California Regional Water Quality Control Board
North Coast Region

Russian River Pathogen TMDL

Onsite Wastewater Treatment System Impact Study Report

July 2013

1.0 INTRODUCTION

The North Coast Regional Water Board staff are developing the Russian River Total Maximum Daily Loads (TMDLs) for pathogen indicators to identify and control contamination. Potential pathogen contamination has been identified in three areas of the lower and middle Russian River watershed (Hydrologic Units 114.10 and 114.20). Identification of the contamination led to the placement of waters within these areas on the federal Clean Water Act Section 303(d) list of impaired waters. The contamination identified has been linked to impairment of the contact recreation (REC-1) and non-contact recreation (REC-2) designated beneficial uses. Health advisories have been published and/or posted by Sonoma County and City of Santa Rosa authorities.

North Coast Regional Water Board staff conducted a source analysis study for the development of the Russian River TMDL. The study was organized into individual tasks to collect information to help address the identified TMDL management questions (NCRWQCB 2012). Based on results of the study, Regional Water Board staff made the following findings:

1. Pathogenic indicator bacteria concentrations were higher during wet periods compared to dry periods
2. Human-source *Bacteroides* bacteria were detected in all sample locations and land use categories throughout the watershed.
3. Stable isotope analysis results showed that the dominant sources of source water for bacteria samples were manure and septic wastes.
4. During wet periods, pathogenic indicator bacteria concentrations were higher in urban sewered areas and areas with septic systems compared to less developed areas.
5. Human-source Bacteroides was higher in onsite septic areas compared to urban sewered areas.

The study appeared to indicate that septic systems were a contributing source of pathogenic indicator bacteria. We wanted to confirm this hypothesis by more focused monitoring. We did this by comparing water samples collected downstream of hydrologic catchments that drain areas with densely situated Onsite Wastewater Treatment System (OWTS) and catchments that drain areas with a relatively low density of OWTS. Additionally, provisions of the recently adopted statewide OWTS Policy require Regional Water Board staff to identify impaired water bodies where septic systems are believed to be source of the impairment and establish additional protections, including supplemental treatment systems, in these areas. These new requirements highlight the need to explicitly identify sources of pathogens from onsite systems.

To address questions arising from the study findings, Regional Water Board staff collected wet-weather water samples from various locations in the lower Russian River watershed during 2012-2013 to identify possible pathogen impacts from catchments that drain areas with a high density of OWTS. A Quality Assurance Project Plan (Butkus 2012a) was developed that detailed the water sample collection and analysis of the *E. coli*, *Enterococcus*, and *Bacteroides* bacteria concentrations. Additional water samples were also collected and analyzed for stable isotopes of nitrate to assess the relative water source differences in oxygen ($\delta^{18}O$) and nitrogen ($\delta^{15}N$).

2.0 MONITORING QUESTION

Pathogenic indicator bacteria can be transported to surface waters from malfunctioning or poorly sited OWTS. An OWTS doesn't have to be malfunctioning to contribute pathogenic indicator bacteria to surface waters. An OWTS can also be poorly sited so that there is insufficient and/or ineffective soil treatment upon effluent dispersal. During dry weather periods, OWTS effluent can travel in shallow groundwater to perennial streams, entering through shallow groundwater. through springs or the stream hyphoreic zone. During storm events, runoff from the landscape surface can flood OSWT systems resulting in the direct transport of untreated human waste to surface waters. This mode of transport can also occur in ephemeral streams that exist only for a short period following a storm event. This study focused sampling efforts during storm events when transport of bacteria to surface waters is most likely to occur.

The OWTS Impact Study was designed to answer the following management question:

- Do catchments with high density of OWTS contribute pathogenic indicator bacteria from human sources?

3.0 WATER SAMPLING LOCATIONS

Regional Water Boards staff selected catchments and sampling locations for the study based on parcel density and the perceived risk of bacterial transport from OWTS in the study area. Parcel data was obtained from the Sonoma County Assessor. The risk of bacterial transport from OWTS systems was assessed using a spatial data model developed by Regional Water Board staff (Fortescue 2012) using factors selected from the Basin Plan's Policy on the Control of Water Quality with Respect to On-Site Waste Treatment and Disposal Practices (NCRWQCB 2011). Landscape analysis of spatial data was conducted to select sampling locations that best represent the identified parcel density and fecal indicator bacteria (FIB) transport risk categories (Tables 1 & 2). Catchments were selected based on the risk of FIB transport to surface waters and the parcel density (Butkus 2012b).

Three sample locations were selected to represent catchments draining each of the following four categories, for a total of twelve sites:

- High parcel-density with a high risk of FIB transport from OWTS
- High parcel-density with a low risk of FIB transport from OWTS
- Low parcel-density with a high risk of FIB transport from OWTS
- Low parcel-density with a low risk of FIB transport from OWTS

In addition, three additional sample locations were selected by Regional Water Board staff to represent catchments that drain areas served by OWTS that have high parcel density and are near a stream. It is hypothesized by Regional Water Board staff that catchments with these characteristics present a high potential to contribute pathogens to the Russian River. Based on these catchment characteristics, additional sampling locations were selected from the Fitch Mountain area near Healdsburg, downtown Monte Rio and Camp Meeker.

Figure 1 presents the parcel density and FIB transport risk for each of the catchments sampled. This figure shows the relative relationship between the categories and the additional catchments of concern between these variables.

Figure 2 through Figure 28 show comparisons of the distribution of sample data between various groups using Box and whisker plots. The horizontal line in each box shows the median value of the data set. The boxes represent the interquartile range and the error bars (i.e. whiskers) represent the 10th and 90th percentiles of the data set.

Figure 2 presents the range of catchment areas for each of the four categories. The figure shows that the catchment areas for low transport risk catchments are larger than those selected to represent a high transport risk. Figure 3 presents the range of parcel densities for selected catchments. The figure confirms the large difference in parcel densities between the high parcel density categories and the low parcel density categories. Figure 4 shows the distribution of FIB transport risk for each category. The figure confirms the large difference in FIB transport risk between the high transport risk categories and the low transport risk categories.

Wet weather water samples were collected from fifteen (15) catchments in the lower Russian River watershed (Table 1). Site number 14 (Monte Rio) was relocated to another location than identified in the Quality Assurance Project Plan. The originally selected location simply did not have runoff to sample that drained from the catchment after a storm event. The sample was collected at a nearby location in Monte Rio that had runoff available to collect.

4.0 MONITORING RESULTS

As described in the Quality Assurance Project Plan (Butkus 2012a), samples for analysis were collected from each location five (5) times during the study period. Despite the occurrence of early storm events in November 2012, the first storm event sampled was not until December 2, 2012, due to logistical reasons. The December 2, 2012 sample represented the largest of all the storm events sampled (Table 3). Water samples were collected at every site during this storm event. However, because subsequent storm events sampled were smaller and did not generate runoff at all locations, not all locations were sampled during every storm event. The locations and the dates sampled are shown in Table 4.

The results of FIB sample analysis are shown in Table 5. The result shown in the table is the median concentration value derived from replicate samples of fecal indicator bacteria at each location. Table 6 presents the ratio of stable isotopes of nitrogen ($\delta^{15}N$) and oxygen ($\delta^{18}O$) in dissolved nitrate. Several of the reported nitrate concentrations were below the level of quantitation. These data were not used in the assessment since isotope values for samples below the limit of quantitation may not be reliable.

Triplicate samples were collected once from each sampling location during the study to assess sampling variability, except at Sites 9 and 14, where samples were not collected due to the lack of runoff. Only one storm event on December 3, 2012 was large enough to generate runoff at these two locations. Table 7 – 10 shows the variability of the triplicate samples of FIB concentrations. The mean coefficient of variation ranges from 18% to 32%. The precision of the sampling was similar to the measurements made from replicate sampling in the Russian River during 2011-2012 which found coefficient of variations of 34% for *E. coli* bacteria and 37% for *Enterococcus* bacteria (NCRWQCB 2012; Butkus 2013).

5.0 ASSESSMENT RESULTS

Assessment Methods

Each of the sampling locations was selected to represent a particular catchment category of parcel density and FIB transport risk (i.e., high parcel density and high transport risk). The measured FIB concentrations were used to assess whether any particular sampling location is significantly different that the other locations selected to represent that category.

Visual comparisons and statistical hypothesis tests were made between different groupings of the measured FIB concentrations and other metrics. Distributions of the measured FIB concentrations are compared visually using box and whisker plots. The boxes represent the interquartile range of the distribution around the median and the whiskers represent the 10th and 90th percentiles. Hypothesis tests were considered statistically significantly different if the resulting probability of rejecting the null hypothesis (H_o)was equal or lower than $\alpha = 0.05$. Nonparametric (i.e., distribution-free) inferential statistical methods were used to assess differences between groups. These hypothesis tests make no assumption about the frequency distributions of the measured data. Nonparametric methods are the most appropriate approach for assessing water quality data, which can have widely varying frequency distributions (Helsel and Hirsch 2002).

The Kruskal-Wallis statistical test was used to assess if any particular sampling location showed a statistical difference in FIB concentrations from the other locations sampled for that catchment category (H_o). The Kruskal-Wallis test is a hypothesis test conducted using ranked data (Helsel and Hirsch 2002). This non-parametric test was used for testing if samples originate from the same distribution by assessing the equality of population medians among the groups. The parametric equivalent of the Kruskal-Wallis test is the one-way analysis of variance. When the Kruskal-Wallis test indicates significant results ($H_o < \alpha$), then at least one of the samples is different from the other samples in the group.

The relationships between FIB concentrations and catchment characteristics were investigated. In addition, the relationship of stable isotope of nitrate and catchment characteristics was also evaluated. Catchment characteristics included the area, parcel density and FIB transport risk.

Water Sample Measurements:
E. coli bacteria concentration
Enterococcus bacteria concentration
All *Bacteroides* bacteria concentration
Human-host *Bacteroides* bacteria concentration
Stable isotopes of oxygen ($\delta^{18}O$)
Stable isotopes of nitrogen ($\delta^{15}N$)

Catchment Characteristics:
Catchment size (acres)
Parcel Density (number of parcel centroids/catchment size)
FIB Transport Risk (index number)

The relationships between these variables were investigated using the Spearman's rank correlation coefficient (ρ) (Helsel and Hirsch 2002). Spearman's rank correlation coefficient is a nonparametric statistical measure of the dependence between two variables. Spearman correlation coefficients approach either plus one ($\rho \sim +1.0$) or minus one ($\rho \sim -1.0$), as the relationship become stronger. A small correlation coefficient (between -0.5

and 0.5) indicates a weak relationship between the variables. For example, a strong relationship means that when *E. coli* bacteria concentration is high in a sample, there is a large likelihood that *Enterococcus* bacteria concentrations will also be high.

Statistical tests were used to evaluate whether there was a significant difference between different catchment categories. The Mann-Whitney U statistical test was applied to assess the difference between the distributions of measured FIB concentrations and stable isotopes of nitrate based on parcel density and FIB transport risk. For example, the test was used to determine if there was a significant difference in E. coli concentrations from catchments with a high parcel density as opposed to catchment with a low parcel density.

The Mann-Whitney U test is a non-parametric hypothesis test for assessing whether two samples of observations come from the same distribution (Helsel and Hirsch 2002). The test null hypothesis is that the two samples are drawn from a single population. The test is similar to performing an ordinary parametric two-sample t test, but is based on ranking the data set. This statistical test is a nonparametric inferential statistical method that makes no assumption about the frequency distributions.

Assessment of Sampling Location influence on FIB Concentrations

Tables 11 – 14 show the results of the Kruskal-Wallis statistical tests between sampling locations for each catchment category. Only three of the tests showed a statistically significant difference between locations. *Enterococcus* bacteria concentrations were different in the high parcel density & high FIB transport risk category (Table 11). Visual observation of the distribution of *Enterococcus* bacteria concentrations show that Site 2 is much higher than the other locations sampled. In addition, the distribution of both *E.coli* and All *Bacteriodes* bacteria concentrations show that Site 10 is much higher than the other locations sampled. These data (i.e., *Enterococcus* bacteria concentrations from Site 2 and both *E.coli* and All *Bacteriodes* bacteria concentrations from Site 10) were excluded from further assessment since they may not be representative of the high parcel density & high FIB transport risk category based on both visual observation and the hypothesis tests.

Relationship between FIB Concentrations and Other Variables

Table 15 presents the matrix of Spearman's rank correlation coefficients between the FIB concentrations and the other variables. Three of the relationships are relatively strong. All *Bacteroides* bacteria concentrations are positively correlated with both human-host *Bacteroides* and *Enterococcus* bacteria concentrations. *Enterococcus* bacteria concentrations are also positively correlated with *E. coli* bacteria concentrations. Neither of the stable isotopes of nitrate was correlated with any of the FIB concentrations. FIB transport showed a weak, negative correlation to all of the FIB concentrations.

Assessment of Catchment Category influence on FIB Concentrations

The Kruskal-Wallis statistical test was also used to assess if there was statistical difference in FIB concentrations and stable isotopes of nitrate between catchment categories. Table 16 presents the results of the hypothesis test that the equality of population medians among the groups is the same. Figures 8 – 11 show the distributions of the FIB concentrations for each catchment category. The results indicate that each of the FIB groups were significantly different between the catchment categories. There was no significant different found between these categories for the stable isotopes of nitrate.

