | 1.2E-2 | <LOD | <LOD | 1.7E-2 | 4.4E-3 | <LOD | <LOD | <LOD | <LOD |
| S11   | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 940 | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 10/30/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8    | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11   | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 940 | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC  | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
|------|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Sub 12 | 11/6/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8    | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11   | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490   | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 11/14/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8    | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11   | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490   | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 11/19/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T12   | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S12   | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S15   | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| T8    | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| S11   | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Marina | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| 490   | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| EOC   | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sub 12 | 11/27/18 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
APPENDIX B. STATISTICAL TESTS

Wilcoxon Rank-Sum Test

FIB Plate Counts

Total *E. coli* vs. total enterococci BHL watershed combined 2018

![Graph showing Wilcoxon Rank-Sum Test results]

**Oneway Analysis of CFU/100ml By FIB**

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level  | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|--------|-------|-----------|----------------|------------|-------------------|
| e. coli| 192   | 34054.0   | 36960.0        | 177.365    | -2.672            |
| mENT   | 192   | 39866.0   | 36960.0        | 207.635    | 2.672             |

**2-Sample Test, Normal Approximation**

| S     | Z     | Prob>|Z| |
|-------|-------|-----|----|
| 39866 | 2.67177 | 0.0075* |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|-------|---|
| 7.1408    | 1  | 0.0075* |
Total *E. coli* comparison between sampling years 2017 and 2018

![One way analysis of E. coli by year](image)

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| 2017  | 119   | 20151.0   | 18564.0        | 169.336    | 2.058             |
| 2018  | 192   | 28365.0   | 29952.0        | 147.734    | -2.058            |

#### 2-Sample Test, Normal Approximation

| S   | Z    | Prob>|Z| |
|-----|------|-----|---|
| 20151 | 2.05850 | 0.0395* |

#### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|-------|
| 4.2401    | 1  | 0.0395* |

Missing Rows 41
Total *E. coli* BHL watershed combined 2017
Total *E. coli* BHL watershed combined 2018

![Graph showing Oneway Analysis of *E. coli* By Crop Status](chart)

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level         | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|---------------|-------|-----------|----------------|------------|-------------------|
| Growing       | 72    | 867950    | 6948.00        | 120.549    | 4.644             |
| Post-harvest  | 72    | 700500    | 6948.00        | 97.292     | 0.152             |
| Pre-Planting  | 48    | 284350    | 4632.00        | 59.240     | -5.363            |

### 1-Way Test, ChiSquare Approximation

ChiSquare 35.0834, DF 2, Prob>ChiSq <.0001*

### Nonparametric Comparisons For Each Pair Using Wilcoxon Method

| Level         | Level     | Score Mean Difference | Std Err Dif | Z     | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|---------------|-----------|-----------------------|-------------|-------|---------|----------------|----------|----------|-----------------|
| Post-harvest  | Growing   | -18.6111              | 6.951876    | -2.67714 | <.0074* | -44.0000       | -90.000  | -9.3043  |
| Pre-Planting  | Post-harvest | -25.2431            | 6.481036    | -3.89491 | <.0001* | -27.3104       | -70.000  | -9.3846  |
| Pre-Planting  | Growing   | -36.8229              | 6.481216    | -5.68148 | <.0001* | -82.8704       | -160.667 | -46.0000 |
Total enterococci BHL watershed combined 2017
Total enterococci BHL watershed combined 2018
Total enterococci S11 vs. ST12 2017

**Oneway Analysis of Ent By Site**

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level  | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|--------|-------|-----------|----------------|------------|-------------------|
| S11    | 14    | 260.500   | 210.000        | 18.6071    | 2.182             |
| Sub 12 | 15    | 174.500   | 225.000        | 11.6333    | -2.182            |

**2-Sample Test, Normal Approximation**

|          | S   | Z   | Prob>|Z|  |
|----------|-----|-----|-----|----|---|
|          | 260.5| 2.18245 | 0.0291* |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|-------|---|
|          | 4.8588 | 1   | 0.0275* |   |

