Bacteria (SOOE Extended)

Additional Discussion

In addition to the data in Figures 15.2 and 15.3 (found in State of Our Estuaries Report), three different fecal indicator bacteria were measured at both sites, including fecal coliforms, *E. coli*, and enterococci. The *E. coli* concentrations at Adams Point show very similar and slightly lower concentrations compared to fecal coliforms, with a similar decreasing trend (Figure 15.4). Enterococci concentrations at Adams Point were rarely above (1% of samples) the State standard of 104/100 ml but showed no temporal trend (Figure 15.5). Fecal coliform and *E. coli* concentrations in the Lamprey River were much higher than at Adams Point but also showed a decreasing trend from elevated concentrations through the 1990s (Figures 15.6 & 15.7). The annual geometric mean fecal indicator concentrations at low tide at these two sites and at sites in the Cocheco River (CR) and the upper Piscataqua River (UPR) showed wide differences between sites and years from 2020 to 2022 (Figure 15.5). CR and UPR were used to allow comparison of indicator levels at sites where sampling and analyses have occurred in the past three years, except that sampling did not occur in 2021 at CR. All three indicators were relatively low at Adams Point and at UPR, with levels increasing in 2022 at UPR. In comparison, indicator levels were substantially higher in the Lamprey and Cocheco rivers, and levels of all three indicators were higher at both of these tidal river sites during 2022 compared to 2020. These findings are consistent with the NH Shellfish Program classification for harvesting shellfish including proximity to wastewater treatment facility outfalls.

The use of bacterial indicators of fecal contamination has been a long-term and effective tool for managing public health risks for a variety of uses of surface waters. The levels of these indicators dictate shellfish harvest classifications and the basis for posting warnings to swimmers and other recreational users about potential health risks, but they provide no information about the source(s) of the detected contamination. An ongoing study involves the use of a commonly used Microbial Source Tracking (MST) method to show what types of fecal-borne bacteria sources are present in the Lamprey River watershed, from the tidal waters in Newmarket to Raymond, NH (Jones, 2021; 2022). MST is useful because it provides information on what is causing detected contamination, and thus allows for focusing resources to mitigate actual sources of pollution. In the ongoing study in the Lamprey River watershed, sources are identified using two methods for detecting source-specific genetic markers: one method, Polymerase Chain Reaction (PCR) detected presence/absence of 9 different sources: human, bird, mammal, dog, cow, horse, Canada goose, sea gull and ruminants. The second method, a semi-quantitative PCR (qPCR) detected relative levels of 3 sources: human, bird, and mammal.

Concentrations of fecal indicator bacteria (*E. coli*, enterococci) were generally low and below State water quality standard thresholds (Table 15.1). At the freshwater sites (Sites 2-6), the *E. coli* State standard was exceeded 7 times (17.5% of samples) at all but Site 6. *E. coli* concentrations exceeded the water quality standard in 7 of the 8 samples at Site 1 but this contamination had no effect on the upstream freshwater sites. The enterococci water quality standard for tidal water recreational use was exceeded 5 times (62.5%) at Site 1. Enterococci levels in the freshwater portion of the watershed were always below the water quality standard, suggesting that the higher level contamination at the tidal site was from nearby sources. These

results are consistent with the long-term monitoring of fecal indicator bacteria in the upper tidal Lamprey River (Figures 15.3, 15.6, 15.7).

The MST analyses showed that all 9 different sources were detected at least once in the watershed (Figure 15.6). The mammal source marker was detected in all samples as it serves as a positive control for the analysis. Bird and dog sources were detected in 39 and 31 of the samples, respectively, with cow and human sources detected in 21 and 12 samples, respectively. The Canada goose, sea gull, other ruminant and horse sources were detected in only 5 or fewer samples. The average number of source types detected were relatively consistent although Site 1 had an average of 5.3 sources per sample date while other sites had between 2.9 and 3.6 sources detected (Table 15.2). Human contamination was detected at each site once, except for Site 1 where it was detected in 7 out of 8 samples (Table 15.2). Data for the human-specific genetic marker using qPCR were related to risk of unacceptable levels of human illness (Boehm et al. 2015). The threshold they reported, 4200 copies of the human marker/100 ml, was exceeded on 6 of the 8 samples dates at Site 1 and once at Site 3 (Table 15.2). These results suggest that human contamination source pollution is consistent and elevated in the vicinity of Site 1. Finally, there was a slight seasonal trend of increasing numbers of sources detected at all 6 sample sites from May to November (Figure 15.7), which is important to understand where contamination from different sources is coming from.