Assessment of Catchment Characteristics Influence on FIB Concentrations

Table 17 presents the results of the Mann-Whitney U test comparing FIB concentrations and stable isotopes of nitrate between catchments with a high parcel density (>0.75 parcels/acre) and those with a low parcel density (<0.12 parcels/acre). A statistically significant difference was observed in both All *Bacteroides* and *E. coli* bacteria concentrations based on parcel density. Visual comparison of the distributions of these concentrations show that higher parcel density is associated with higher concentrations of both All *Bacteroides* and *E. coli* bacteria (Figures 12 & 13).

Table 18 presents the results of the Mann-Whitney U test comparing FIB concentrations and stable isotopes of nitrate between catchments with a high FIB transport risk (index ≥10) and those with a low parcel density. (index <10). A statistically significant difference was observed in all FIB concentrations based on transport risk. Visual comparison of the distributions of these concentrations show that lower transport risk is associated with higher FIB concentrations (Figures 14-17). These results and observations support the previous finding that FIB transport is negatively correlated to FIB concentrations.

Assessment of Catchment Transport Risk influence on FIB Concentrations

The FIB transport risk index was evaluated further to determine why there appears to be a negative relationship between the index value and measured FIB concentrations. Each of the four (4) elements of the index was assumed to have a positive relationship to FIB transport. This assumption appears to be invalid for the set of catchments selected for this study. The index was separated into each of the elements for the study catchments. The spatial data used as input to the index were area-weighted for each study catchment (Table 19). Both the setback rank and the hydrologic group rank very little variability between the study sites. These two elements have relatively little influence on the ability of the index to discern differences between the groups and were excluded from the assessment. Therefore, the assessment was focused only on the effect of the remaining two elements, hill slope rank and soil depth rank, on the index values.

The Mann-Whitney U statistical test was applied to assess the difference between the distributions of measured FIB concentrations based on soil depth rank and hill slope rank. Table 20 shows that no significant differences were observed in all FIB concentrations between catchments with a high soil depth rank (>3.0) and those with a low soil depth rank (<3.0). Table 21 shows that highly significant differences were observed in all FIB concentrations between catchments with a high hill slope rank (>3.5) and those with a low hill slope rank (<3.5). Visual comparison of the distributions of these concentrations shows that lower hill slope is associated with higher FIB concentrations (Figures 18-21). These results and observations support the finding that hill slope index is not positively correlated with FIB concentrations for the set of catchments selected for this study. The assumption that there was a positive correlation between hill slope and FIB concentrations is invalid.

Assessment of Catchment Transport Risk influence on the Stable Isotopes of Nitrate

Measurements of the stable isotopes of oxygen ($\delta^{18}O$) and nitrogen ($\delta^{15}N$) were assessed to help identify the source of the water associated with the bacteria samples. The results were compared to typical values of $\delta^{18}O$ and $\delta^{15}N$ of nitrate (Figure 22).

- Samples with $\delta^{15}N$ values between 2‰ and 8‰ and $\delta^{18}O$ values below 15‰ are derived from soil sources, likely from stormwater erosion.
- Samples with $\delta^{18}O$ values above 15‰ are largely runoff processes.
- Samples with $\delta^{15}N$ values below 5‰ are typically ammonium from in situ processes such as wastewater treatment.
- Samples with $\delta^{15}N$ values above 5‰ are manure and septic waste.

Most of the samples fell within the range of a soil source of nitrate derived from ammonia through nitrification (Table 22 and Figure 23). These sources of nitrate were likely derived from erosion caused by storm events. Relatively few of the samples had $\delta^{15}N$ values above 10‰ or below 5‰. There were no significant differences found in stable isotope values based on parcel density (Table 17) or FIB transport risk (Table 18). These results were similar to the values found in other wet period water samples collected in the Russian River watershed (NCRWQCB 2012).

Assessment of FIB Concentrations in the Study Areas of Concern

Three sample locations were sampled that represent catchments draining areas of concern for OWTS impacts. The sampling locations were selected from catchments from the Fitch Mountain area near Healdsburg (Site 13), downtown Monte Rio (Site 14) and Camp Meeker (Site 15). These areas generally have a high parcel density on OWTS. The distribution of FIB concentrations from these catchments of concern were compared to the other catchments sampled (Figures 24- 28). Only a single storm event was sampled at Site 14 due to a lack of runoff so the results may not be representative of the catchment. However, this storm event showed much higher FIB concentration the other catchment samples. The other two catchments of concern (Sites 13 & 15) showed similar range of FIB concentrations as the other catchments sampled.

6.0 FINDINGS

Based on the assessments of FIB concentrations presented in this report, Regional Water Board staff can make the following findings:

- Triplicate samples were collected to assess sampling variability. The mean coefficient of variation ranges from 18% to 32%.
- *Enterococcus* bacteria concentrations from Site 2 (River Road culvert, Monte Rio) were much higher than the other locations sampled. In addition, both *E.coli* and All *Bacteriodes* bacteria concentrations from Site 10 (Fredson Road, Healdsburg) were also much higher than the other locations sampled. These data were excluded from further assessment since they may not be representative of the catchment category they were placed.
- All *Bacteroides* bacteria concentrations were positively correlated with both human-host *Bacteroides* and *Enterococcus* bacteria concentrations. *Enterococcus* bacteria concentrations were also positively correlated with *E. coli* bacteria concentrations. This means that as bacteria concentrations increase the other indicators also

likely increase. For example, one is likely to measure high *E. coli* bacteria concentrations in a water sample with high *Enterococcus* bacteria concentrations

- Neither of the stable isotopes of nitrate was correlated with any of the FIB concentrations.
- FIB transport risk showed a weak, negative correlation to all of the FIB concentrations. This means that the higher the assumed risk, the lower the FIB concentrations were likely to be measured in a water sample.
- Each of the FIB groups was significantly different between the catchment categories.
- There was no significant difference found between the catchment categories for the stable isotopes of nitrate. Most of the samples fell within the range of a soil source of nitrate derived from ammonia through nitrification. These sources of nitrate were likely derived from erosion caused by storm events. These results were similar to the values found in other wet period water samples collected in the Russian River watershed.
- A higher parcel density is associated with higher concentrations of both All *Bacteroides* and *E. coli* bacteria.
- No significant differences were observed in FIB concentrations between catchments with different soil depths.
- The FIB transport risk index is invalid for the set of catchments selected for this study. Lower transport risk is associated with higher FIB concentrations. This anomaly was caused by the incorrect assumption that hill slope index is positively correlated with FIB concentrations
- There were no significant differences found in stable isotope values based on parcel density or FIB transport risk. The results indicate the source of nitrate is soil likely derived from the storm event causing erosion. The stable isotope values were similar to the values found in other wet period water samples collected in the Russian River watershed.
- The catchments of concern showed similar range of FIB concentrations as the other catchments sampled.

7.0 CITATIONS

Butkus, S. 2012a. Russian River Human Impact Study Quality Assurance Project Plan. North Coast Regional Water Quality Control Board, Santa Rosa, CA.

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Helsel, D.R. and R.M. Hirsch. 2002. Statistical Methods in Water Resources, Techniques of Water Resources Investigations, Book 4, Chapter A3, 510 p. U.S. Geological Survey, Washington DC. Available at http://water.usgs.gov/pubs/twri/twri4a3/

Michener, R. and K. Lajtha. 2007. Stable Isotopes in Ecology and Environmental Science. Chpater 12, pp. 375-435.

NCRWQCB 2011. Water Quality Control Plan for the North Coast Region. North Coast Regional Water Quality Control Board, Santa Rosa, CA.

NCRWQCB 2012. Russian River Pathogen TMDL 2011-2012 Monitoring Report. North Coast Regional Water Quality Control Board. Santa Rosa, CA.

7.0 TABLES

Table 1. Study Sampling Locations

Category	Site ID	SWAMP ID	Latitude	Longitude	Location Description
High Parcel Density High Risk	Site 1	114DFMR68	38.6131	-122.8410	1740 Fitch Mtn Road - west of Villa Anna (Healdsburg)
	Site 2	114C01EDR	38.4776	-122.9762	River Road - culvert 100' east of Duncan Road (Monte Rio)
	Site 3	114C02SPR	38.5063	-121.0735	River Drive at Summerhome Park Road (Forestville)
High Parcel Density Low Risk	Site 4	114C03OMR	38.4781	-121.0018	19375 Old Monte Rio Road (across street from Northwood golf course)
	Site 5	114CO4TRF	38.4903	-121.1022	8612 Trenton Road (Forestville)
	Site 6	114DDRC59	38.4978	-121.0979	Along west shoulder of Del Rio Court (Forestville)
Low Parcel Density High Risk	Site 7	114C05MNS	38.4581	-122.9891	9632 Main Street (Monte Rio)
	Site 8	114C06VRG	38.5059	-121.0423	12656 River Road at Von Renner Grading (near Rio Nido)
	Site 9	114C07MRC	38.4575	-122.9531	Moscow Road box culvert - 100' west of 'Right Curve' sign (near Cassini Campgound)
Low Parcel Density Low Risk	Site 10	114CO8FRS	38.6561	-121.1264	Fredson Road south of Salvation Army driveway (Healdsburg)
	Site 11	114C09WDC	38.6467	-121.0805	3654 West Dry Creek Road (Healdsburg)
	Site 12	114C10AVR	38.6509	-121.1316	148 Alexander Valley Road (Healdsburg)
Areas of Concern	Site 13	114C11RDH	38.6238	-122.8452	West end of Redwood Drive (Healdsburg)
	Site 14	114C12FSM	38.4697	-123.0124	Foothill Drive at B Street (Monte Rio)
	Site 15	114C13LSA	38.4252	-121.0399	Lakeside Ave at Market Street (Camp Meeker)

Table 2. Catchment Characteristics

Category	Site ID	Catchment Area (acres)	Parcel Density (# per acre)	FIB Transport Risk Index
High Parcel Density High Risk	Site 1	34.7	2.25	12.4
	Site 2	4.6	3.88	11.0
	Site 3	45.3	1.90	10.0
High Parcel Density Low Risk	Site 4	74.0	3.37	8.7
	Site 5	167.0	0.76	7.9
	Site 6	90.6	2.91	9.6
Low Parcel Density High Risk	Site 7	82.6	0.01	10.8
	Site 8	43.0	0.02	10.9
	Site 9	16.4	0.06	10.6
Low Parcel Density Low Risk	Site 10	108.8	0.04	6.4
	Site 11	113.5	0.05	7.3
	Site 12	36.8	0.11	8.2
Areas of Concern	Site 13	30.9	0.39	10.2
	Site 14	6.7	2.54	9.7
	Site 15	6.3	7.84	10.2

Table 3. Precipitation during samples storm events as measured in Santa Rosa (CDEC Station STA at latitude 38.479, longitude -122.712)

Storm Event Dates	Two-day Antecedent Total Precipitation (inches)
12/3/2012	1.39
2/19/2013	0.16
3/6/2013	0.38
3/20/2013	0.54
4/4/2013	1.00

Table 4. Storm event dates sampled by location
* No sample collected due to a lack of runoff flow

Location	Date Sampled				
	Storm Event 1	Storm Event 2	Storm Event 3	Storm Event 4	Storm Event 5
Site 1	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 2	12/3/2012	3/20/2013	4/4/2013	*	*
Site 3	12/3/2012	3/6/2013	3/20/2013	4/4/2013	*
Site 4	12/3/2012	3/6/2013	3/20/2013	4/4/2013	*
Site 5	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 6	12/3/2012	3/20/2013	4/4/2013	*	*
Site 7	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 8	12/3/2012	3/20/2013	4/4/2013	*	*
Site 9	12/3/2012	*	*	*	*
Site 10	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 11	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 12	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 13	12/3/2012	2/19/2013	3/6/2013	3/20/2013	4/4/2013
Site 14	12/3/2012	*	*	*	*
Site 15	12/3/2012	3/6/2013	4/4/2013	*	*

Table 5. Median Fecal Indicator Bacteria Concentration Results

Location	**Collection Date**	**All *Bacteroides* (16SrRNA genes/100mL)**	**Human *Bacteroides* (16SrRNA genes/100mL)**	***E. coli* (MPN/100mL)**	***Enterococcus* (MPN/100mL)**
Site 1	12/3/2012	7,880	98	20	173
	2/19/13	29,682	349	109	61
	3/6/13	19,978	2,700	3,179	220
	3/20/13	15,413	<60	51	20
	4/4/13	37,600	238	84	10
Site 2	12/3/2012	12,100	217	1,019	384
	3/20/13	128,069	490	152	>24,196
	4/4/13	162,916	<60	187	5,172
Site 3	12/3/2012	2,150	178	158	295
	3/6/13	52,036	11,200	160	432
	3/20/13	158,524	27,700	3,654	216
	4/4/13	74,930	4,750	146	613
Site 4	12/3/2012	7,278	624	3,255	1,046
	3/6/13	169,775	39,200	2,613	12,997
	3/20/13	290,952	11,000	1,050	1,396
	4/4/13	322,490	48,800	2,481	2,603
Site 5	12/3/2012	45,667	5,644	1,376	1,236
	2/19/13	68,502	48,200	393	86
	3/6/13	531,524	220,000	1,664	3,873
	3/20/13	221,299	46,600	749	4,611
	4/4/13	487,550	167,400	4,892	4,950
Site 6	12/3/2012	10,800	2,131	246	211
	3/20/13	79,321	3,460	8,164	>24,196
	4/4/13	2,796,000	135,600	2,755	41,060
Site 7	12/3/2012	813	<60	52	10
	2/19/13	2,087	166	<10	<10
	3/6/13	3,824	523	80	21
	3/20/13	19,239	2,740	10	10
	4/4/13	10,373	2,260	31	275
Site 8	12/3/2012	6,409	<60	62	171
	3/20/13	35,711	1,450	836	1,450
	4/4/13	78,628	5,750	1,695	3,551
Site 9	12/3/2012	5,043	<60	327	85

