# Use of Sucralose and Caffeine as Tracers of Human Waste in a Coral Reef Ecosystem

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## Abstract

Over the past ten years, divers have noted a decrease in healthy coral cover and an increase in benthic algae in Vatia Bay, on the island of Tutuila, American Samoa. The cause for this is unknown, but one hypothesis is that nutrient pollution from the local village may be driving the coral decline. Excess nutrients (especially nitrogen and phosphorus) can impact corals directly by lowering fertilization success, and reducing both photosynthesis and calcification rates, or indirectly such as through stimulation of the growth of benthic algae. Declining coral health adversely affects the biodiversity of the Bay and likely decreases ecosystem services. The objectives of this study were to determine the nutrient status of Vatia Bay (i.e. are levels elevated) and attempt to use caffeine and sucralose as tracers to assess the potential importance of human waste to the nutrient budget of the system.

Water samples were collected monthly at sixteen sites, selected using a stratified random design, for analysis of nitrate, nitrite, ammonium, urea, total nitrogen, orthophosphorus, total phosphorus, silica and salinity. These data confirm that nutrient concentrations are elevated in the Bay, when compared to territorial water quality standards (total nitrogen<0.15 mg N/L; total phosphorus<0.02 mg P/L). Land based contributions of phosphorus and reactive nitrogen can enter the environment from a variety of sources, but in Vatia the most likely sources are piggeries and septic systems. Analysis of water samples for tracers of human waste (caffeine and sucralose) confirmed that human derived nutrients are contributing to the nutrient budget of the Bay. Caffeine was detected in 82% of samples and sucralose was detected in 51% of samples, definitively confirming that human waste is reaching the Bay. Additionally, sucralose concentrations are significantly correlated with some nutrient constituents, including urea, a major component of waste. These data are useful not only to enhance the understanding of the role that anthropogenic nutrients play in the biodiversity and ecosystem health of the Bay, but also serve as an important “baseline” against which to measure future change.

**Keywords:** Coral reefs, nutrient pollution, water quality, sucralose, caffeine

# 1. Introduction

### *1.1 Background*

Coral reefs have the potential to be adversely affected by a variety of water quality problems, including toxics, sedimentation and over enrichment of nutrients. Nutrients (particularly nitrogen and phosphorus) are critical to ecosystem primary productivity, but excess amounts can lead to macroalgal and benthic algal blooms, which can overgrow or outcompete the corals (Kuffner et al. 2006; Hughes and Tanner 2000; D'Angelo and Wiedenmann 2014). These impacts can be exacerbated by disturbance events, such as physical disturbance (e.g. tsunami or storm), a bleaching or disease event, or a crown of thorns outbreak (Szmant 2002; Fabricius 2005). Additionally, excess nutrients can directly affect corals by reducing calcification and photosynthesis rates (Marubini and Davis, 1996), and by lowering fertilization success (Harrison and Ward, 2001).

Like any pollutant, effective management of nutrient pollution requires accurate source identification. This can be problematic because the number of potential sources is large. Chemical fertilizers (both agricultural and non-agricultural uses), industrial sources, animal waste, and human waste can all contribute both nitrogen and phosphorus to the coastal environment (Galloway et al., 2003). Additionally, atmospheric deposition can be an additional pathway of nitrogen flux, originating from fossil fuel combustion and ammonia volatilization from agriculture; Mathews et al., 2002).

Historically, source identification of nutrients has relied on a variety of techniques including: modeling (Castro et al. 2001; Whitall et al. 2004), microbial source tracking (Scott et al. 2002) or stable nitrogen isotopes (Xue et al. 2009). However, each of these techniques has its drawbacks, and none are suitable for all applications. For example, stable nitrogen isotopes can be very useful in discriminating between chemical fertilizers and human/animal waste sources, but cannot discriminate between human and animal sources. In recent years, researchers have been measuring caffeine and sucralose in the environment as chemical proxies (or tracers) for human waste (Mead et al. 2009; Knee et al. 2010). Both of these compounds are persistent through the human gut and septic systems, and they tend to persist in the aqueous environment. Because these compounds exist only in the human diet, they can be utilized to detect the presence of human waste in nearshore marine systems.

