most, only minor refinement of results in these cases" (Attachment 1). PhyloChip® analysis performed in nearby watersheds (the Ogunquit River in Ogunquit, ME) has successfully identified human fecal contamination in samples using the same SourceTracker library (FBE, 2022), suggesting that the methodology is applicable to coastal watersheds in northern New England. Note that despite two wet-weather sampling dates, the watershed was experiencing an exceptional drought in 2022 when samples were collected (D1 in July and October and D2 for August and September according to the U.S. Drought Monitor; NOAA, 2023), which may have resulted in an atypical transport pattern for fecal waste (for human, pets, and wildlife).

2. The high concentrations of particulates in the samples could have caused matrix interference that impacted the results. As is typical for tidal creeks in salt marshes, the water samples collected in Parsons Creek contained a high level of particulates that were retained on the water filters along with the DNA material of interest after filtration. At ACPS005-U35 there was so much particulate matter in the samples that filtering took substantially longer than the other sites, and in two cases, only 100 mL could be filtered (Attachment 2). The presence of this additional substrate could have impacted laboratory processes or the DNA on the filter directly; however, Veracet confirmed that there was only one potential issue with DNA preparation (PC-OUT on 10/4/2022 had low DNA after multiple extractions; Attachment 1) and that all other samples had "thousands of bacterial species that would be commonly found in environmental water" (Attachment 1). Together, this suggests that although possible, matrix interference is unlikely to have impacted the results.

3. It is possible that, because of watershed management efforts such as the replacement of failed septic systems and the implementation of the town pump out ordinance, human fecal contamination in Parsons Creek has been reduced to the point where the human signature is undetectable at the five sampling sites. If these results were interpreted without the knowledge of previous water quality data and trends in Parsons Creek, this explanation would seem like the most likely scenario. However, given that human fecal contamination has been identified as a diffuse source throughout the watershed via other methods (canine scent detection and DNA ribotyping) and that Enterococci levels at the five sites have increased or remained high in recent years, it would be reasonable to doubt this conclusion. To better resolve these conflicting results, it is recommended that additional source tracking data be collected using alternative methods (e.g., mitochondrial DNA markers).

4. The sensitivity of the PhyloChip® method may not be high enough to detect the diffuse levels of human fecal contamination in Parsons Creek. Since the method is centered around matching the microbial composition of known fecal sources to the microbial community found in water samples, it is possible that results indicate no human sources if the microbial community is altered in transit from its source(s) to the sampling site in such a way that it becomes unrecognizable among the fecal source assemblages listed in Veracet's SourceTracker database. Both the degree to which the microbial community changes as it travels through the environment and the sensitivity of the PhyloChip® method to these changes are difficult to quantify. One study in New England has shown that habitat characteristics have a greater impact on microbial community composition in salt marshes than external, human-derived inputs such as nutrients (Bowen et al., 2009). This conclusion aligns well with the microbiological paradigm that "everything is everywhere, but the environment selects" (Bass-Becking, 1934), and suggests that the salt marsh area surrounding Parsons Creek could be actively dampening any human waste signal from septic systems by amplifying or reducing specific bacterial taxa based on environmental conditions.

Additionally, there is precedent for the PhyloChip® method appearing less sensitive than other fecal source tracking methods, as recent sampling conducted by FBE in Rocky Neck State Park in East Lyme, CT yielded multiple positive hits for human waste via canine scent detection but no human sources via