Table 5. Median Fecal Indicator Bacteria Concentration Results *continued*

Location	**Collection Date**	**All *Bacteroides*** **(16SrRNA genes/100mL)**	**Human *Bacteroides*** **(16SrRNA genes/100mL)**	***E. coli*** **(MPN/100mL)**	***Enterococcus*** **(MPN/100mL)**
Site 10	12/3/2012	32,700	81	323	410
	2/19/13	570,924	6,730	5,827	20
	3/6/13	574,218	12,950	10,099	8,686
	3/20/13	172,543	8,580	1,137	2,098
	4/4/13	528,882	17,500	11,199	7,701
Site 11	12/3/2012	49,667	1,156	154	205
	2/19/13	32,558	4,280	598	128
	3/6/13	63,479	4,040	857	2,247
	3/20/13	53,642	5,070	373	1,565
	4/4/13	25,925	2,720	2,755	7,701
Site 12	12/3/2012	4,143	<60	171	139
	2/19/13	31,979	1,920	31	15
	3/6/13	31,298	2,143	132	288
	3/20/13	26,291	1,610	201	52
	4/4/13	164,674	5,560	121	2,310
Site 13	12/3/2012	9,450	698	327	384
	2/19/13	19,045	4,380	377	10
	3/6/13	22,678	2,310	789	233
	3/20/13	35,295	14,100	122	98
	4/4/13	66,357	2,280	3,076	12,997
Site 14	12/3/2012	1,640,000	371,000	2,489	2,481
Site 15	12/3/2012	24,000	2,680	96	563
	3/6/13	56,827	17,700	31	41
	3/20/13	47,050	1,530	238	605
	4/4/13	56,045	15,500	31	83

Table 6. Stable Isotope Analysis of Nitrate Results

* Indicates samples are below the limit of quantitation.
Isotope values for samples below the limit of quantitation may not be reliable.

Location	**Collection Date**	**δ^{15}N**	**δ^{18}O**	**Nitrate-N (mg/L)**
Site 1	12/3/2012	6.10	3.82	0.40
	2/19/2013	6.87	6.44	0.33
	3/6/2013	8.15	4.66	0.14
	3/20/2013	8.04	3.80	0.23
	4/4/2013	6.76	3.42	0.1m
Site 2	12/3/2012	9.61	6.24	0.03
	3/20/2013	16.26*	18.84*	0.06
	4/4/2013	6.54*	12.13*	<0.01
Site 3	12/3/2012	7.05	3.54	1.45
	3/6/2013	6.74	1.95	0.69
	3/20/2013	7.65	3.07	0.94
	4/4/2013	6.44	1.75	0.71
Site 4	12/3/2012	11.61	7.32	1.07
	3/6/2013	4.15	0.99	0.74
	3/20/2013	1.55	5.25	0.12
	4/4/2013	4.20	0.57	0.23
Site 5	12/3/2012	8.68	6.08	0.99
	2/19/2013	10.83	5.26	0.24
	3/6/2013	7.45	1.84	0.72
	3/20/2013	8.16	6.09	0.26
	4/4/2013	6.49	0.41	0.38
Site 6	12/3/2012	8.20	3.83	2.58
	3/20/2013	18.26	12.46	0.66
	4/4/2013	12.25	6.46	0.18
Site 7	12/3/2012	5.76*	10.81*	0.05
	2/19/2013	26.70*	18.36*	<0.01
	3/6/2013	20.95*	14.96*	<0.01
	3/20/2013	18.93*	21.70*	<0.01
	4/4/2013	12.91*	22.47*	<0.01
Site 8	12/3/2012	4.21	3.69	0.74
	3/20/2013	8.81	15.56	0.07
	4/4/2013	8.68	10.28	0.09
Site 9	12/3/2012	2.81	3.89	0.69

Table 6. Stable Isotope Analysis of Nitrate Results *continued*

* Indicates samples are below the limit of quantitation.
Isotope values for samples below the limit of quantitation may not be reliable.

Location	Collection Date	$\delta^{15}N$	$\delta^{18}O$	Nitrate-N (mg/L)
Site 10	12/3/2012	10.78	9.65	0.58
	2/19/2013	12.13*	13.18*	<0.01
	3/6/2013	7.65	3.17	0.10
	3/20/2013	8.86*	22.84*	<0.01
	4/4/2013	4.01*	6.02*	<0.01
Site 11	12/3/2012	3.66	4.84	0.80
	2/19/2013	6.48	7.61	0.11
	3/6/2013	7.83	-0.75	0.88
	3/20/2013	7.60	5.69	0.11
	4/4/2013	9.83	2.34	0.69
Site 12	12/3/2012	7.26	1.98	1.07
	2/19/2013	8.59	2.93	1.24
	3/6/2013	10.70	2.17	0.64
	3/20/2013	8.98	6.33	1.25
	4/4/2013	10.85	6.84	0.22
Site 13	12/3/2012	7.42	3.91	1.10
	2/19/2013	8.54	6.34	0.20
	3/6/2013	4.80	2.09	0.25
	3/20/2013	8.81	4.15	0.13
Site 14	12/3/2012	9.70	5.04	4.27
Site 15	12/3/2012	8.05	4.98	4.25
	3/6/2013	7.23	0.38	7.20
	3/20/2013	9.60	2.62	0.97
	4/4/2013	6.06	-0.29	4.38

Table 7 – Replicate Sample Variability for *E. coli* Bacteria Concentrations

Location	**Collection Date**	***E. coli* Bacteria Concentration (MPN/100mL)**			**Coefficient of Variation (%)**
		Replicate 1	**Replicate 2**	**Replicate 3**	
Site 1	12/3/2012	20	50	20	58%
Site 2	12/3/2012	1019	1017	1274	13%
Site 3	12/3/2012	156	158	160	1%
Site 4	3/6/2013	3076	2613	2481	11%
Site 5	3/6/2013	1723	1624	1664	3%
Site 6	3/20/2013	8664	7701	8164	6%
Site 7	3/6/2013	86	97	31	50%
Site 8	3/20/2013	836	581	984	25%
Site 10	3/20/2013	882	1137	1374	22%
Site 11	3/20/2013	292	495	373	26%
Site 12	3/20/2013	231	201	132	27%
Site 13	3/20/2013	84	171	122	35%
Site 15	3/6/2013	31	52	20	47%
Mean Variability					25%

Table 8 – Replicate Sample Variability for *Enterococcus* Bacteria Concentrations

Location	**Collection Date**	***Enterococcus* Bacteria Concentration (MPN/100mL)**			**Coefficient of Variation (%)**
		Replicate 1	**Replicate 2**	**Replicate 3**	
Site 1	12/3/2012	185	135	173	16%
Site 2	12/3/2012	295	384	432	19%
Site 3	12/3/2012	243	295	359	19%
Site 4	3/6/2013	12997	10462	14136	15%
Site 5	3/6/2013	3076	3873	4106	15%
Site 6	3/20/2013	>24196	>24196	>24196	-
Site 7	3/6/2013	10	97	31	99%
Site 8	3/20/2013	1450	1354	2987	47%
Site 10	3/20/2013	2098	2098	2143	1%
Site 11	3/20/2013	1565	1935	1201	23%
Site 12	3/20/2013	63	10	52	67%
Site 13	3/20/2013	98	109	85	12%
Site 15	3/6/2013	31	75	41	47%
Mean Variability					32%

Table 9 – Replicate Sample Variability for All *Bacteriodes* Bacteria Concentrations

Location	**Collection Date**	**All *Bacteroides* Bacteria Concentration (16SrRNA genes/100mL)**			**Coefficient of Variation (%)**
		Replicate 1	**Replicate 2**	**Replicate 3**	
Site 1	12/3/2012	7,880	11,100	7,570	22%
Site 2	12/3/2012	12,100	12,526	10,313	10%
Site 3	12/3/2012	2,537	2,060	2,150	11%
Site 4	3/6/2013	165,210	169,775	234,262	20%
Site 5	3/6/2013	68,502	56,317	68,802	11%
Site 6	3/20/2013	72,940	80,789	79,321	5%
Site 7	3/6/2013	5,373	3,824	3,291	26%
Site 8	3/20/2013	29,927	35,722	35,711	10%
Site 10	3/20/2013	141,008	172,543	260,919	32%
Site 11	3/20/2013	53,642	54,365	43,647	12%
Site 12	3/20/2013	24,063	31,466	26,291	14%
Site 13	3/20/2013	31,932	41,662	35,295	14%
Site 15	3/6/2013	56,827	83,452	29,923	47%
Mean Variability					18%

Table 10 – Replicate Sample Variability for Human-host *Bacteroides* Bacteria Concentrations

Location	**Collection Date**	**Human-host *Bacteroides* Bacteria Concentration (16SrRNA genes/100mL)**			**Coefficient of Variation (%)**
		Replicate 1	**Replicate 2**	**Replicate 3**	
Site 1	12/3/2012	98	69	156	41%
Site 2	12/3/2012	217	381	128	53%
Site 3	12/3/2012	178	178	127	18%
Site 4	3/6/2013	39,200	36,400	50,750	18%
Site 5	3/6/2013	50,600	42,500	48,200	9%
Site 6	3/20/2013	2,080	4,080	3,460	32%
Site 7	3/6/2013	557	293	523	31%
Site 8	3/20/2013	1,600	1,450	1,250	12%
Site 10	3/20/2013	4,680	8,580	8,620	31%
Site 11	3/20/2013	6,310	5,070	4,390	19%
Site 12	3/20/2013	1,610	1,140	2,020	28%
Site 13	3/20/2013	16,300	14,100	11,100	19%
Site 15	3/6/2013	17,300	23,800	17,700	19%
Mean Variability					32%

Table 11. Kruskal-Wallis Statistical Test for a difference in FIB concentrations between sampling locations in the high parcel density - high FIB transport risk category (i.e., Sites 1, 2 & 3).

Constituent	**Kruskal-Wallis Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	3.503	0.174	No
Enterococcus bacteria	8.060	0.018	**Yes**
All *Bacteroides* bacteria	2.060	0.357	No
Human-host *Bacteroides* bacteria	3.534	0.171	No
$\delta^{15}N$	2.651	0.266	No
$\delta^{18}O$	5.864	0.053	No

Table 12. Kruskal-Wallis Statistical Test for a difference in FIB concentrations between sampling locations in the high parcel density -low FIB transport risk category (i.e., Sites 4, 5 & 6).

Constituent	**Kruskal-Wallis Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	0.799	0.671	No
Enterococcus bacteria	1.041	0.594	No
All *Bacteroides* bacteria	0.179	0.914	No
Human-host *Bacteroides* bacteria	2.388	0.303	No
δ^{15}N	4.754	0.093	No
δ^{18}O	1.938	0.379	No

Table 13. Kruskal-Wallis Statistical Test for a difference in FIB concentrations between sampling locations in the low parcel density - high FIB transport risk category (i.e., Sites 7, 8 & 9).

Constituent	**Kruskal-Wallis Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	4.912	0.086	No
Enterococcus bacteria	4.708	0.095	No
All *Bacteroides* bacteria	3.271	0.195	No
Human-host *Bacteroides* bacteria	1.453	0.484	No
δ^{15}N	3.000	0.180	No
δ^{18}O	2.000	0.655	No

Table 14. Kruskal-Wallis Statistical Test for a difference in FIB concentrations between sampling locations in the low parcel density - low FIB transport risk category (i.e., Sites 10, 11 & 12).

Constituent	**Kruskal-Wallis Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	9.380	0.009	**Yes**
Enterococcus bacteria	2.289	0.318	No
All *Bacteroides* bacteria	7.220	0.027	**Yes**
Human-host *Bacteroides* bacteria	4.340	0.114	No
$\delta^{15}N$	2.908	0.234	No
$\delta^{18}O$	1.185	0.553	No

Table 15. Spearman's Rank Correlation Matrix

ρ	**All *Bacteroides* bacteria**	**Human-host *Bacteroides* bacteria**	***E. coli* bacteria**	***Enterococcus* bacteria**
All *Bacteroides* bacteria	1.00			
Human-host *Bacteroides* bacteria	0.77	1.00		
***E. coli* bacteria**	0.48	0.46	1.00	
***Enterococcus* bacteria**	0.64	0.50	0.73	1.00
$\delta^{15}N$	0.06	0.06	0.25	0.21
$\delta^{18}O$	-0.08	-0.18	0.20	0.05
Parcel Density	0.38	0.16	0.20	0.17
FIB Transport Risk	-0.38	-0.49	-0.39	-0.43
Catchment Size	0.26	0.58	0.33	0.40

Table 16. Kruskal-Wallis Statistical Test for a difference between the four categories.