Caffeine ($C_8H_{10}N_4O_2$) is a common dietary stimulant found in a variety of foods and beverages, including coffee, soda, energy drinks, and chocolate, as well as over-the-counter medications. Caffeine is excreted in human urine and can persist in the natural environment. Previous studies have quantified caffeine in aquatic systems (Edwards et al. 2015) and found correlations with other indicators of human waste (Knee et al. 2010). The half-life of caffeine in surface waters has been estimated at between ten and 20,000 years (Edwards et al. 2015).

Sucralose (4-chloro-4-deoxy-α,D-galactopyranosyl-1,6-dichloro-1,6-didexoy-β,D-fructofuranoside) is a chlorinated disaccharide derived from sucrose. It is used as an artificial sweetener in diet beverages, candies and as a stand-alone additive (i.e. under the brand name Splenda). Most ingested sucralose is not metabolized in the human body and is excreted in urine and feces (Torres et al 2011). It is relatively stable in the environment which makes its use as a conservative tracer appealing. It has previously been quantified in freshwater (Spoelstra et al.

2013) and marine (Mead et al. 2009) ecosystems. It has also been shown to outperform other chemical sewage tracers in field assessments (Oppenheimer et al. 2011). The half-life of sucralose in surface waters has been estimated to be on the order of several years (Lubick 2008). Because caffeine is more prevalent in human diets, using both analytes in tandem as tracers may have some utility.

### ***1.2 Study Site Description***

Vatia Bay is located on the north shore of the island of Tutuila, the largest and most populous island of the U.S. territory of American Samoa (Figure 1). American Samoa's reefs are considered to be among the most pristine in the United States (Birkeland et al. 2008). These reefs host approximately 950 species of fish, 240 species of algae, 330 species of coral and many other species of invertebrates (Birkeland et al. 2008). At its widest point, the roughly horseshoe shaped Bay is approximately 750 meters wide and 1 kilometer long, with the opening to the ocean oriented to the northeast. There have been local concerns about the impacts of land based sources of pollution and water quality on the coral reef ecosystems of Vatia Bay (NOAA CRCP 2012), which consists of a mixture coral, crustose coralline algae (CCA), and algae (Vargas-Angel and Schumacher 2018).

Three perennial streams, as well as some intermittent streams, bring freshwater inflows from the surrounding watershed into the Bay. Additionally, groundwater may play a significant role in the freshwater influxes to the Bay (Shuler et al, 2019). The land adjacent to the Bay consists of the small village of Vatia (6.5 km$^2$ in area), which is made up 116 housing units and 640 residents. The population of the village is relatively stable with a loss of 8 residents reported between the

2000 and 2010 Census (US Census, 2010). The land surrounding the village is part of the National Park of American Samoa. The village contains an elementary school and multiple churches, but no businesses or industry. The village has no centralized sewage treatment system. Household sewage is treated via septic systems or cesspits. In some cases, household waste may go untreated. There is no large-scale crop agriculture in the watershed, although there are some small backyard vegetable gardens, and a slightly larger (approximately one hectare) cleared area in which bananas and taro are grown. Additionally, village residents operate small scale, backyard piggeries, whose standing stock varies (see Supplementary Material).

Based on the available land use data (population, number of animals, etc.) and literature values for nutrient flux (see modeling work contained in Castro et al. 2001), it is hypothesized that human waste and waste from the piggeries are likely to be the dominant sources of nutrients to the Bay. Discriminating between human and animal sources of waste is an important data need for coastal managers. This study sought to determine the ambient levels of nutrients in the Bay and if human waste was contributing to that nutrient load.

## 2. Materials and Methods

### *2.1 Water Quality Sampling Design*

In order to compare sections of the Bay, a stratified random sampling design was employed. After operationally defining four strata within the Bay based on proximity to the stream/shore and geography (Inner, Central, North, and South), four sites were randomly selected (using ArcGIS) in each stratum (see Figure 2). This approach allows for statistical comparisons among the articulated strata. Additionally, one targeted site was selected near the mouth of the largest stream entering the Bay (just upstream from the largest bridge in the village). Details about each site, including latitude and longitude are shown in Table S1 (Supplementary Material). Field personnel accessed the sites by either sea kayak or wading. At wading sites, care was taken to sample on an incoming swell/wave and away from the person's body to minimize the potential for contamination. Water was collected both from the surface (0.1 m below surface) and bottom (via Niskin bottle, just above bottom); exceptions to this were very shallow sites (<1 m depth, sites IN3, IN4, SB10, SB11, SB12, NB19, NB20) at which only surface water was sampled, and one site (NB17) which consistently had high wave energy, making deploying the Niskin bottle from the sea kayak unsafe.