Missing Rows 7
Total *E. coli* S11 vs. ST12 2018

| Level   | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|---------|-------|-----------|----------------|------------|-------------------|
| S11     | 33    | 1269.50   | 1105.50        | 38.4697    | 2.097             |
| Sub 12  | 33    | 941.500   | 1105.50        | 28.5303    | -2.097            |

**2-Sample Test, Normal Approximation**

| S       | Z        | Prob>|Z| |
|---------|----------|------|
| 941.5   | -2.09691 | 0.0360* |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|------|
| 4.4240    | 1  | 0.0354* |
Total enterococci T8 vs. S15 2017

**Oneway Analysis of Ent By Site**

![Graph showing comparison between S15 and T8 sites with Enterococci counts.]

**Kruskal-Wallis Tests (Rank Sums)**

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|------------------|
| S15   | 18    | 411.500   | 333.000        | 22.8611    | 2.469            |
| T8    | 18    | 254.500   | 333.000        | 14.1389    | -2.469           |

**2-Sample Test, Normal Approximation**

| S   | Z   | Prob>|Z| |
|-----|-----|-----|
| 254.5 | -2.46876 | 0.0136* |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|
| 6.1732    | 1  | 0.0130* |
Total enterococci T8 vs. S15 vs. EOC 2018

**Oneway Analysis of Ent By Site**

| Site | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean)/Std0 |
|------|-------|-----------|----------------|------------|------------------|
| EOC  | 23    | 1329.50   | 954.500        | 57.8043    | 3.866            |
| S15  | 33    | 1377.50   | 1369.50        | 41.7424    | 0.071            |
| T8   | 26    | 696.000   | 1079.00        | 26.7692    | -3.812           |

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| ChiSquare | DF | Prob > ChiSq |
|-----------|----|--------------|
| 20.7365   | 2  | <.0001*      |

**Nonparametric Comparisons For Each Pair Using Wilcoxon Method**

| Level  | Level | Score Mean Difference | Std Err Diff | Z     | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|--------|-------|-----------------------|--------------|-------|---------|----------------|----------|----------|-----------------|
| T8     | S15   | -12.1713              | 4.502697     | -2.70312 | 0.0069* | -128.000       | -229.00  | -19.000  |                 |
| S15    | EOC   | -12.4690              | 4.429271     | -2.81514 | 0.0049* | -508.000       | -1690.00 | -40.000  |                 |
| T8     | EOC   | -16.7977              | 4.089843     | -4.10716 | <.0001* | -733.000       | -1896.00 | -211.000 |                 |
Total *E. coli* T8 vs. S15 2017

### Oneway Analysis of *E. coli* By Site

![Oneway Analysis Graph](image)

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Score-Mean)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| S15   | 17    | 361.000   | 297.500        | 21.2353    | 2.170             |
| T8    | 17    | 234.000   | 297.500        | 13.7647    | -2.170            |

#### 2-Sample Test, Normal Approximation

| S | Z | Prob>|Z| |
|---|---|-------|
| 234 | -2.17044 | 0.0300* |

#### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-------|
| 4.7859    | 1  | 0.0287* |

Missing Rows 2
Total *E. coli* T8 vs. S15 vs. EOC 2018
Percent resistant tetracycline BHL watershed combined 2017

**Oneway Analysis of % TET Res By Crop Status**

| Crop Status  | Count | Score Sum | Score | Score Mean | (Mean-Mean0)/Std0 |
|--------------|-------|-----------|-------|------------|-------------------|
| Growing      | 41    | 2314.00   | 2050.00 | 56.4390    | 1.872             |
| Post-harvest | 51    | 2444.00   | 2550.00 | 47.9216    | -0.739            |
| Pre-planting | 7     | 192.00    | 350.000 | 27.4286    | -2.150            |