Constituent	**Kruskal-Wallis Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	15.974	0.001	**Yes**
Enterococcus bacteria	13.195	0.004	**Yes**
All *Bacteroides* bacteria	14.912	0.002	**Yes**
Human-host *Bacteroides* bacteria	17.576	0.001	**Yes**
$\delta^{15}N$	2.629	0.452	No
$\delta^{18}O$	2.725	0.436	No

Table 17. Mann-Whitney U Statistical Test for a difference between a high and low parcel density

Constituent	**Mann-Whitney U Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	327.5	0.015	**Yes**
Enterococcus bacteria	312	0.172	No
All *Bacteroides* bacteria	335	0.009	**Yes**
Human-host *Bacteroides* bacteria	357	0.154	No
$\delta^{15}N$	158	0.595	No
$\delta^{18}O$	149	0.425	No

Table 18. Mann-Whitney U Statistical Test for a difference between a high and low FIB transport risk

Constituent	**Mann-Whitney U Statistic**	**Probability Value**	**Statistically Significant?**
E. coli bacteria	110	0.003	**Yes**
Enterococcus bacteria	105.5	0.001	**Yes**
All *Bacteroides* bacteria	117	0.006	**Yes**
Human-host *Bacteroides* bacteria	112.5	<0.001	**Yes**
$\delta^{15}N$	115	0.109	No
$\delta^{18}O$	172	0.904	No

Table 19. Area-weighted Index Component Rank Scores

Location	FIB Transport Risk Index	Hill Slope Rank	Hydrologic Soil Group Rank	Soil Depth Rank	Setback Rank
Site 1	12.4	4.49	3.00	4.76	0.00
Site 2	11.0	4.92	3.00	3.00	0.00
Site 3	10.0	3.90	3.00	3.04	0.01
Site 4	8.7	1.41	3.00	3.00	0.00
Site 5	7.9	1.91	3.00	3.00	0.00
Site 6	9.6	3.79	3.00	2.81	0.00
Site 7	10.8	4.88	3.00	3.00	0.23
Site 8	10.9	4.65	3.00	3.28	0.00
Site 9	10.6	4.61	3.00	3.00	0.00
Site 10	6.4	1.58	3.00	1.81	0.00
Site 11	7.3	3.27	3.00	1.00	0.00
Site 12	8.2	2.29	3.05	1.12	0.00

Table 20. Mann-Whitney U Statistical Test for a difference between a high and low soil depth rank

Constituent	Mann-Whitney U Statistic	Probability Value	Statistically Significant?
E. coli bacteria	58	0.277	No
Enterococcus bacteria	76.5	0.182	No
All *Bacteroides* bacteria	63	0.415	No
Human-host *Bacteroides* bacteria	75	0.162	No

Table 21. Mann-Whitney U Statistical Test for a difference between a high and low hill slope rank

Constituent	Mann-Whitney U Statistic	Probability Value	Statistically Significant?
E. coli bacteria	117	0.006	**Yes**
Enterococcus bacteria	112.5	<0.001	**Yes**
All *Bacteroides* bacteria	110	0.003	**Yes**
Human-host *Bacteroides* bacteria	105.5	0.001	**Yes**

Table 22. Median Values of the Stable Isotopes by Category

Category	**Median $\delta^{15}N$**	**Median $\delta^{18}O$**
High Parcel Density – High FIB Transport Risk	7.0	3.7
High Parcel Density – Low FIB Transport Risk	8.2	5.3
Low Parcel Density – High FIB Transport Risk	6.4	7.1
Low Parcel Density – Low FIB Transport Risk	8.2	4.0
Areas of Concern	8.1	3.9
All Locations	7.8	3.9

8.0 FIGURES

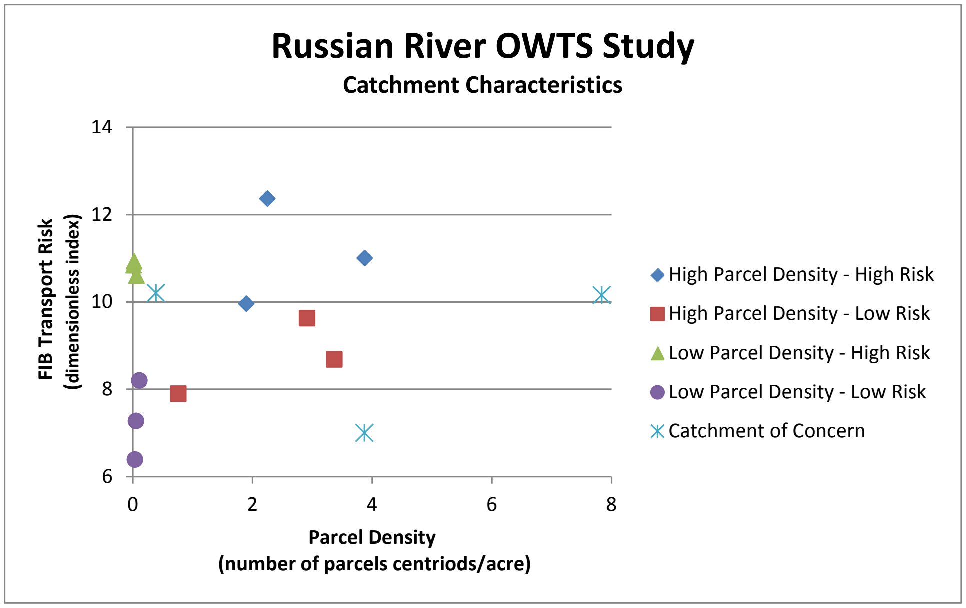

Figure 1. Characteristics of the catchments studied

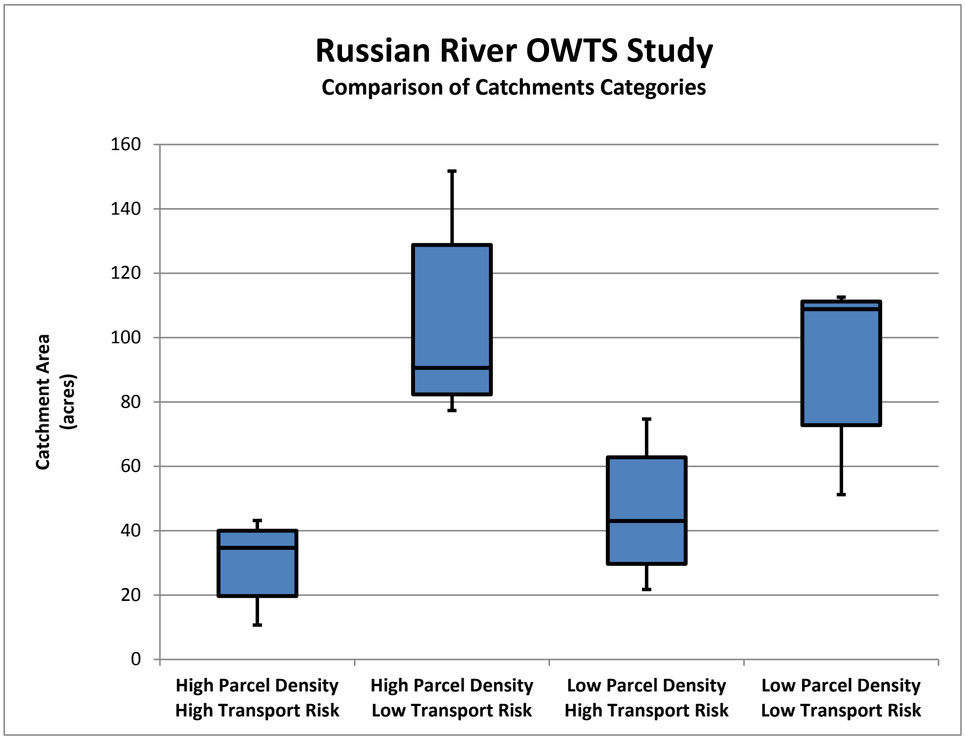

Figure 2. Comparison of the drainage areas between catchment categories

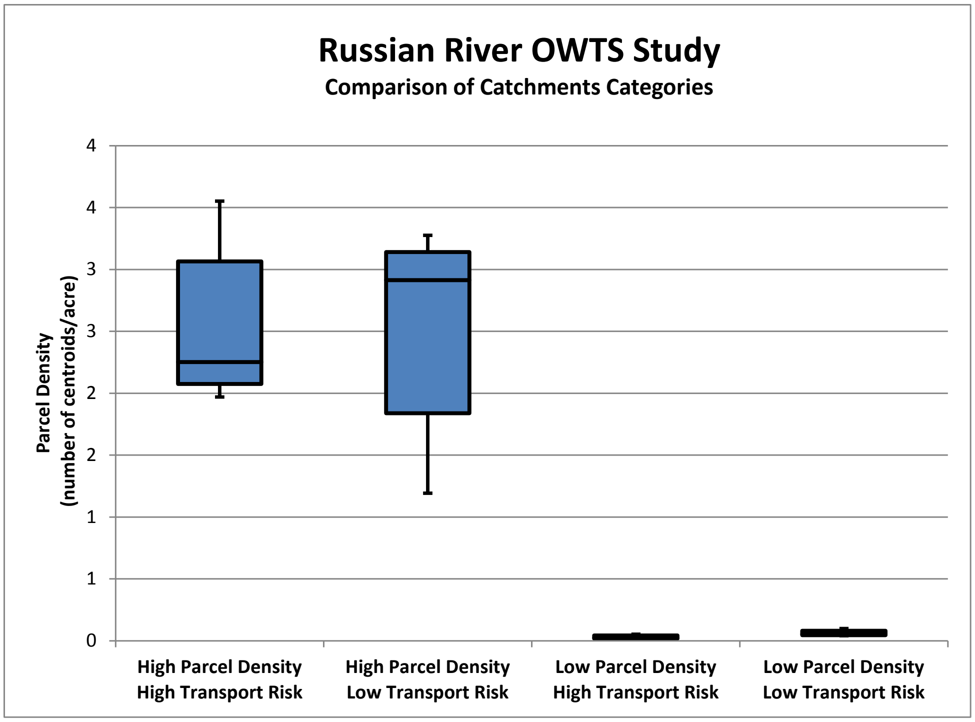

Figure 3. Comparison of the parcel density between catchment categories

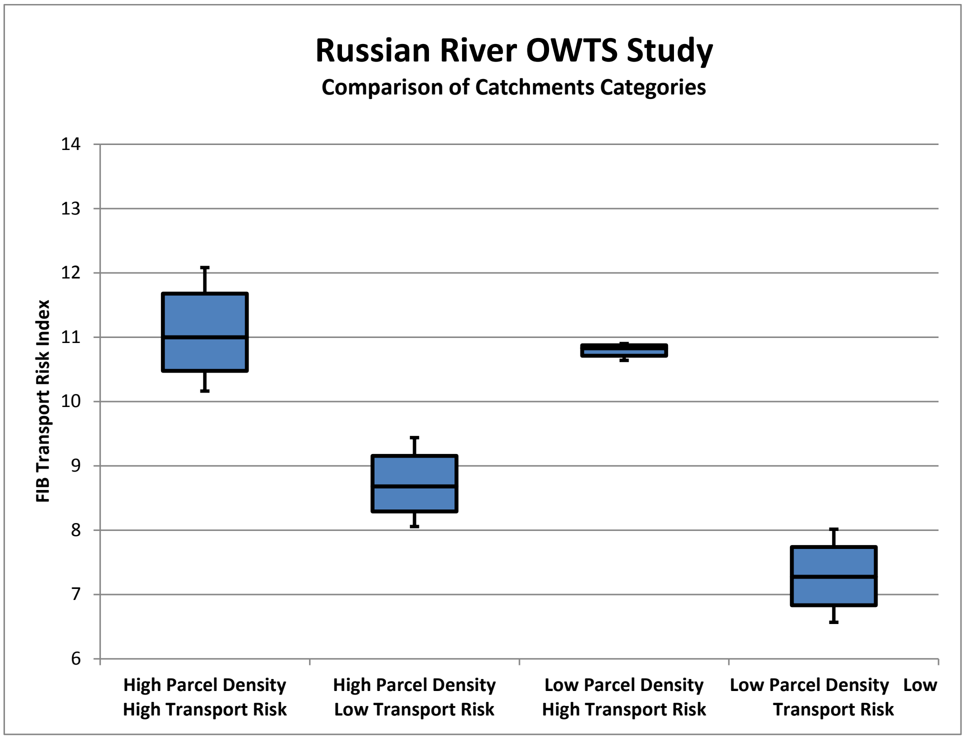

Figure 4. Comparison of the transport risk index between catchment categories

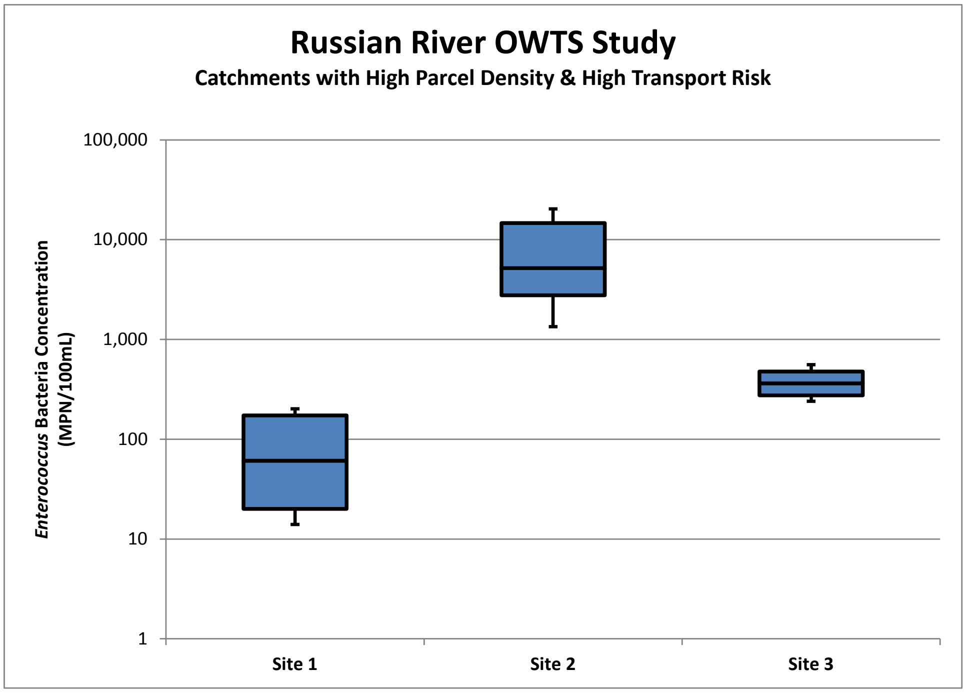

Figure 5. Comparison of *Enterococcus* bacteria concentrations from catchments with a high parcel density and a high FIB transport risk