From 2015 to 2017, each of these sites was visited monthly to collect grab samples. In 2018, sampling efforts focused on capturing precipitation events, so the sampling was conducted at less regular intervals (i.e. not every month). A total of 27 sampling trips were conducted.

High density polyethylene (HDPE) bottles were used for nutrient collections. The bottles were rinsed three times with site water prior to sampling. Nitrile or latex gloves were worn by field personnel to avoid contamination of the samples during handling. Samples were stored on ice, in the dark while in the field, frozen at -20°C upon returning to the lab and not thawed until immediately prior to analysis. Samples were not filtered so that total nutrient levels could be analyzed, rather than only dissolved levels. Samples were analyzed for: nitrate, nitrite, ammonium, urea, total nitrogen, orthophosphate, total phosphorus and silica. During some sampling months (eight total), extra sample volume was collected (into amber glass vials) for analysis of caffeine and sucralose. These were sampled concurrently with the nutrient samples at the same sites.

### *2.2 Analytical Methods Used for the Analysis of Nutrients*

Nutrient laboratory analyses were performed by a NOAA contract lab (Geochemical and Environmental Research Group, Texas A&M University, sub-contract to TDI Brooks). Water samples were analyzed for a standard suite of nutrient analytes: nitrate ($NO_3^-$), nitrite ($NO_2^-$), orthophosphate ($HPO_4^=$), ammonium ($NH_4^+$), urea ($(NH_2)_2CO$), total nitrogen (TN), total phosphorus (TP) and silica.

Nitrate and nitrite analyses were based on the methodology of Armstrong et al (1967). Orthophosphate was measured using the methodology of Bernhardt and Wilhelms (1967) with the modification of hydrazine as reductant. Silicate determination was accomplished using the methods of Armstrong et al (1967) using stannous chloride. Ammonium analysis was based on the method of Harwood and Kuhn (1970) using dichloro-isocyanurate as the oxidizer. Urea was measured using diacetyl-monoximine and themicarbozide. The total concentrations of nitrogen and phosphorus were determined after an initial decomposition step. This method involves persulfate oxidation while heating the sample in an autoclave (115°C, 20 minutes) (Hansen and Koroleff 1999). After oxidation of the samples, nutrient determination was conducted on the Astoria Pacific analyzer for nitrate and orthophosphate.

### *2.3 Analytical Methods Used for the Analysis of Tracers*

Tracers (caffeine and sucralose) were quantified at Florida International University (sub-contract to TDI Brooks) using previously published methods (Wang 2012; Batchu et al 2015). These methods resulted in method detection limits (MDLs) of 1.1 and 12.1 ng/L, for caffeine and sucralose respectively. More detail on these methods is presented in the Supplemental Materials.

### *2.4 Method Detection Limits*

Method detection limits (MDL) for all analytes are shown in Table 1. Analytical values that were below the MDL were treated with the statistical methods described in Flynn (2010). Briefly, the dataset for each analyte was transformed using a natural log transform and the below MDL data was then fitted to the curve below the MDL cutoff using the following bootstrapping approach. The Shapiro Wilk W statistic was maximized using an iterative solving process, which results in an assigned "dummy" value for each occurrence below the detection limit, ranging from zero to the detection limit. This creates a dataset in which the data that are below the MDL have unique values with the same statistical distribution as the dataset as a whole and can therefore be analyzed statistically without biasing the data (e.g. without assigning all below MDL data to one half of the MDL value).

### *2.5 Statistical Analysis of Water Quality Data*

Because the datasets were not perfectly normal, even with transformation, non-parametric statistics were used to evaluate relationships within the dataset. A Wilcoxon test, with post-hoc Dunn's analysis was used to examine differences among strata. Spearman correlations were used to examine relationships between analytes. Summary statistics (e.g. mean, maximum, standard error) were also calculated for each site. Relevant statistical findings are discussed in the text below, and presented in tabular form. JMP statistical software was used for all statistical analysis.