The leading cause of seafood-borne illnesses are *Vibrio* species. These bacteria are naturally occurring and tend to proliferate and persist in warm areas, or in the Northeast during warm summer months. Because of this, they require separate ongoing assessment and monitoring because their presence and concentrations do not correlate with fecal-borne indicator bacteria. *Vibrio parahaemolyticus* was first detected in Great Bay in 1970 (Bartley and Slanetz 1971), while *V. vulnificus* was first detected in 1989 (O'Neill et al. 1990) and *V. cholerae* in 2008 (Schuster et al. 2011).

More recent ongoing monitoring for all three of these most significant public health threats has resulted in relatively long-term (2007 to present) databases for levels of these *Vibrio* species in oysters, water, plankton and sediments in the Great Bay estuary (Hartwick et al. 2019; 2021). Each species occupies specific niches in the estuarine ecosystem of the NH Seacoast. Their average monthly occurrence over the past 5 years (2018-22) shows that *Vibrio parahaemolyticus* is detected earlier and later, and reaches higher concentrations compared to *V. vulnificus* and *V. cholerae* in the Oyster River and at Nannie Island in Great Bay, and the latter two are detected at much lower concentrations at Nannie Island compared to the Oyster River (Figures 15.8 & 9). The average monthly *Vibrio parahaemolyticus* concentrations at Nannie Island over 3-year time spans from 2014 to 2022 showed relatively similar patterns for both 2014-16 and 2020-22, while concentrations were higher during August and September during the middle period, 2017-19 (Figure 15.10).

The results show only the total concentrations of these potentially pathogenic *Vibrio* species, whereas local studies have shown that hypervirulent strains that are most commonly associated with human illness in the Northeast are not detectable or present only at extremely low levels in the NH Seacoast estuarine ecosystems (Xu et al. 2015). Total populations are critical monitoring

targets as higher populations associated with warming of the Gulf of Maine and coastal New Hampshire will increase the potential for the emergence of virulent strains.

Methods and Data Sources

The methods used for detection and quantification of fecal indicator bacteria are summarized in the Great Bay Estuary Water Quality Monitoring Program Quality Assurance Project Plan 2018, specifically in Appendix F: Quality Assurance Plan: Microbiology Laboratory at UNH-Jackson Estuarine Laboratory, and Appendix G: SOPs for Detection of Total Coliforms, Fecal Coliforms, *Escherichia coli* and Enterococci from Environmental Samples. The general approach for detection and quantification of all three fecal indicator bacteria in the surface waters of the NH Seacoast is to filter measured volumes of water and collect target bacteria in membrane filters that are then placed on selective agar media plates that are incubated under conditions to select for growth of the different bacterial indicators and inhibit the growth of non-target bacteria. After a day of incubation, the individual bacterial indicator cells grow into visible colonies that are differentiated from other bacteria by color due to indicator-specific reactions that cause dyes to indicate a positive response. The number of colonies is recorded and expressed as colony-forming units per 100 ml.

In the ongoing Microbial Source Tracking study in the Lamprey River watershed (Jones 2022), sources are identified using two methods for detecting source-specific genetic markers (Rothenheber 2017; Rotheheber and Jones, 2018): one method (polymerase chain reaction; PCR) was used to detect the presence/absence of different sources and a semi-quantitative method (qPCR) was usedto detect relative levels, expressed as copy number of the target genes, of different sources.

The methods used for detection and quantification of potentially pathogenic *Vibrio* species are based on FDA protocols (Kaysner and DePaola 2004) and summarized in several more recent sources (Hartwick et al. 2019; Whistler et al. 2015).

Data Sources

The GBNERR SWMP and the PREP Monitoring Programs, along with UNH, provided data for the bacterial indicators of fecal contamination. The Jackson Estuarine Laboratory, the Center for Vibrio Disease and Ecology and the Cheryl Whistler laboratory at UNH provided data for the MST and Vibrio aspects of this report.