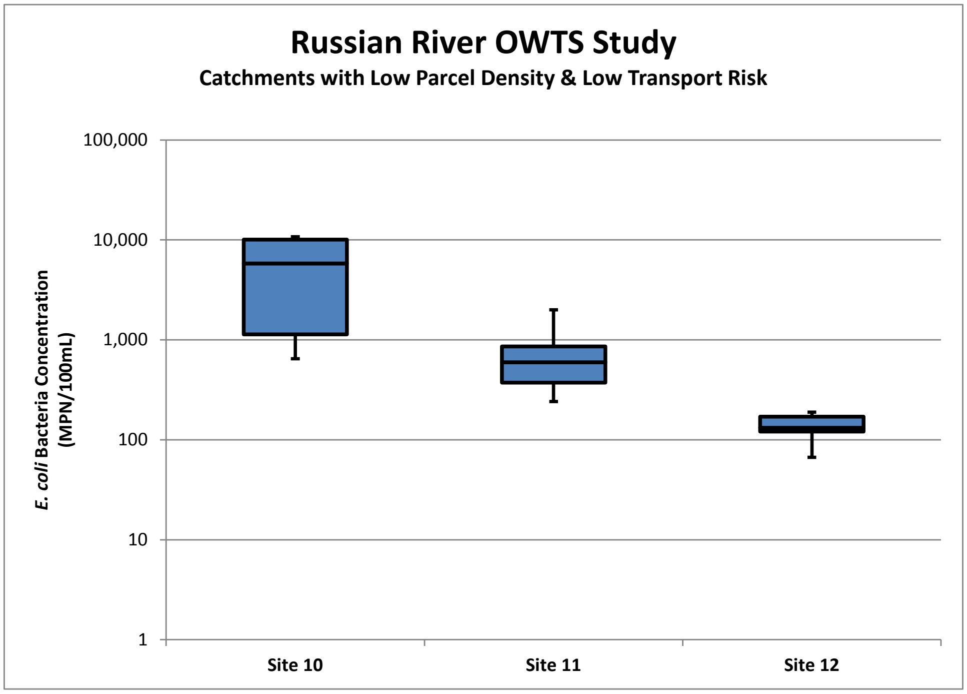

Figure 6. Comparison of *E. coli* bacteria concentrations from catchments with a low parcel density and a low FIB transport risk

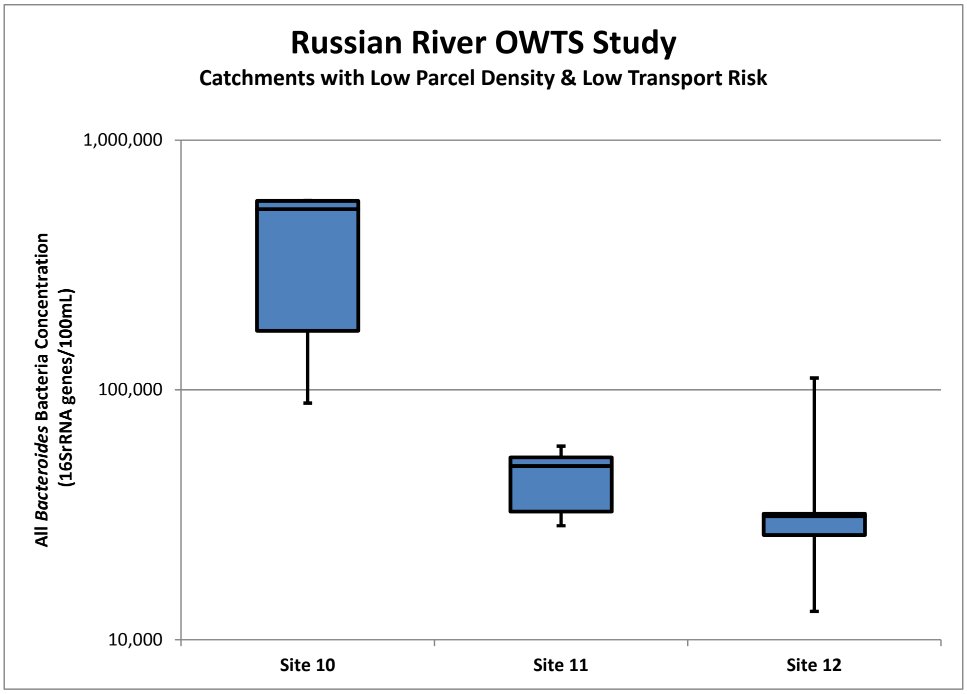

Figure 7. Comparison of All *Bacteroides* bacteria concentrations from catchments with a low parcel density and a low FIB transport risk

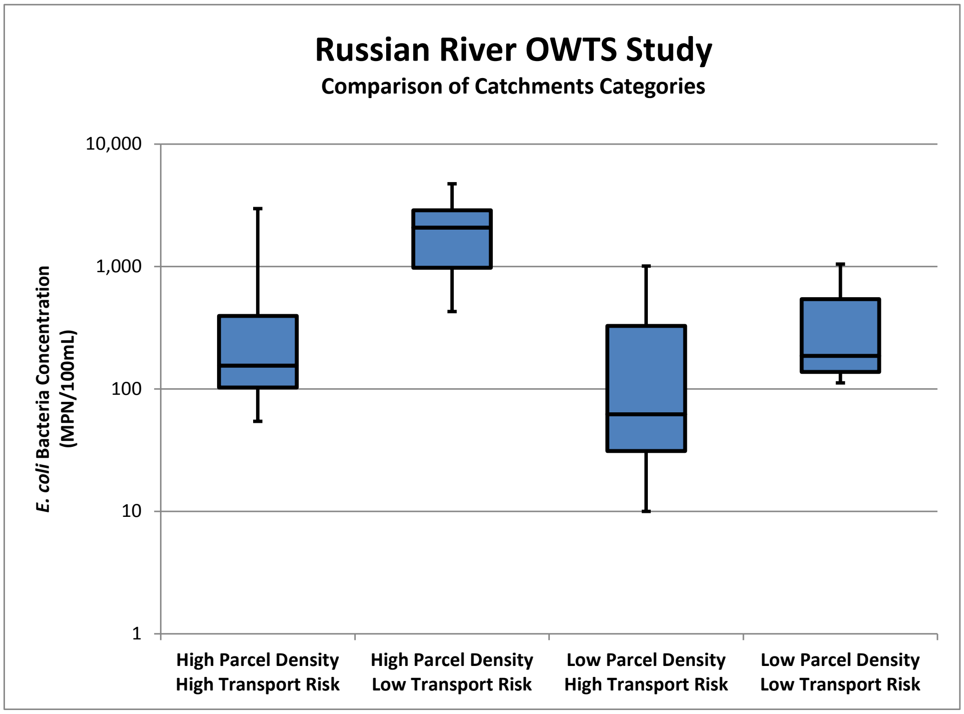

Figure 8. Comparison of *E. coli* bacteria concentrations between catchment categories

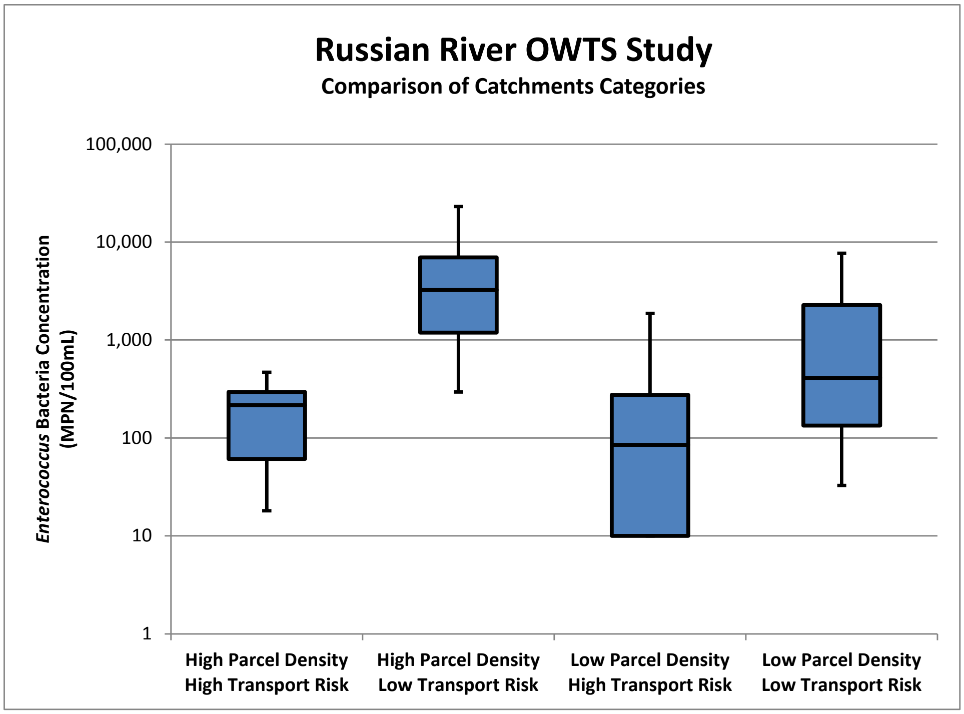

Figure 9. Comparison of *Enterococcus* bacteria concentrations between catchment categories

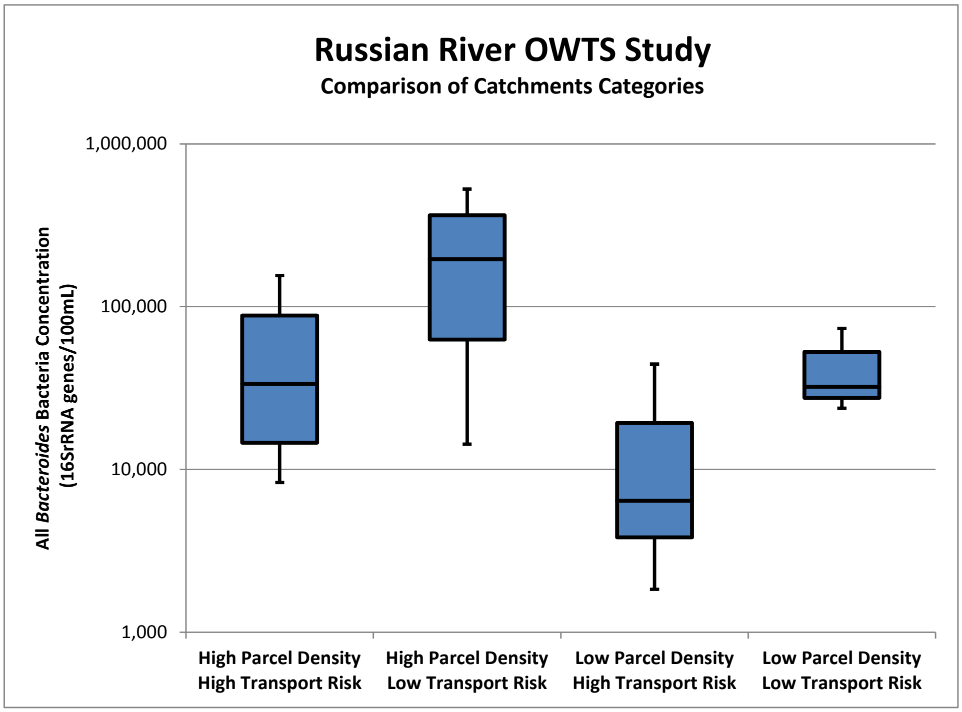

Figure 10. Comparison of All *Bacteroides* bacteria concentrations between catchment categories