Table 1: Method detection limits for the laboratories used in this study.

| Analyte | MDL |
|---|---|
| Silicate | 0.00196 mg-N/L |
| Nitrate | 0.00154 mg-N/L |
| Nitrite | 0.000168 mg-N/L |
| Ammonium | 0.000798 mg-N/L |
| Urea | 0.012 mg-N/L |
| Orthophosphate | 0.00035 mg-P/L |
| Total Nitrogen | 0.00154 mg-N/L |
| Total Phosphorus | 0.00035 mg-P/L |
| Sucralose | 12.1 ng/L |
| Caffeine | 1.10 ng/L |

## 3. Results and Discussion

All data and metadata from this study are available for download through NOAA's National Centers for Environmental Information (NCEI; https://data.nodc.noaa.gov/cgi-bin/iso?id=gov.noaa.nodc:0208020).

Table 2 shows the mean, maximum values, and standard error of the mean for each nutrient species for each site for both surface and bottom. There are significant differences among the strata for nitrate, silica and total phosphorus (Wilcoxon with post-hoc Dunn's test, $\alpha$=0.05), with silica and total phosphorus being highest in the Inner and Central strata and nitrate being highest in the South stratum. This suggests that groundwater, overland flow and the stream all contribute to the nutrient budget of the Bay.

In order to put the nutrient data from this study into a larger (regional) context, they were compared with water quality data (nitrate, ammonium) from a joint study by American Samoa Environmental Protection Agency (ASEPA) and Division of Marine and Wildlife Resources (sites described in Comeros-Raynal et al 2017; unpublished data) study which quantified water quality on the reef flat in twenty six drainage areas across the island of Tutuila, monthly in 2016 and 2017. These data provide valuable context for what was observed in Vatia (Figures 3 and 4). It should be noted that these twenty-six drainage areas included some more developed areas (e.g. Nu'uuli, Faga'alu) compared to Vatia, which is a relatively small village. Also included in this comparison were limited data that we collected in Tafeu, which is a small Bay in an uninhabited watershed on the north shore of the island. This site is useful as an unimpacted/pristine reference site. The overall means and medians (island wide) of the ASEPA/DMWR studies are similar to what was observed in this study in Vatia (Figures 3 and 4), with the exception of nitrite which is higher in Vatia. As expected, Tafeu has relatively low concentrations compared to Vatia.

Water quality standards for the territory of American Samoa have been enacted by USEPA/ASEPA (USEPA 2013). For embayments such as Vatia Bay, there are nutrient criteria for TP and TN, specifically that the median cannot exceed 0.02 mg/L and 0.15 mg/L respectively. Median values of TP and TN measured in this study (Table 3) indicate that all sites are in exceedance of these water quality standards for TN, and all sites except for SB11 (bottom) exceed the standard for TP. It should be noted that the median for SB11 (bottom) is 0.019 mg/L, which is just barely below the standard. Based on these water quality standards, in combination with observed benthic prevalence of algae, we conclude that Vatia Bay is under nutrient stress.

Table 2a: Summary statistics (maximum, mean, median, Interquartile Range (IQR)) by site for inorganic nitrogen species (nitrate, nitrite, ammonium) for all data across months and depths. All units are in mg-N/L. N=27.