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level       | Count | Score Sum | Score | Score Mean | (Mean-Mean0)/Std0 |
|-------------|-------|-----------|-------|------------|-------------------|
| Growing     | 41    | 2314.00   | 2050.00 | 56.4390    | 1.872             |
| Post-harvest| 51    | 2444.00   | 2550.00 | 47.9216    | -0.739            |
| Pre-planting| 7     | 192.00    | 350.000 | 27.4286    | -2.150            |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob > ChiSq |
|-----------|----|--------------|
| 6.6533    | 2  | 0.0359*      |

**Nonparametric Comparisons For Each Pair Using Wilcoxon Method**

| Level       | - Level       | Score Mean Difference | Std Err Diff | Z     | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------------|---------------|-----------------------|--------------|-------|---------|----------------|----------|----------|-----------------|
| Post-harvest| Growing       | -8.4036               | 5.600496     | -1.50052 | 0.1335  | -1.94643       | -3.42304 | 0.5370370 |                |
| Pre-planting| Growing       | -12.0418              | 5.717806     | -2.10602 | 0.0352* | -3.75000       | -7.16485 | 0.0000000 |                |
| Pre-planting| Post-harvest  | -13.8095              | 6.804891     | -2.02947 | 0.0424* | -1.62162       | -7.36464 | 0.0000000 |                |

Missing Rows: 26
Percent resistant tylosin BHL watershed combined 2018

Oneway Analysis of % TYL Res By Crop Status

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level       | Count | Score Sum | Score Mean | Expected Score | (Mean-Mean0)/Std0 |
|-------------|-------|-----------|------------|----------------|------------------|
| Growing     | 71    | 8346.00   | 6709.50    | 117.549        | 4.882            |
| Post-harvest| 71    | 5709.50   | 6709.50    | 80.415         | -2.983           |
| Pre-Planting| 46    | 3710.50   | 4347.00    | 80.663         | -2.140           |

1-Way Test, ChiSquare Approximation

ChiSquare  DF  Prob>ChiSq
23.8479   2   <.0001*

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

| q*  | Alpha |
|-----|-------|
| 1.95996 | 0.05 |

| Level          | - Level   | Score Mean Difference | Std Err Dif | Z     | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|----------------|-----------|-----------------------|-------------|-------|---------|----------------|----------|----------|-----------------|
| Pre-Planting   | Post-harvest | -0.4657              | 5.198787    | -0.08958 | 0.9286 | 0.000000       | 0.000000 | 0.000000 |
| Pre-Planting   | Growing   | -22.3002              | 6.179656    | -3.60865 | 0.0003* | -0.222222      | -0.446429 | -0.038462 |
| Post-harvest   | Growing   | -28.5352              | 6.564701    | -4.34677 | <.0001* | -0.222222      | -0.425532 | -0.068966 |
Percent resistant tetracycline S11 vs. ST12 2017

### Oneway Analysis of %Tet Res By Site

| Site | %Tet Res |
|------|----------|
| S11  |          |
| Sub 12 |        |

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level  | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|--------|-------|-----------|----------------|------------|-------------------|
| S11    | 10    | 180.000   | 130.000        | 18.0000    | 2.748             |
| Sub 12 | 15    | 145.000   | 195.000        | 9.6667     | -2.748            |

### 2-Sample Test, Normal Approximation

| S  | Z  | Prob>|Z| |
|----|----|-----|---|
| 180| 2.74788 | 0.0060* |

### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq|
|-----------|----|------|--------|
| 7.7042    | 1  | 0.0055* |

Missing Rows 11
Percent resistant tylosin S11 vs. ST12 2017

### Oneway Analysis of %Tyl Res By Site

![Graph showing the comparison of %Tyl Res between S11 and Sub 12 sites.]