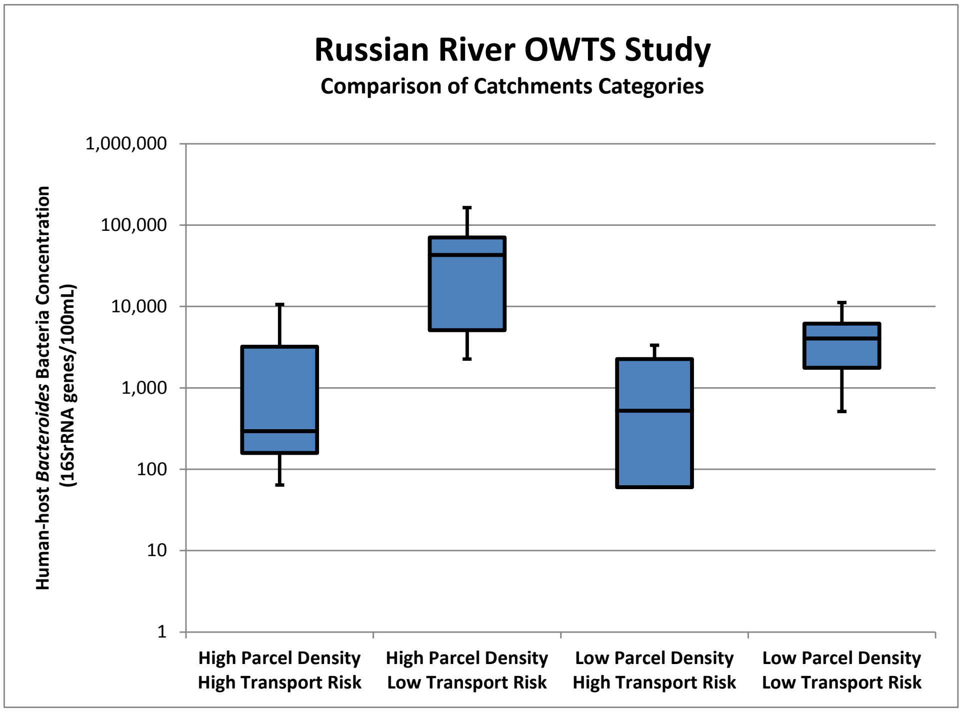

Figure 11. Comparison of Human-host *Bacteroides* bacteria concentrations between catchment categories

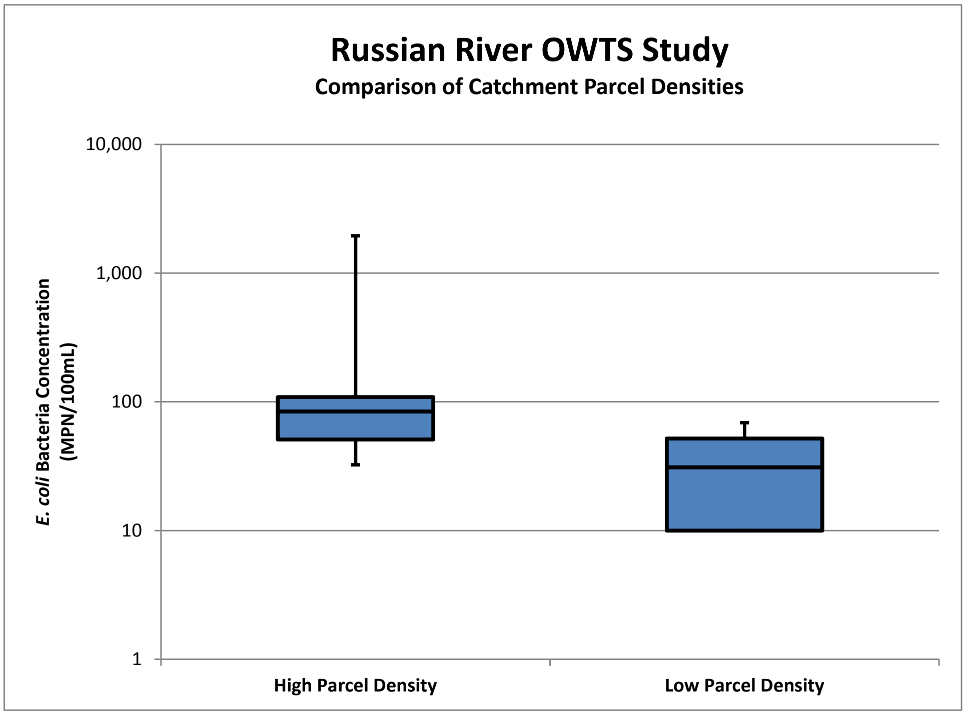

Figure 12. Comparison of *E. coli* bacteria concentrations based on catchment parcel density.

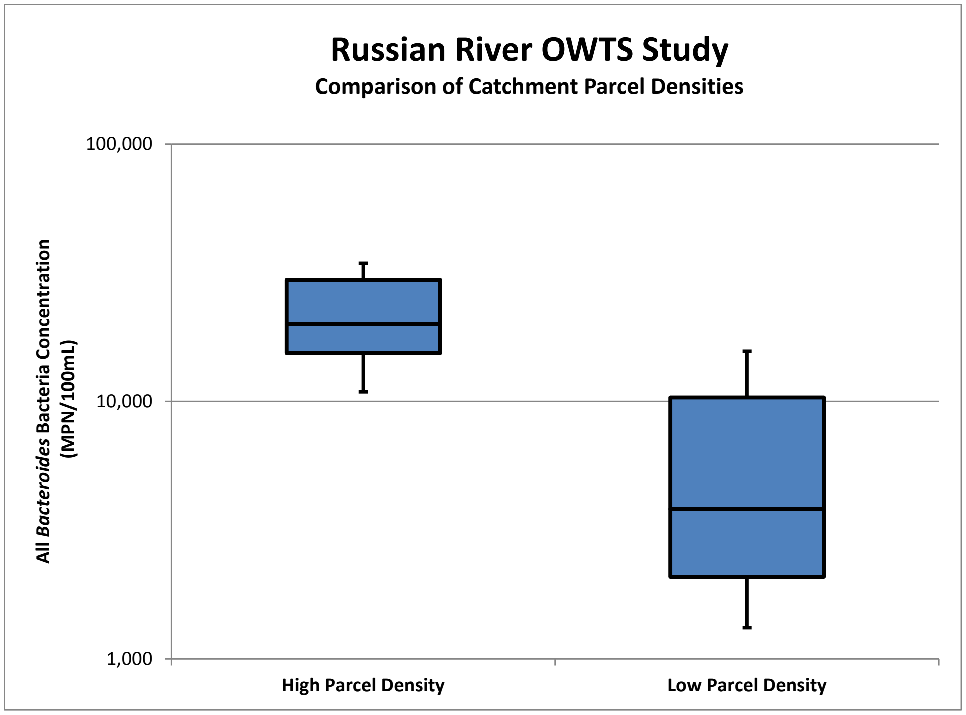

Figure 13. Comparison of All *Bacteroides* bacteria concentrations based on catchment parcel density.

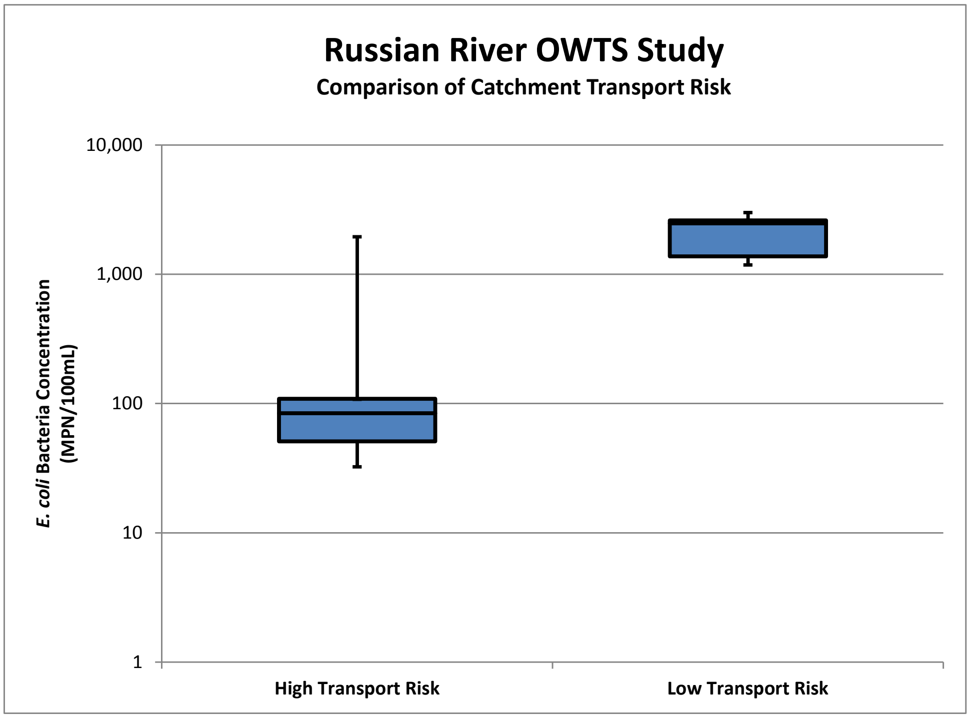

Figure 14. Comparison of *E. coli* bacteria concentrations based on catchment FIB transport risk.

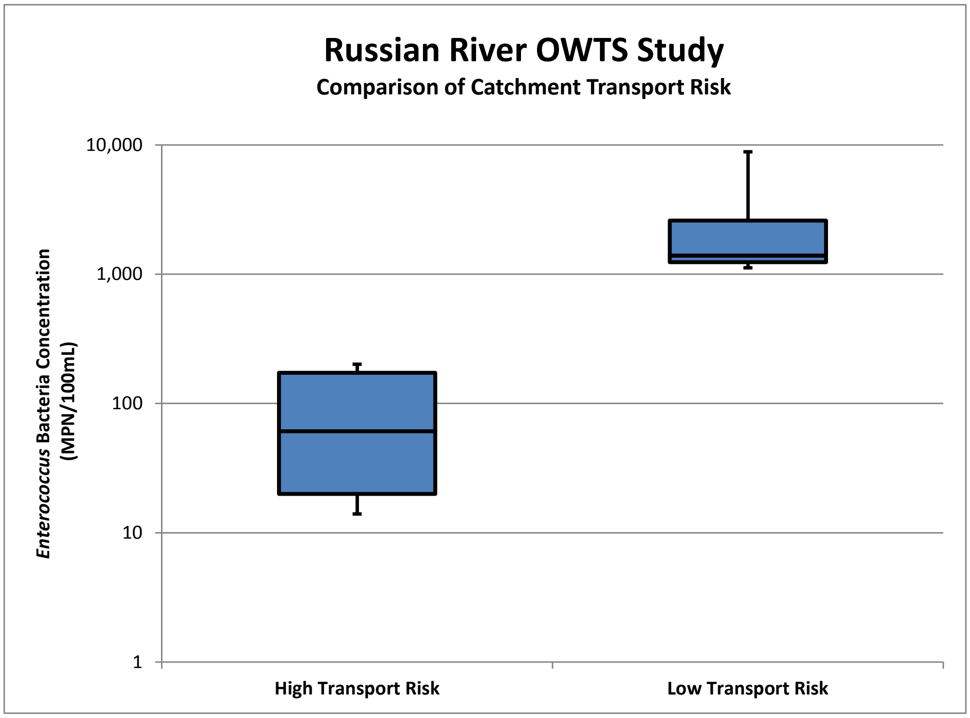

Figure 15. Comparison of *Enterococcus* bacteria concentrations based on catchment FIB transport risk.

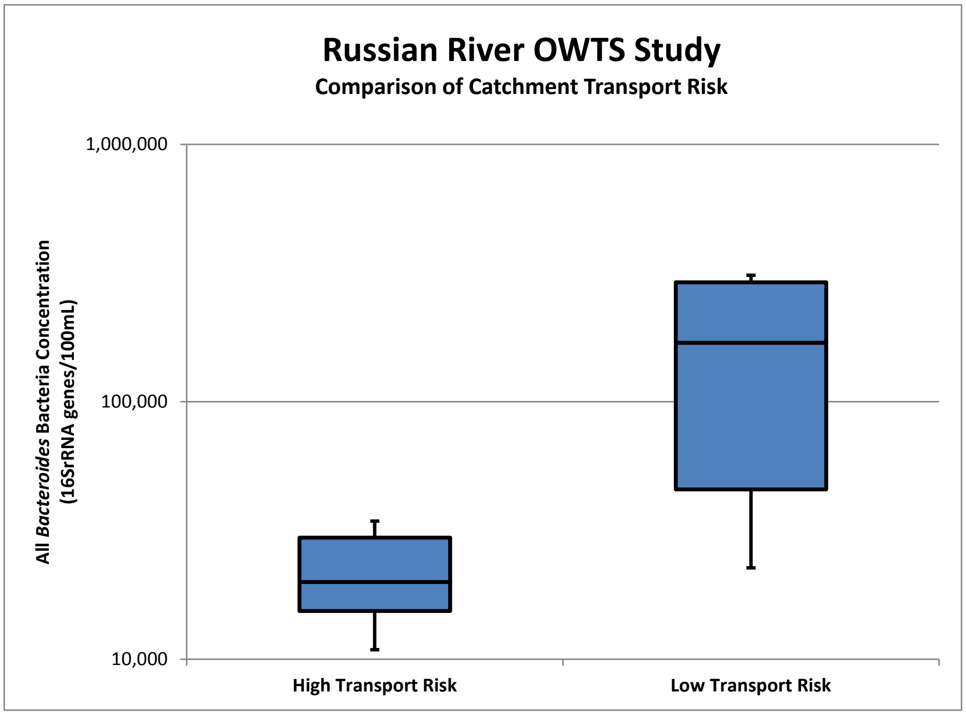

Figure 16. Comparison of All *Bacteroides* bacteria concentrations based on catchment FIB transport risk.