Additional Data Tables and Graphs

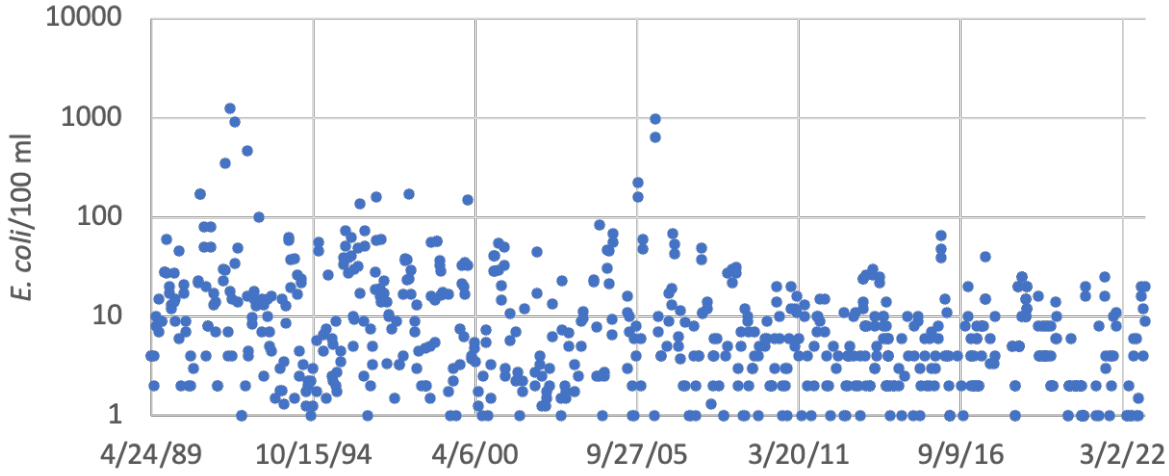

Figure 15.4. *E. coli* concentrations at Adams Point.

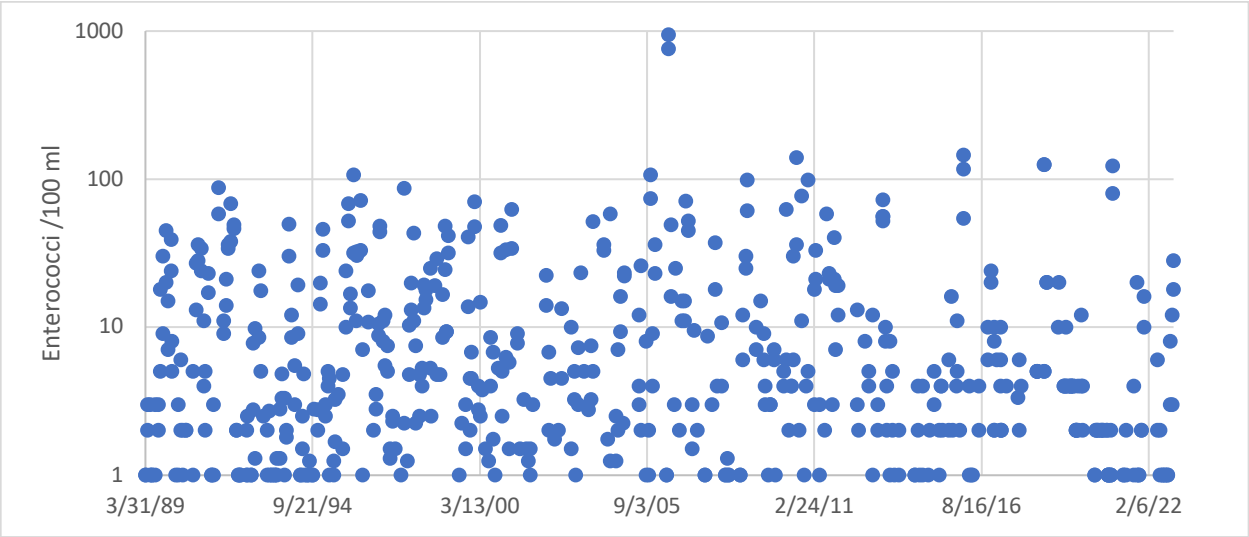

Figure 15.5. Enterococci concentrations at Adams Point.