| | **Nitrate** | | | | **Nitrite** | | | | **Ammonium** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Mean** | **Median** | **Max** | **IQR** | **Mean** | **Median** | **Max** | **IQR** | **Mean** | **Median** | **Max** | **IQR** |
| **Stream** | 0.104 | 0.075 | 0.524 | 0.057 | 0.030 | 0.015 | 0.287 | 0.018 | 0.024 | 0.009 | 0.101 | 0.036 |
| **IN1B** | 0.003 | 0.001 | 0.021 | 0.004 | 0.008 | 0.001 | 0.053 | 0.012 | 0.006 | 0.002 | 0.032 | 0.010 |
| **IN1S** | 0.010 | 0.004 | 0.056 | 0.012 | 0.012 | 0.002 | 0.063 | 0.022 | 0.006 | 0.002 | 0.019 | 0.011 |
| **IN2B** | 0.005 | 0.003 | 0.042 | 0.006 | 0.010 | 0.002 | 0.074 | 0.012 | 0.010 | 0.005 | 0.061 | 0.011 |
| **IN2S** | 0.008 | 0.005 | 0.040 | 0.011 | 0.009 | 0.002 | 0.060 | 0.013 | 0.009 | 0.002 | 0.053 | 0.008 |
| **IN3S** | 0.004 | 0.002 | 0.030 | 0.005 | 0.012 | 0.002 | 0.077 | 0.012 | 0.008 | 0.004 | 0.051 | 0.009 |
| **IN4S** | 0.008 | 0.002 | 0.067 | 0.009 | 0.013 | 0.002 | 0.092 | 0.015 | 0.008 | 0.005 | 0.047 | 0.011 |
| **NB17S** | 0.006 | 0.005 | 0.026 | 0.008 | 0.011 | 0.001 | 0.072 | 0.007 | 0.007 | 0.004 | 0.043 | 0.008 |
| **NB18B** | 0.009 | 0.007 | 0.062 | 0.009 | 0.009 | 0.002 | 0.060 | 0.016 | 0.008 | 0.006 | 0.041 | 0.007 |
| **NB18S** | 0.009 | 0.008 | 0.028 | 0.008 | 0.011 | 0.002 | 0.060 | 0.008 | 0.007 | 0.004 | 0.037 | 0.008 |
| **NB19S** | 0.007 | 0.002 | 0.048 | 0.008 | 0.010 | 0.001 | 0.057 | 0.009 | 0.008 | 0.006 | 0.043 | 0.011 |
| **NB20S** | 0.007 | 0.005 | 0.028 | 0.010 | 0.013 | 0.002 | 0.140 | 0.009 | 0.008 | 0.003 | 0.054 | 0.012 |
| **SB10S** | 0.004 | 0.002 | 0.021 | 0.003 | 0.010 | 0.002 | 0.064 | 0.008 | 0.011 | 0.005 | 0.054 | 0.015 |
| **SB11B** | 0.009 | 0.009 | 0.009 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.017 | 0.017 | 0.017 | 0.000 |
| **SB11S** | 0.018 | 0.002 | 0.266 | 0.006 | 0.011 | 0.002 | 0.067 | 0.015 | 0.010 | 0.004 | 0.054 | 0.010 |
| **SB12S** | 0.010 | 0.005 | 0.070 | 0.011 | 0.012 | 0.002 | 0.074 | 0.007 | 0.008 | 0.002 | 0.050 | 0.012 |
| **SB9B** | 0.004 | 0.003 | 0.017 | 0.005 | 0.010 | 0.002 | 0.068 | 0.007 | 0.007 | 0.002 | 0.036 | 0.013 |
| **SB9S** | 0.018 | 0.007 | 0.311 | 0.010 | 0.010 | 0.002 | 0.079 | 0.011 | 0.007 | 0.002 | 0.049 | 0.007 |
| **CB25B** | 0.008 | 0.000 | 0.116 | 0.004 | 0.011 | 0.001 | 0.070 | 0.011 | 0.009 | 0.004 | 0.048 | 0.012 |
| **CB25S** | 0.006 | 0.003 | 0.024 | 0.007 | 0.011 | 0.002 | 0.063 | 0.021 | 0.006 | 0.002 | 0.047 | 0.008 |
| **CB26B** | 0.015 | 0.001 | 0.311 | 0.006 | 0.008 | 0.001 | 0.051 | 0.011 | 0.006 | 0.001 | 0.054 | 0.011 |
| **CB26S** | 0.009 | 0.006 | 0.047 | 0.011 | 0.015 | 0.002 | 0.145 | 0.007 | 0.017 | 0.005 | 0.167 | 0.017 |
| **CB27B** | 0.006 | 0.002 | 0.045 | 0.005 | 0.010 | 0.001 | 0.074 | 0.009 | 0.007 | 0.002 | 0.058 | 0.008 |
| **CB27S** | 0.007 | 0.005 | 0.025 | 0.009 | 0.011 | 0.002 | 0.061 | 0.011 | 0.008 | 0.003 | 0.052 | 0.012 |
| **CB28B** | 0.009 | 0.001 | 0.136 | 0.004 | 0.011 | 0.001 | 0.062 | 0.016 | 0.006 | 0.002 | 0.040 | 0.007 |
| **CB28S** | 0.006 | 0.003 | 0.021 | 0.009 | 0.010 | 0.002 | 0.064 | 0.013 | 0.007 | 0.003 | 0.049 | 0.011 |