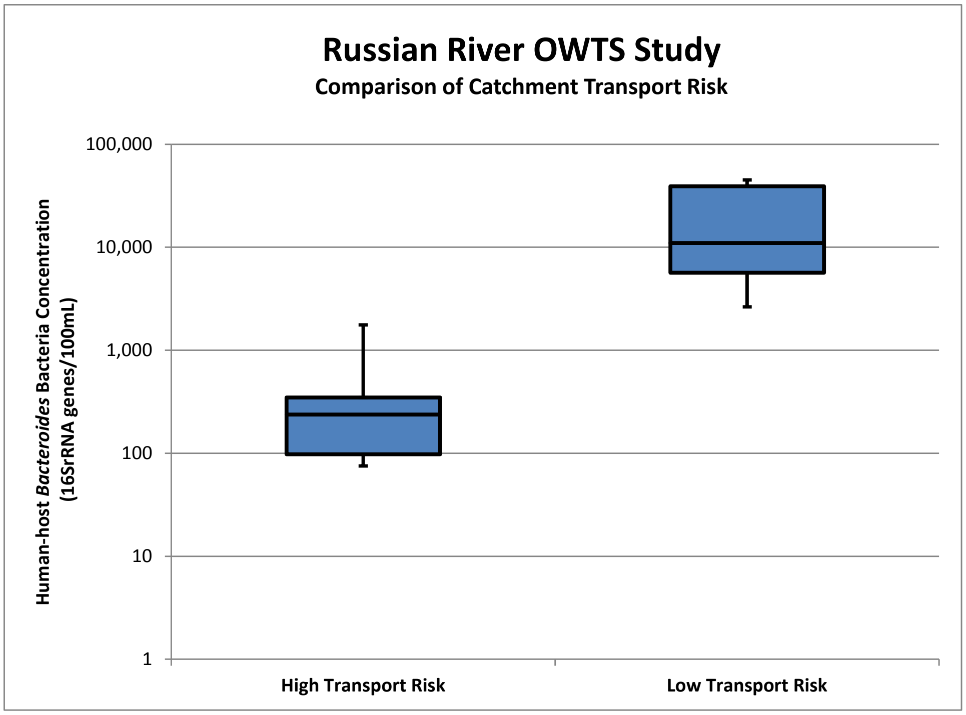

Figure 17. Comparison of Human-host *Bacteroides* bacteria concentrations based on catchment FIB transport risk.

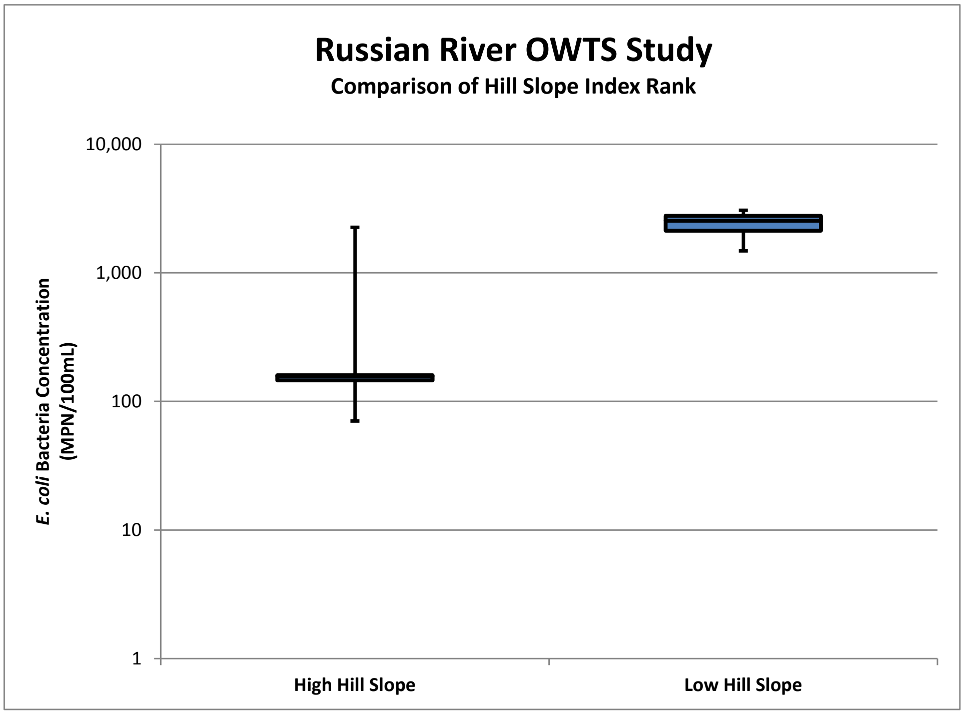

Figure 18. Comparison of *E. coli* bacteria concentrations based on catchment hill slope index rank.

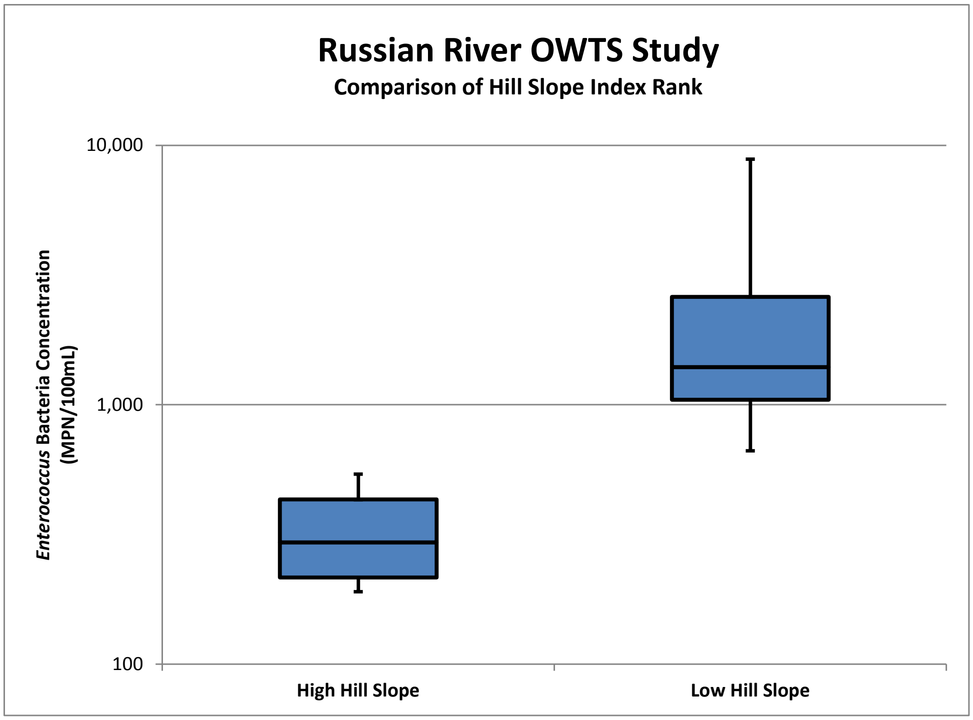

Figure 19. Comparison of *Enterococcus* bacteria concentrations based on catchment hill slope index rank.

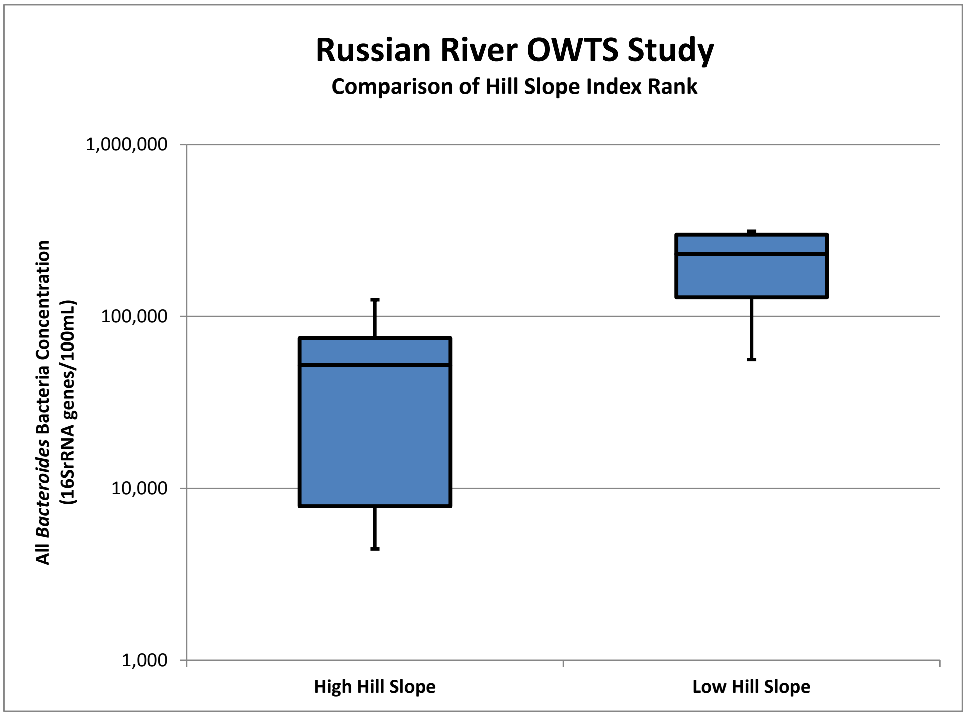

Figure 20. Comparison of All *Bacteroides* bacteria concentrations based on catchment hill slope index rank.

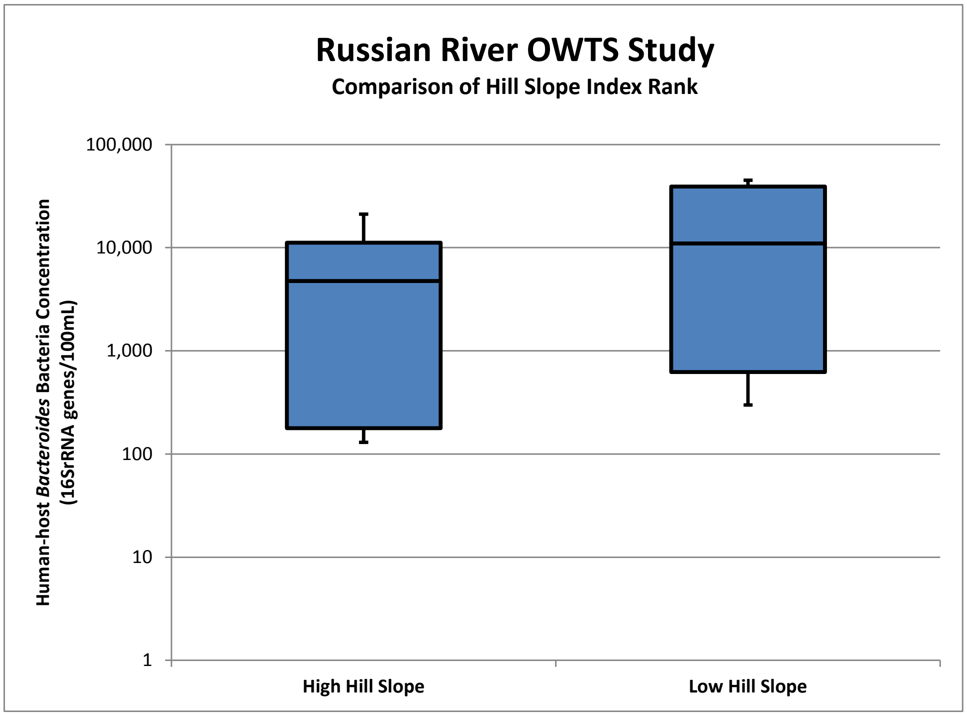

Figure 21. Comparison of Human-host *Bacteroides* bacteria concentrations based on catchment hill slope index rank

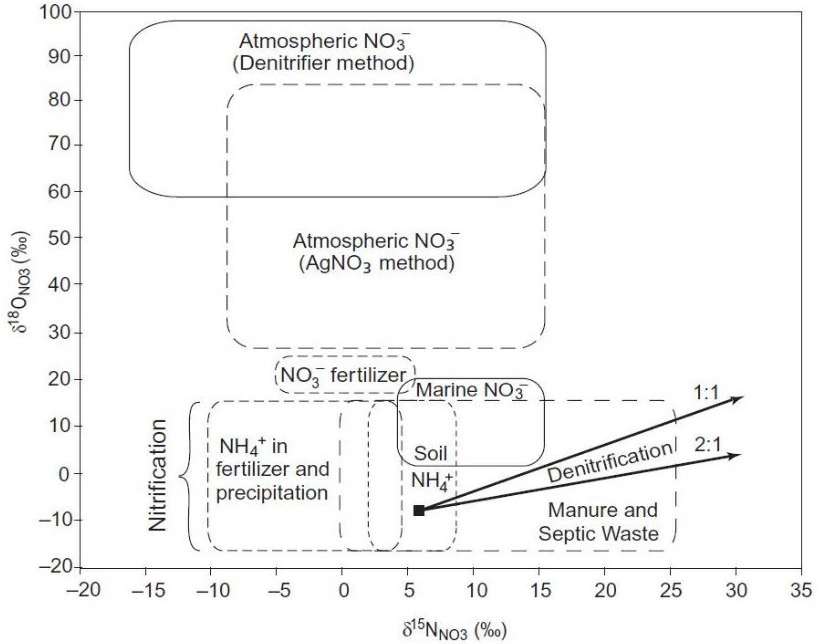

Figure 22. Typical values of the Stable Isotopes of oxygen ($\delta^{18}O$) and nitrogen ($\delta^{15}N$) of nitrate derived from various sources (diagram from Michener and Lajtha, 2007).