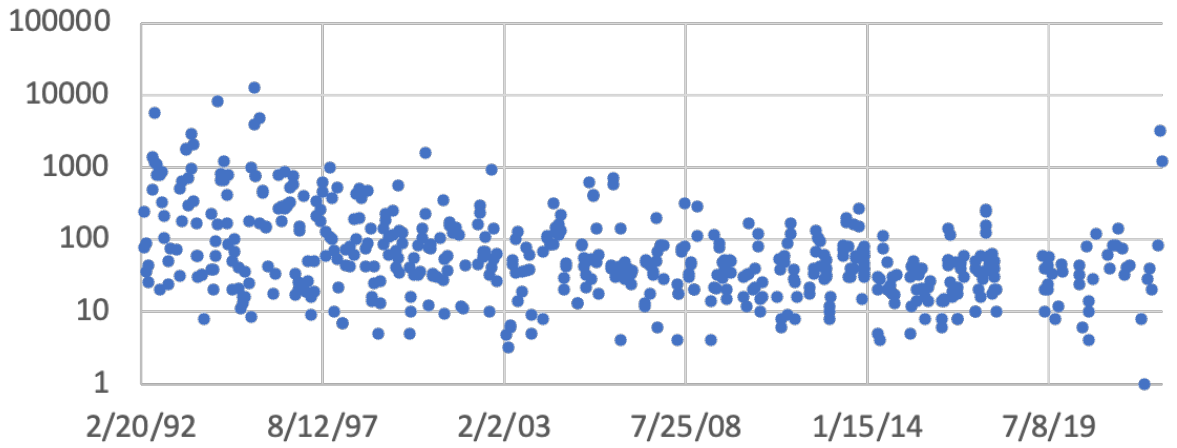

Figure 15.6. Fecal coliform concentrations in the Lamprey River.

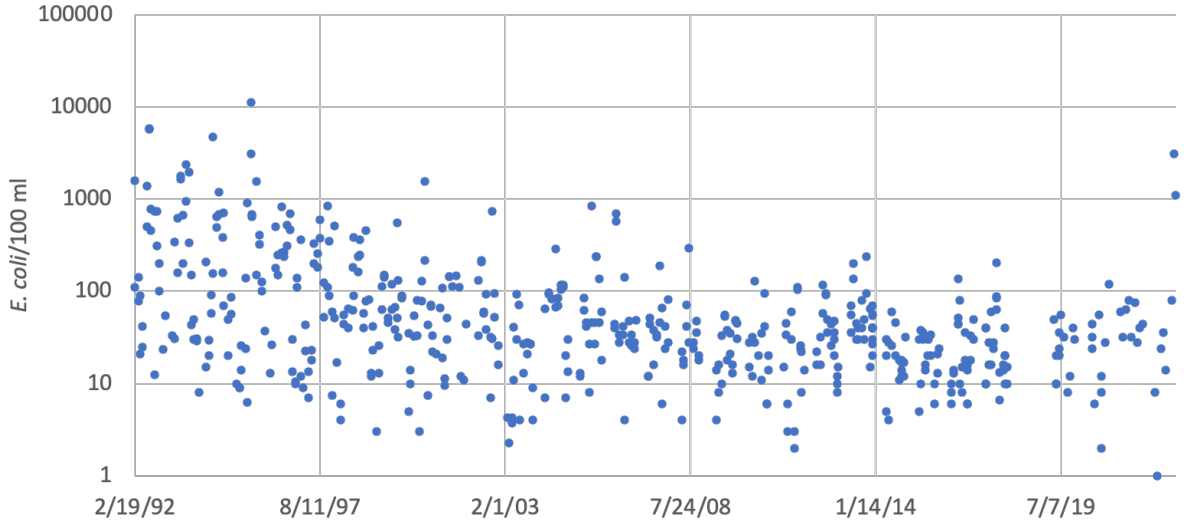

Figure 15.7. *E. coli* concentrations in the Lamprey River.