Both tracers of human waste (caffeine and sucralose) were detected in the Bay. Sucralose was detected in 51% of samples analyzed (97 out of 192) and caffeine was detected in 82% of the samples analyzed (157 out of 192). Concentrations of these tracers ranged from below limits of detection to 370 and 343 ng/L for sucralose and caffeine, respectively. Because human waste is the only potential source of these compounds, this definitively shows that human waste is reaching Vatia Bay. The higher occurrence of caffeine compared to sucralose is interesting because caffeine is actually less environmentally persistent than sucralose. This pattern could be

Table 2b: Summary statistics (maximum, mean, median, Interquartile Range (IQR)) by site for urea and total nitrogen. All units are in mg-N/L. N=27.

|  | **Urea** |  |  |  | **Total N** |  |  |  |
|---|---|---|---|---|---|---|---|---|
| **Site** | **Mean** | **Median** | **Max** | **IQR** | **Mean** | **Median** | **Max** | **IQR** |
| **Stream** | 0.013 | 0.006 | 0.048 | 0.011 | 0.297 | 0.261 | 0.638 | 0.220 |
| **IN1B** | 0.005 | 0.004 | 0.014 | 0.006 | 0.274 | 0.235 | 0.603 | 0.192 |
| **IN1S** | 0.006 | 0.006 | 0.015 | 0.006 | 0.256 | 0.247 | 0.482 | 0.227 |
| **IN2B** | 0.006 | 0.005 | 0.012 | 0.006 | 0.251 | 0.222 | 0.503 | 0.196 |
| **IN2S** | 0.006 | 0.006 | 0.020 | 0.005 | 0.266 | 0.224 | 0.529 | 0.226 |
| **IN3S** | 0.008 | 0.007 | 0.028 | 0.006 | 0.244 | 0.209 | 0.444 | 0.179 |
| **IN4S** | 0.006 | 0.005 | 0.012 | 0.006 | 0.254 | 0.225 | 0.574 | 0.219 |
| **NB17S** | 0.005 | 0.004 | 0.012 | 0.008 | 0.271 | 0.221 | 0.658 | 0.263 |
| **NB18B** | 0.005 | 0.004 | 0.014 | 0.006 | 0.264 | 0.242 | 0.473 | 0.213 |
| **NB18S** | 0.005 | 0.005 | 0.012 | 0.006 | 0.271 | 0.235 | 0.638 | 0.203 |
| **NB19S** | 0.005 | 0.004 | 0.015 | 0.004 | 0.284 | 0.261 | 0.611 | 0.234 |
| **NB20S** | 0.005 | 0.006 | 0.014 | 0.006 | 0.302 | 0.264 | 0.726 | 0.262 |
| **SB10S** | 0.006 | 0.006 | 0.022 | 0.006 | 0.293 | 0.234 | 0.898 | 0.266 |
| **SB11B** | 0.001 | 0.001 | 0.001 | 0.000 | 0.267 | 0.247 | 0.590 | 0.136 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **SB11S** | 0.006 | 0.006 | 0.019 | 0.006 | 0.251 | 0.213 | 0.587 | 0.176 |
| **SB12S** | 0.005 | 0.006 | 0.017 | 0.007 | 0.248 | 0.245 | 0.470 | 0.183 |
| **SB9B** | 0.005 | 0.005 | 0.018 | 0.006 | 0.247 | 0.215 | 0.522 | 0.172 |
| **SB9S** | 0.005 | 0.005 | 0.014 | 0.005 | 0.260 | 0.224 | 0.524 | 0.151 |
| **CB25B** | 0.006 | 0.006 | 0.018 | 0.006 | 0.235 | 0.196 | 0.540 | 0.118 |
| **CB25S** | 0.005 | 0.005 | 0.015 | 0.007 | 0.240 | 0.200 | 0.718 | 0.123 |
| **CB26B** | 0.005 | 0.005 | 0.015 | 0.007 | 0.386 | 0.386 | 0.386 | 0.000 |
| **CB26S** | 0.006 | 0.006 | 0.016 | 0.009 | 0.248 | 0.213 | 0.670 | 0.163 |
| **CB27B** | 0.005 | 0.006 | 0.012 | 0.007 | 0.287 | 0.229 | 1.399 | 0.149 |
| **CB27S** | 0.005 | 0.005 | 0.017 | 0.006 | 0.238 | 0.202 | 0.613 | 0.153 |
| **CB28B** | 0.005 | 0.004 | 0.015 | 0.007 | 0.253 | 0.203 | 0.622 | 0.242 |
| **CB28S** | 0.006 | 0.006 | 0.017 | 0.006 | 0.540 | 0.470 | 1.144 | 0.373 |