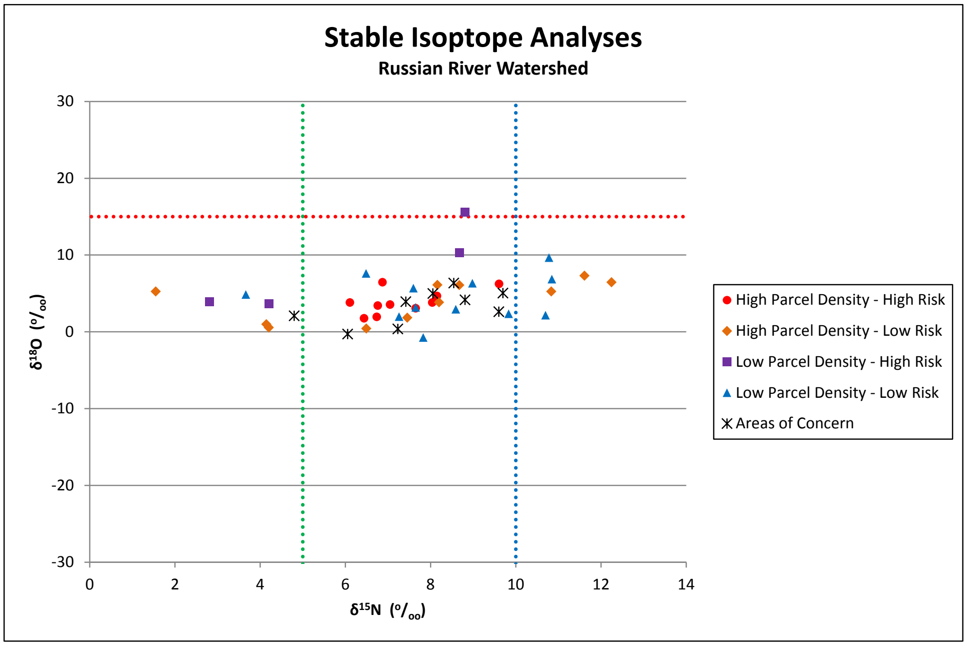

Figure 23. Comparison of the stable isotopes of nitrogen based on catchment category

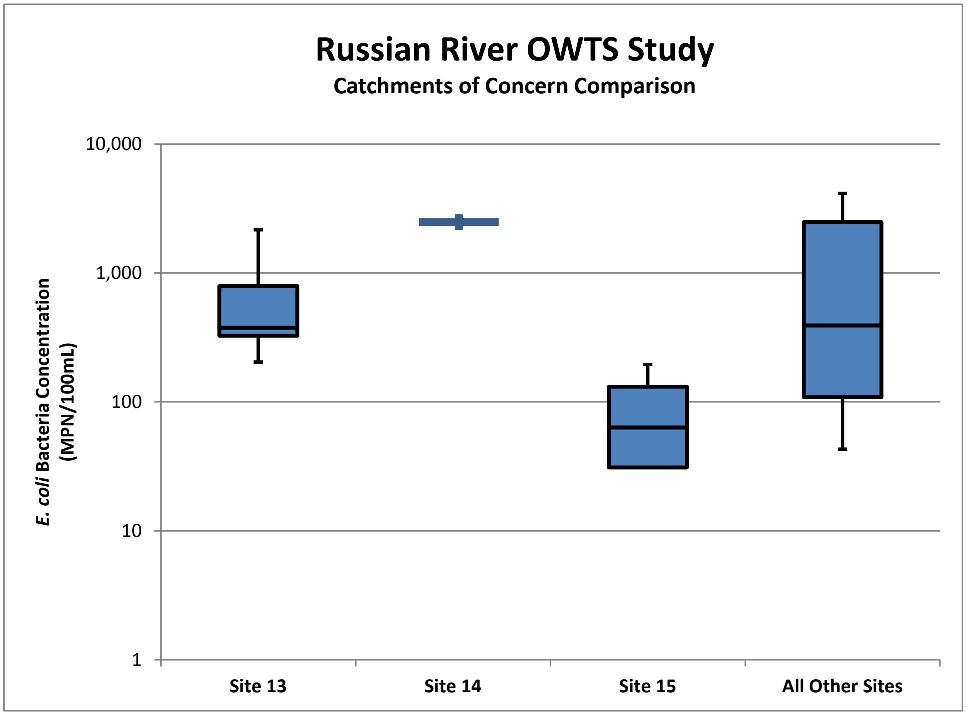

Figure 24. Comparison of *E. coli* bacteria concentrations from the catchments of concern

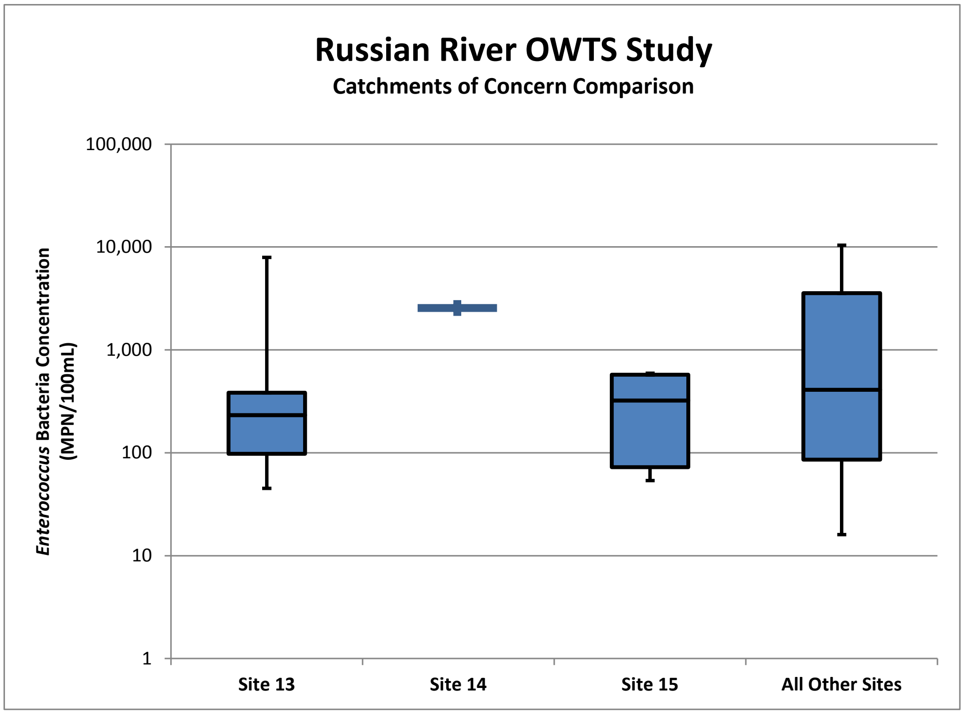

Figure 25. Comparison of *Enterococcus* bacteria concentrations from the catchments of concern

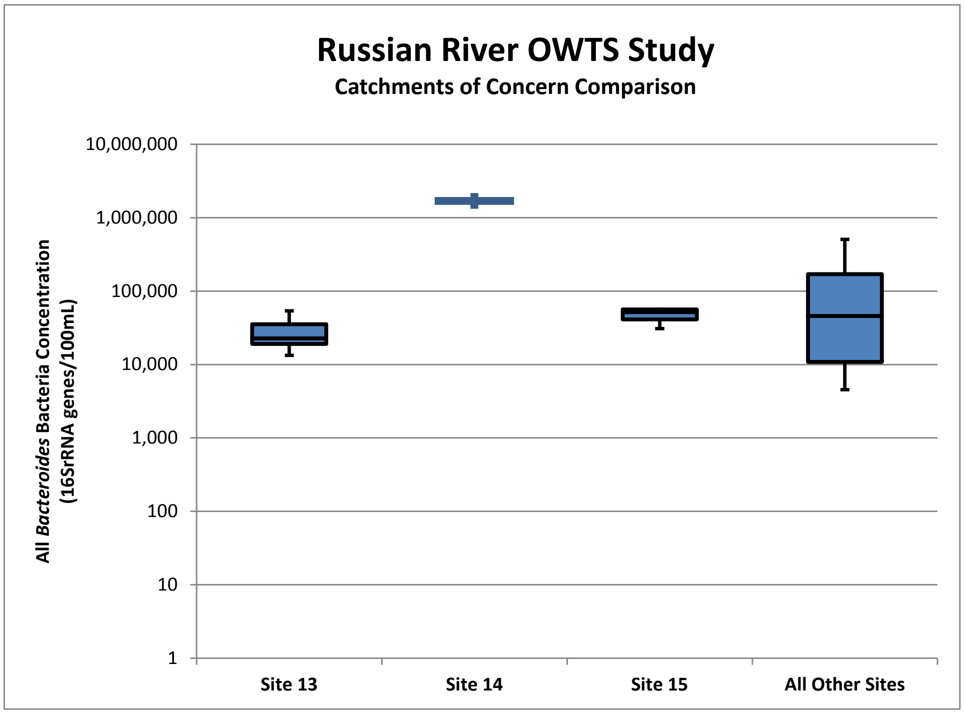

Figure 26. Comparison of All *Bacteroides* bacteria concentrations from the catchments of concern

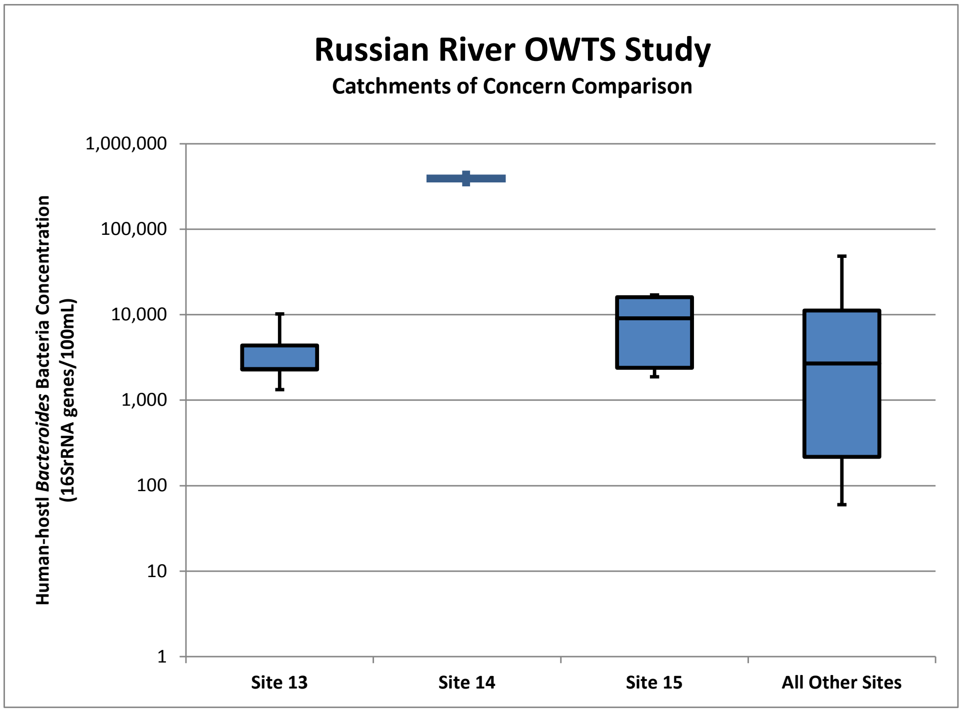

Figure 27. Comparison of Human-host *Bacteroides* bacteria concentrations from the catchments of concern

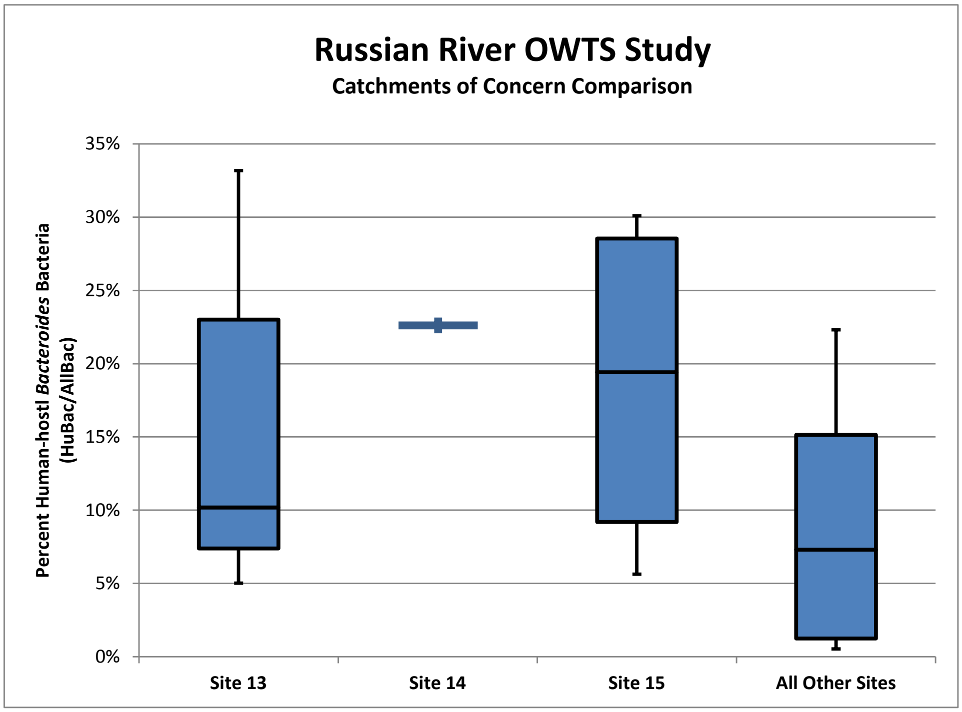

Figure 28. Comparison of the percent of Human-host *Bacteroides* bacteria concentrations from the catchments of concern