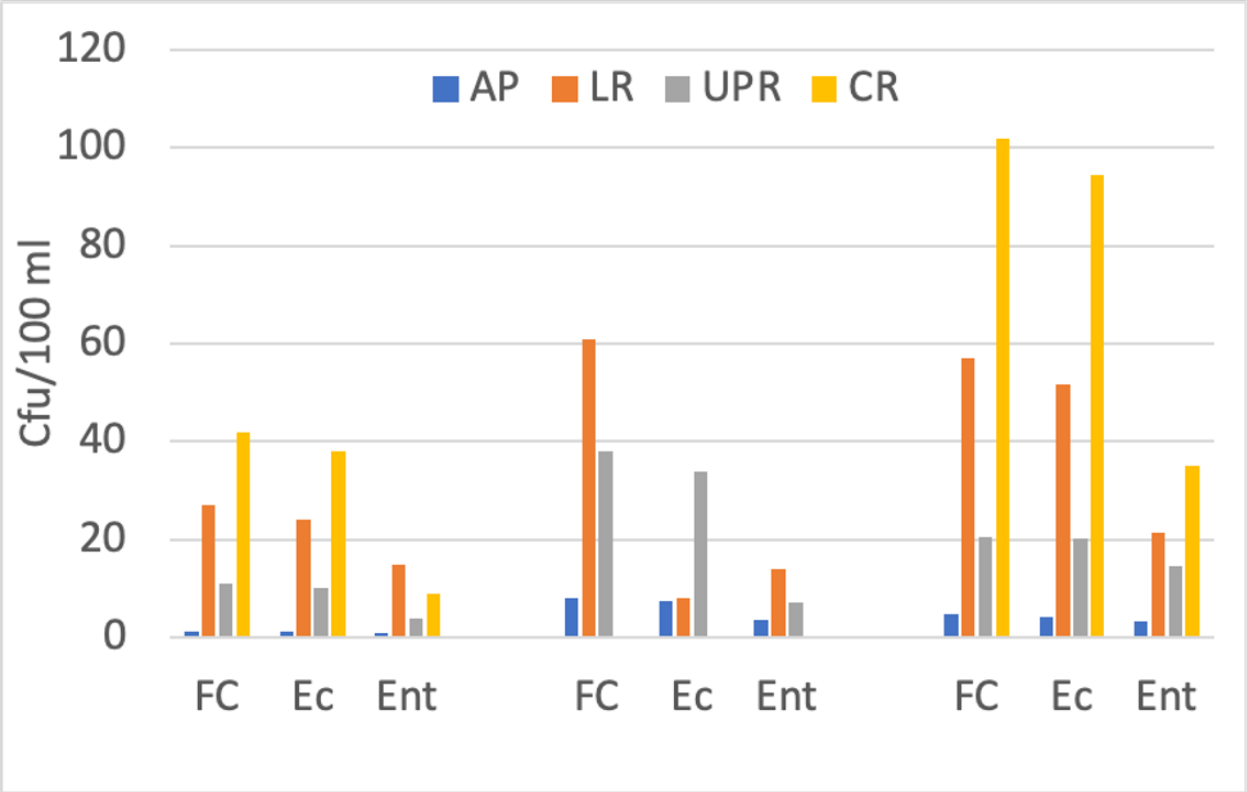

Figure 15.8. Annual geometric mean fecal coliform (FC), *E. coli* (Ec) and enterococci (Ent) concentrations at low tide at Adams Point (AP), Lamprey River (LR), upper Piscataqua River (UPR) and Cocheco River (CR): 2020 (left), 2021 (middle) and 2022 (right).

Site	Enterococci >104/100 ml	*E. coli* >158/100ml
1	5	7
2	0	2
3	0	1
4	0	2
5	0	2
6	0	0
Total	**5**	**7**
% samples	**63%**	**15%**

Table 15.1. Frequency of exceedance of State water quality standards at 6 sites in the Lamprey River watershed: 2022. Tidal water related data are highlighted in yellow, freshwater data are highlighted in blue.