Table 2c: Summary statistics (maximum, mean, median, Interquartile Range (IQR)) by site for orthophosphate, total phosphorus and silica. All units are in mg-N/L. N=27.

| | **Orthophosphate** | | | | **Total P** | | | | **Silica** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Mean** | **Median** | **Max** | **IQR** | **Mean** | **Median** | **Max** | **IQR** | **Mean** | **Median** | **Max** | **IQR** |
| **Stream** | 0.099 | 0.066 | 0.365 | 0.035 | 0.163 | 0.109 | 0.585 | 0.136 | 20.861 | 20.788 | 33.949 | 11.068 |
| **IN1B** | 0.013 | 0.012 | 0.031 | 0.006 | 0.041 | 0.030 | 0.184 | 0.019 | 0.148 | 0.101 | 0.874 | 0.159 |
| **IN1S** | 0.017 | 0.015 | 0.027 | 0.011 | 0.046 | 0.030 | 0.192 | 0.015 | 0.992 | 0.546 | 4.358 | 0.956 |
| **IN2B** | 0.014 | 0.013 | 0.028 | 0.006 | 0.047 | 0.031 | 0.258 | 0.019 | 0.328 | 0.171 | 2.114 | 0.253 |
| **IN2S** | 0.016 | 0.015 | 0.042 | 0.008 | 0.045 | 0.030 | 0.226 | 0.015 | 0.980 | 0.371 | 7.370 | 0.895 |
| **IN3S** | 0.021 | 0.022 | 0.041 | 0.013 | 0.053 | 0.035 | 0.231 | 0.032 | 2.420 | 1.101 | 7.263 | 3.769 |
| **IN4S** | 0.013 | 0.013 | 0.045 | 0.007 | 0.039 | 0.029 | 0.191 | 0.010 | 1.568 | 1.019 | 8.521 | 1.519 |
| **NB17S** | 0.012 | 0.013 | 0.020 | 0.006 | 0.036 | 0.024 | 0.197 | 0.014 | 0.167 | 0.127 | 0.706 | 0.110 |
| **NB18B** | 0.014 | 0.013 | 0.026 | 0.008 | 0.032 | 0.028 | 0.077 | 0.016 | 0.232 | 0.175 | 0.784 | 0.277 |
| **NB18S** | 0.017 | 0.016 | 0.042 | 0.008 | 0.029 | 0.028 | 0.050 | 0.009 | 0.560 | 0.294 | 2.486 | 0.884 |
| **NB19S** | 0.012 | 0.013 | 0.024 | 0.010 | 0.030 | 0.026 | 0.081 | 0.012 | 1.152 | 0.830 | 6.002 | 1.244 |
| **NB20S** | 0.015 | 0.012 | 0.036 | 0.009 | 0.031 | 0.028 | 0.080 | 0.012 | 0.182 | 0.139 | 0.640 | 0.232 |
| **SB10S** | 0.015 | 0.014 | 0.023 | 0.009 | 0.029 | 0.028 | 0.063 | 0.017 | 0.423 | 0.114 | 6.613 | 0.171 |
| **SB11B** | 0.014 | 0.014 | 0.014 | 0.000 | 0.019 | 0.019 | 0.019 | 0.000 | 0.368 | 0.368 | 0.368 | 0.000 |
| **SB11S** | 0.017 | 0.015 | 0.052 | 0.006 | 0.031 | 0.026 | 0.075 | 0.016 | 1.464 | 0.371 | 14.286 | 1.007 |
| **SB12S** | 0.015 | 0.014 | 0.048 | 0.008 | 0.031 | 0.027 | 0.079 | 0.012 | 0.145 | 0.088 | 0.762 | 0.122 |
| **SB9B** | 0.012 | 0.012 | 0.024 | 0.009 | 0.029 | 0.024 | 0.063 | 0.014 | 0.129 | 0.079 | 0.624 | 0.078 |
| **SB9S** | 0.016 | 0.015 | 0.029 | 0.010 | 0.030 | 0.030 | 0.055 | 0.017 | 0.589 | 0.123 | 4.844 | 0.494 |
| **CB25B** | 0.015 | 0.013 | 0.041 | 0.009 | 0.037 | 0.030 | 0.081 | 0.026 | 0.217 | 0.133 | 1.496 | 0.179 |
| **CB25S** | 0.016 | 0.014 | 0.040 | 0.010 | 0.037 | 0.030 | 0.092 | 0.016 | 0.888 | 0.503 | 5.305 | 0.562 |
| **CB26B** | 0.014 | 0.012 | 0.028 | 0.009 | 0.033 | 0.032 | 0.087 | 0.018 | 0.124 | 0.070 | 0.660 | 0.117 |
| **CB26S** | 0.016 | 0.014 | 0.041 | 0.007 | 0.034 | 0.033 | 0.063 | 0.018 | 0.810 | 0.297 | 7.623 | 0.767 |
| **CB27B** | 0.014 | 0.013 | 0.030 | 0.006 | 0.033 | 0.028 | 0.123 | 0.015 | 0.091 | 0.070 | 0.413 | 0.112 |
| **CB27S** | 0.014 | 0.013 | 0.037 | 0.006 | 0.036 | 0.027 | 0.134 | 0.019 | 0.378 | 0.118 | 2.642 | 0.399 |
| **CB28B** | 0.012 | 0.011 | 0.027 | 0.007 | 0.040 | 0.029 | 0.207 | 0.020 | 0.093 | 0.050 | 0.522 | 0.103 |
| **CB28S** | 0.014 | 0.014 | 0.031 | 0.009 | 0.036 | 0.028 | 0.193 | 0.016 | 0.456 | 0.245 | 1.647 | 0.658 |