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level  | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|--------|-------|-----------|----------------|------------|-------------------|
| S11    | 11    | 204.000   | 148.500        | 18.5455    | 2.915             |
| Sub 12 | 15    | 147.000   | 202.500        | 9.8000     | -2.915            |

### 2-Sample Test, Normal Approximation

| S   | Z    | Prob>|Z| |
|-----|------|------|
| 204 | 2.91492 | 0.0036* |

### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|------|------|
| 8.6519    | 1  | 0.0033* |

Missing Rows 10
Percent resistant tetracycline T8 vs. S15 2017

### One-way Analysis of %Tet Res by Site

![Oneway Analysis of %Tet Res by Site](image)

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| S15   | 17    | 215.500   | 297.500        | 12.6765    | -2.807            |
| T8    | 17    | 379.500   | 297.500        | 22.3235    | 2.807             |

#### 2-Sample Test, Normal Approximation

| S | Z   | Prob>|Z| |
|---|-----|-----|
| 379.5 | 2.80736 | 0.0050* |

#### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|
| 7.9783    | 1  | 0.0047* |

Missing Rows 2
Percent resistant tetracycline T8 vs. S15 vs. EOC 2018

### Oneway Analysis of % TET Res By Site

![Graph showing % TET Res by site](Image)

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| EOC   | 22    | 358,000   | 792,000        | 16.2727    | -5.391            |
| S15   | 27    | 1111.50   | 972,000        | 41.1667    | 1.647             |
| T8    | 22    | 1086.50   | 792,000        | 49.3864    | 3.656             |

#### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob > ChiSq |
|-----------|----|--------------|
| 31.0574   | 2  | <.0001*      |

### Nonparametric Comparisons For Each Pair Using Wilcoxon Method

| Level | Level | Score Mean Difference | Std Err Dif | Z    | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------|-------|-----------------------|--------------|------|---------|----------------|----------|----------|-----------------|
| S15   | EOC   | 19.71549              | 4.103486     | 4.804571 | <.0001* | 5.84391        | 3.60526  | 8.21875  |                 |
| T8    | EOC   | 17.63636              | 3.870799     | 4.556259 | <.0001* | 16.49807       | 10.37234 | 23.80503 |                 |
| T8    | S15   | 8.20791               | 4.102229     | 2.000842 | 0.0454* | 8.45358        | 0.00000  | 15.62882 |                 |

Missing Rows: 11
Percent resistant tylosin T8 vs. S15 vs. EOC 2018
16S rRNA, *ermB, ermF, tetM* Gene Concentrations

16S rRNA BHL watershed combined 2018
16S rRNA S11 vs. ST12 2017

### Oneway Analysis of 16S copy number/100mL By Site

![Graph showing 16S copy number/100mL by site with data points for S11 and Sub 12.]

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level  | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|--------|-------|-----------|----------------|------------|-------------------|
| S11    | 16    | 385.000   | 280.000        | 24.0625    | 3.607             |
| Sub 12 | 18    | 210.000   | 315.000        | 11.6667    | -3.607            |

#### 2-Sample Test, Normal Approximation

| S   | Z   | Prob>|Z| |
|-----|-----|-----|---|
| 385 | 3.60669 | 0.0003* |

#### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|------|---|
| 13.1330   | 1  | 0.0003* |
16S rRNA T8 vs. S15 2017

**Oneway Analysis of 16S copy number/100mL By Site**

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| S15   | 19    | 1054.00   | 636.500        | 55.4737    | 6.073             |
| T8    | 47    | 1157.00   | 1574.50        | 24.6170    | -6.073            |

**2-Sample Test, Normal Approximation**

| S   | Z     | Prob>|Z| |
|-----|-------|------|-----|
| 1054| 6.07269| <.0001*|

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|------|-------|
| 36.9660   | 1  | <.0001*|