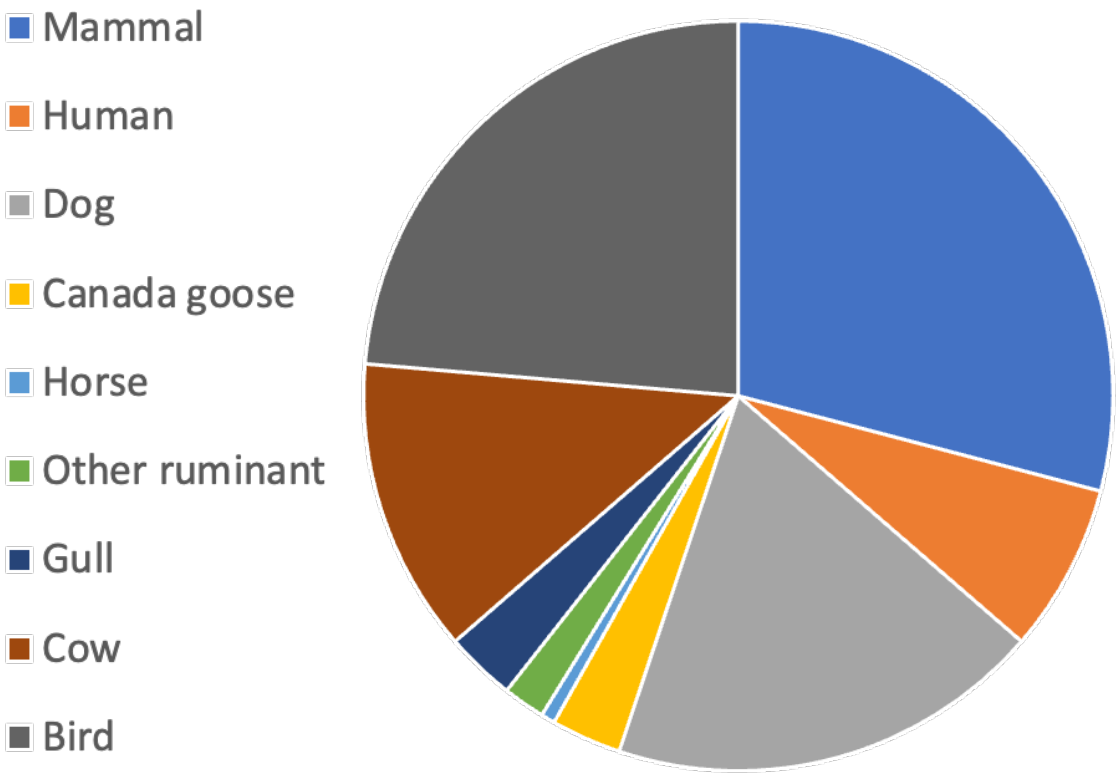

Figure 15.8. Relative frequency of identified fecal contamination sources in the Lamprey River watershed: May-November 2022.

Site #	Ave. # of source types detected	Human source detection	Human source >threshold
1	**5.3**	**7**	**6**
2	3.1	1	0
3	3.6	1	1
4	3.4	1	0
5	2.9	1	0
6	3.6	1	0

Table 15.2. Average fecal source types detected, total times the Human source was detected and when a public health safety threshold concentration (copy number/100 ml sample) was exceeded at 6 sites in the Lamprey River watershed. May-November 2022.

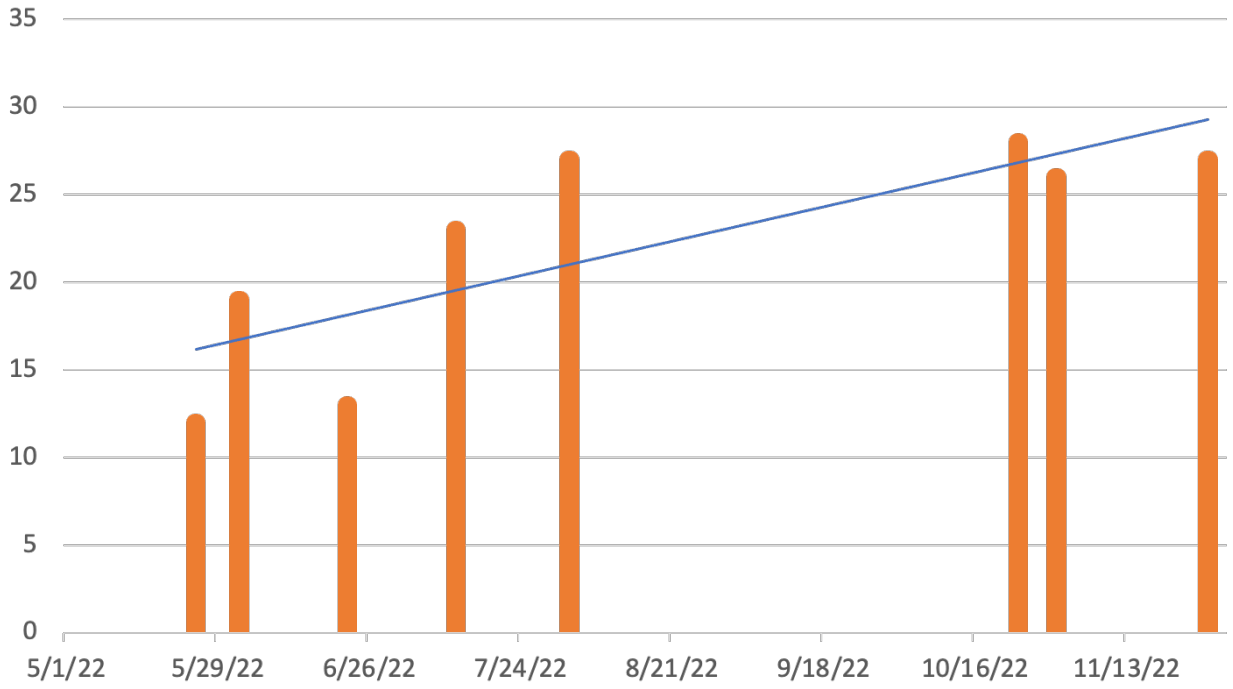

Figure 15.9. The number of fecal contamination sources at all 6 sample sites in the Lamprey River watershed: May-November 2022.