**Nonparametric Comparisons For Each Pair Using Wilcoxon Method**

| q* | Alpha |
|----|-------|
| 1.95956 | 0.05 |

| Level | Score Mean | Std Err Dif | Z   | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------|------------|-------------|-----|---------|----------------|----------|----------|-----------------|
| T8    | -30.8197   | 5.075135    | -6.07269 | <.0001* | -3988993       | -5553941 | -2652615 |                 |
16S rRNA T8 vs. S15 2018

Oneway Analysis of 16S/100mL By Site

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|---------------|------------|-------------------|
| S15   | 33    | 1297.00   | 1105.50       | 39.3030    | 2.484             |
| T8    | 33    | 914.000   | 1105.50       | 27.6970    | -2.484            |

2-Sample Test, Normal Approximation

| S | Z   | Prob>|Z| |
|---|-----|-----|---|
| 914 | -2.48420 | 0.0130* |

1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq |
|-----------|----|-----|--------|
| 6.2036    | 1  | 0.0127* |
ErmB BHL watershed combined 2017

**One-way Analysis of erm(B)/100mL by Crop Status**

![Graph showing erm(B)/100mL by Crop Status with categories: Growing, Post-harvest, Pre-planting.]

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level          | Count | Score Sum | Score | Score Mean | (Mean-Mean0)/Std0 |
|----------------|-------|-----------|-------|------------|-------------------|
| Growing        | 25    | 1062.00   | 1217.50 | 42.4800   | -2.112            |
| Post-harvest   | 54    | 2882.00   | 2673.00 | 53.3704   | 2.205             |
| Pre-planting   | 19    | 907.000   | 940.500 | 47.7368   | -0.439            |

1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>ChiSq |
|-----------|----|------------|
| 5.6955    | 2  | 0.0580     |

**Nonparametric Comparisons for Each Pair Using Wilcoxon Method**

| q*         | Alpha |
|------------|-------|
| 1.95996    | 0.05  |

| Level       | - Level       | Score Mean Difference | Std Err Diff | Z         | p-Value  | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------------|---------------|-----------------------|--------------|-----------|----------|----------------|----------|----------|----------------|
ErmB BHL watershed combined 2018

**Oneway Analysis of erm(B) copies/16S copies By Planting harvest**

![Graph showing erm(B) copies/16S copies by planting harvest phases: Growing, Post-harvest, Pre-Planting.]

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level       | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------------|-------|-----------|----------------|------------|-------------------|
| Growing     | 73    | 6769.00   | 7081.00        | 92.726     | -1.500            |
| Post-harvest| 72    | 6759.00   | 6984.00        | 93.875     | -1.084            |
| Pre-Planting| 48    | 5193.00   | 4656.00        | 108.188    | 2.899             |

**1-Way Test, ChiSquare Approximation**

ChiSquare: 8.4676, DF: 2, Prob>ChiSq: 0.0145 *

**Nonparametric Comparisons For Each Pair Using Wilcoxon Method**

| q* | Alpha |
|----|-------|
| 1.95996 | 0.05  |

| Level       | - Level          | Score Mean Difference | Std Err Dif | Z     | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------------|------------------|-----------------------|-------------|-------|---------|----------------|----------|----------|-----------------|
| Pre-Planting| Growing          | 9.513556              | 3.836942    | 2.479463 | 0.0132* |                | 0        | 0        |                 |
| Pre-Planting| Post-harvest     | 9.045139              | 3.930194    | 2.301448 | 0.0214* |                | 0        | 0        |                 |
| Post-harvest| Growing          | 0.979357              | 3.202760    | 0.305785 | 0.7598  |                | 0        | 0        |                 |
*ermB* T8 vs. S15 2017

**Oneway Analysis of *erm*(B)/100mL By Site**

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|--------------------|
| S14   | 19    | 748.000   | 636.500        | 39.3684    | 2.264              |
| T8    | 47    | 1463.00   | 1574.50        | 31.1277    | -2.264             |

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-----------|----------------|------------|--------------------|
| S     | 748       | 636.500        | 39.3684    | 2.264              |
| T8    | 1463      | 1574.50        | 31.1277    | -2.264             |

**2-Sample Test, Normal Approximation**

| Z     | Prob>|Z| |
|-------|------|
| 2.26373 | 0.0236* |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|------|------|
| 5.1708    | 1  | 0.0230* |
**ErmB T8 vs. S15 2018**

**Oneway Analysis of erm(B)/100mL By Site**

| Site | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|------|-------|-----------|----------------|------------|-------------------|
| S15  | 33    | 1240.00   | 1105.50        | 37.5758    | 2.404             |
| T8   | 33    | 971.000   | 1105.50        | 29.4242    | -2.404            |

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

**2-Sample Test, Normal Approximation**

| S    | Z    | Prob>|Z| |
|------|------|-----|---|
| 971  | -2.40402 | 0.0162*|

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|-----|------|---|
| 5.8225    | 1  | 0.0158*|
TetM S11 vs. ST12 2018

Oneway Analysis of tet(M)/100mL By Site

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| S11   | 33    | 1204.50   | 1105.50        | 36.5000    | 2.533             |
| Sub 12| 33    | 1006.50   | 1105.50        | 30.5000    | -2.533            |

2-Sample Test, Normal Approximation

| S     | Z     | Prob>|Z| |
|-------|-------|----------|
| 1006.5| -2.53291| 0.0113*  |

1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|----------|
| 6.4809    | 1  | 0.0109*  |
Relative Abundance

Relative Abundance BHL watershed combined 2017

### Oneway Analysis of Absolute Relative Abundance By Crop-Status

![Graph showing absolute relative abundance by crop-status]

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level          | Count | Score Sum | Score | Score Mean | (Mean-Mean0)/Std0 |
|----------------|-------|-----------|-------|------------|-------------------|
| Growing        | 216   | 87960.0   | 94392.0 | 407.222    | -2.632            |
| Post-harvest   | 468   | 207905    | 204516 | 444.241    | 1.200             |
| Pre-planting   | 189   | 85636.0   | 82593.0 | 453.101    | 1.305             |

### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>ChiSq |
|-----------|----|------------|
| 7.2169    | 2  | 0.0271*    |

### Nonparametric Comparisons For Each Pair Using Wilcoxon Method

| Level | - Level | Score Mean Difference | Std Err Dif | Z    | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------|---------|-----------------------|-------------|------|---------|----------------|----------|----------|-----------------|
| Post-harvest | Growing | 28.16506 | 12.08862 | 2.330230 | 0.0198* | 0 | 0 | 0 |
| Pre-planting | Growing | 22.50496 | 8.72629 | 2.578685 | 0.0099* | 0 | 0 | 0 |
| Pre-planting | Post-harvest | 5.74537 | 12.80192 | 0.448790 | 0.6536 | 0 | 0 | 0 |
Relative Abundance BHL watershed combined 2018

**One Way Analysis of Absolute Relative Abundance By Crop Status**

![Graph showing absolute relative abundance by crop status](image)

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level       | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------------|-------|-----------|----------------|------------|-------------------|
| Growing     | 657   | 577039    | 570933         | 878.293    | 1.099             |
| Post-harvest| 648   | 541132    | 563111         | 835.080    | -3.967            |
| Pre-planting| 432   | 391283    | 375408         | 905.747    | 3.205             |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob>ChiSq |
|-----------|----|------------|
| 18.3353   | 2  | 0.001 *    |

**Nonparametric Comparisons For Each Pair Using Wilcoxon Method**

| q*   | Alpha |
|------|-------|
| 1.95996 | 0.05  |

| Level          | - Level       | Score Mean Difference | Std Err Diff | Z   | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|----------------|---------------|-----------------------|--------------|-----|---------|----------------|----------|----------|-----------------|
| Pre-planting   | Post-harvest  | 44.5120               | 10.37248     | 4.29135 | <.0001 * | 0              | 0        | 0        |                 |
| Pre-planting   | Growing       | 16.6367               | 11.63805     | 1.42951 | 0.1529  | 0              | 0        | 0        |                 |
| Post-harvest   | Growing       | -32.0061              | 10.69201     | -2.99346 | 0.0028 *| 0              | 0        | 0        |                 |