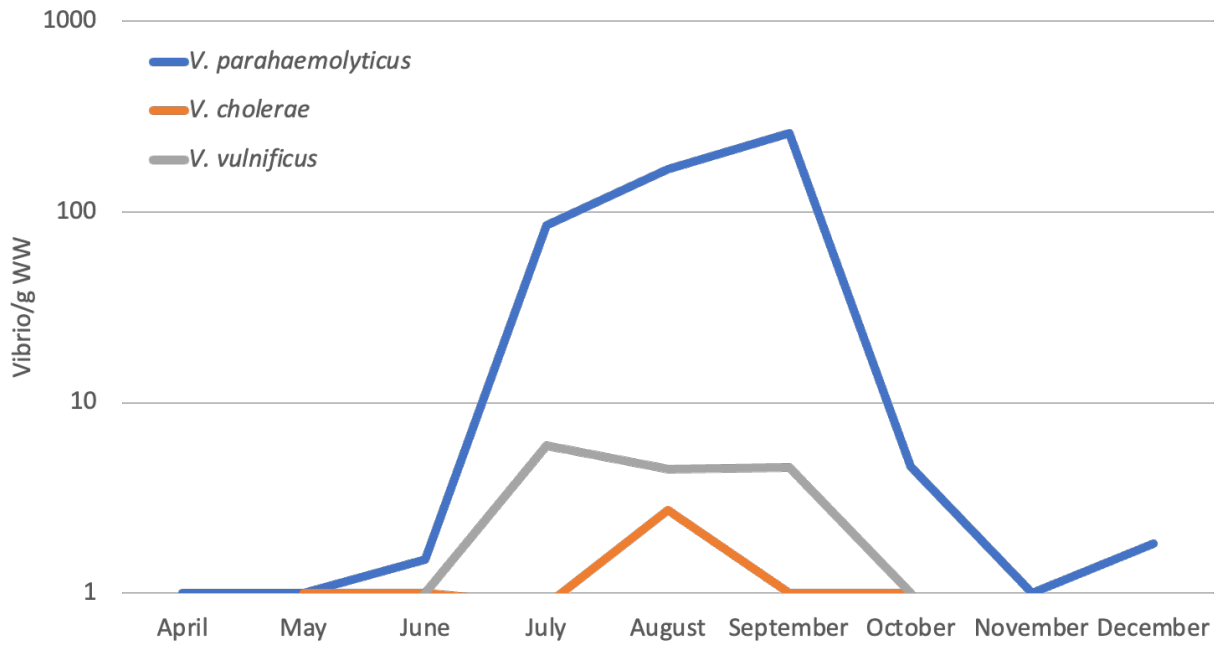

Figure 15.10. Average monthly concentrations of *Vibrio* species in Oyster River oysters: 2018-2022.