Missing Rows 160
Relative Abundance S11 vs. ST12 2018

### Oneway Analysis of Absolute Relative Abundance By Site

![Graph showing relative abundance comparison between S11 and ST12](image)

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| S11   | 297   | 90257.0   | 88357.5        | 303.896    | 2.168             |
| ST12  | 297   | 86458.0   | 88357.5        | 291.104    | -2.168            |

### 2-Sample Test, Normal Approximation

| S     | Z     | Prob>|Z| |
|-------|-------|------|
| 86458 | -2.16770 | 0.0302* |

### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|------|
| 4.7014    | 1  | 0.0301* |
Relative Abundance T8 vs. S15 2017

### Oneway Analysis of Absolute Relative Abundance By Site

| Site | Absolute Relative Abundance | Score Mean | (Mean-Mean0)/Std0 |
|------|-----------------------------|------------|--------------------|
| S15  | 0.005                       | 155.392    | -4.129             |
| T8   | 0.044                       | 195.578    | 4.129              |

### Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| S15   | 171   | 26572.0   | 30096.0        | 155.392    | -4.129            |
| T8    | 180   | 35204.0   | 31680.0        | 195.578    | 4.129             |

### 2-Sample Test, Normal Approximation

| S     | Z     | Prob>|Z| |
|-------|-------|------|
| S15   | -4.12898 | <.0001* |

### 1-Way Test, ChiSquare Approximation

| ChiSquare | DF | Prob>|ChiSq| |
|-----------|----|------|
| 17.0533   | 1  | <.0001* |

### Nonparametric Comparisons For Each Pair Using Wilcoxon Method

| q* | Alpha |
|----|-------|
| 1.95956 | 0.05 |

| Level | Level | Score Mean | Std Err Dif | Z   | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|-------|-------|------------|-------------|-----|---------|----------------|----------|----------|-----------------|
| T8    | S15   | 40.18026   | 9.731286    | 4.128978 | <.0001* | 0              | 0        | 0.0001011 |                 |
Relative Abundance T8 vs. S15 vs. EOC 2018

**Oneway Analysis of Absol Rel Abun By Site**

**Wilcoxon / Kruskal-Wallis Tests (Rank Sums)**

| Level | Count | Score Sum | Expected Score | Score Mean | (Mean-Mean0)/Std0 |
|-------|-------|-----------|----------------|------------|-------------------|
| EOC   | 253   | 91685.0   | 107272         | 362.391    | -7.394            |
| S15   | 297   | 129595    | 125928         | 436.347    | 1.668             |
| T8    | 297   | 137848    | 125928         | 464.135    | 5.424             |

**1-Way Test, ChiSquare Approximation**

| ChiSquare | DF | Prob > ChiSq |
|-----------|----|--------------|
| 59.2535   | 2  | <.0001*      |

**Nonparametric Comparisons For Each Pair Using Wilcoxon Method**

| q* | Alpha |
|----|-------|
| 1.95996 | 0.05 |

| Level - Level | Score Mean Difference | Std Err Diff | Z   | p-Value | Hodges-Lehmann | Lower CL | Upper CL | Difference Plot |
|---------------|-----------------------|--------------|-----|---------|----------------|----------|----------|-----------------|
| T8 - EOC      | 63.62172              | 8.29218      | 7.672499 | <.0001* | 0              | 0        | 0        |                 |
| S15 - EOC     | 50.46113              | 7.67333      | 6.576175 | <.0001* | 0              | 0        | 0        |                 |
| T8 - S15      | 21.73064              | 10.33549     | 2.102527 | 0.0355* | 0              | 0        | 0        |                 |