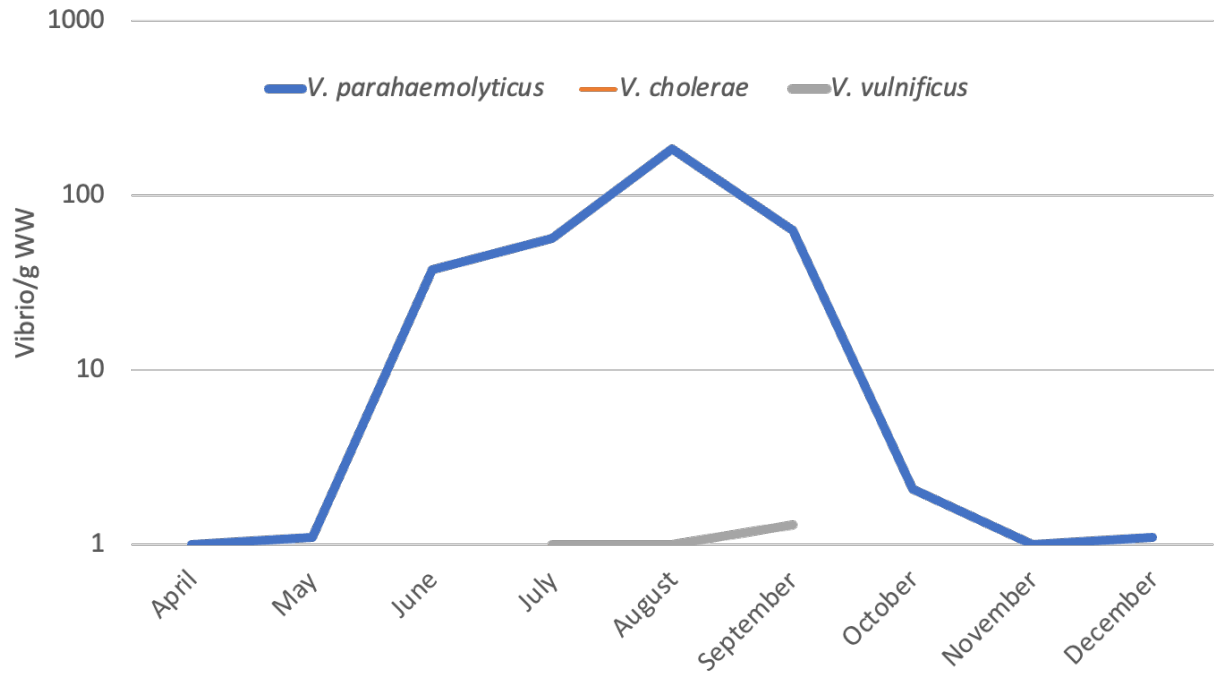

Figure 15.11. Average monthly concentrations of *Vibrio* species in Nannie Island oysters: 2018-2022.

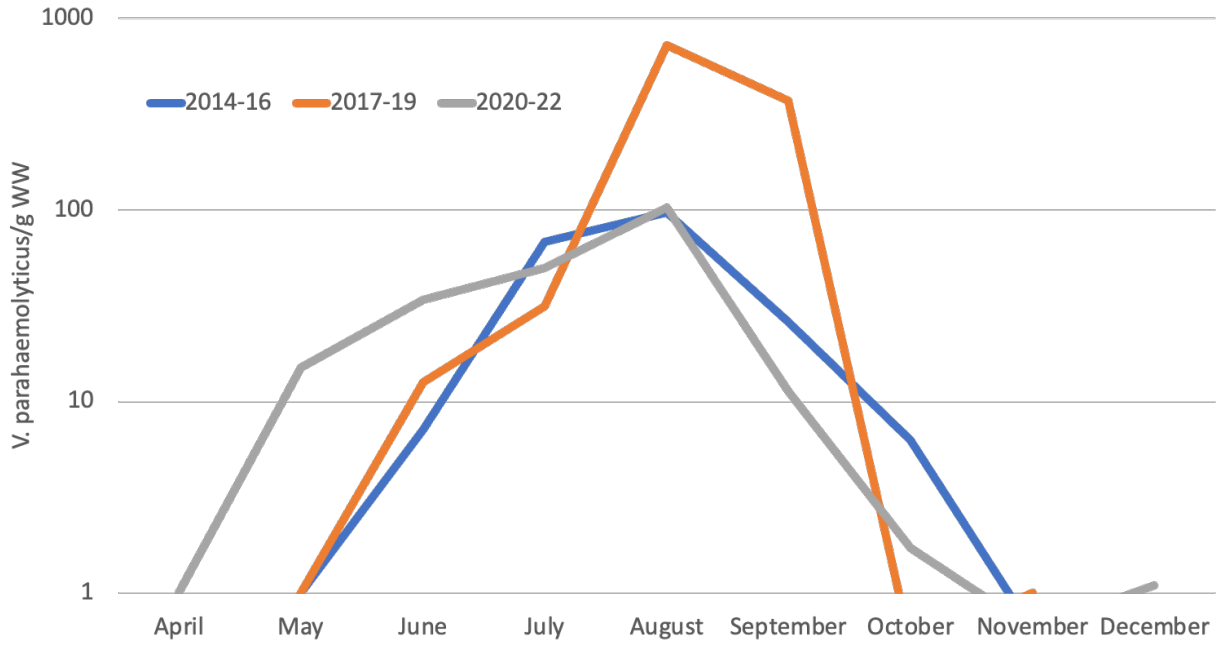

Figure 15.12. Average monthly concentrations of *Vibrio parahaemolyticus* species in Nannie Island oysters over 3 consecutive 3-year time spans: 2014-2022.

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