due to higher usage of caffeine by residents of the village, as it is in more food products, and products that may be consumed in larger quantities, than sucralose, or may be related to differences in analytical MDL between the two compounds; the MDL for caffeine is an order of magnitude lower than for sucralose.

There were no statistically significant differences (Wilcoxon with post-hoc Dunn's test, $\alpha=0.05$) between strata for sucralose and caffeine concentrations. While not included in the statistical analysis (because the stream is a targeted site, not part of the stratified random design), concentrations of these tracers were generally higher in the stream than in the Bay. Although dilution is obviously occurring as stream water reaches the Bay, elevated stream concentrations suggests that watershed sources play a role in tracer flux, and therefore indicative of sewage related inputs.

Tracer concentrations from eight unique sampling dates were significantly correlated with multiple nutrient analyte concentrations (Table 4), which consistent with correlations reported in other systems by other researchers (e.g. Oppenheimer et al 2011). Spearman rho coefficients were generally below 0.40 (indicating relatively weak relationships), with the exception of sucralose and urea, which were slightly more strongly correlated in the North and South strata (rho=0.47 and 0.55, respectively). This would be consistent with leaking septic systems (or complete lack of sewage treatment) contributing a flux of nutrients (as urea, a primary component of human waste) via groundwater or direct overland flow. Unfortunately, there were not enough stream data points to statistically assess the correlations in the stream itself. Scatterplots of the tracers versus individual nutrients are shown in Figure 5.

Table 3: Median bottom water values for Vatia Bay. Territorial water quality standards are 0.15 mg N/L total N and 0.02 mg P/L total P. All sites exceeded the standard for TN and only one site (SB11) did not exceed the standard for TP (highlighted in bold italics).

| Site Name | Median TN (mg N/L) | Median TP (mg P/L) |
|---|---|---|
| **CB25** | 0.261 | 0.030 |
| **CB26** | 0.247 | 0.032 |
| **CB27** | 0.224 | 0.028 |
| **CB28** | 0.225 | 0.029 |
| **IN1** | 0.242 | 0.030 |
| **IN2** | 0.261 | 0.031 |
| **NB18** | 0.245 | 0.028 |
| **SB11** | 0.386 | ***0.019*** |
| **SB9** | 0.202 | 0.024 |

suggest that this same relationship with nutrients may be present in the stream, especially for sucralose. Interestingly, in the Bay itself, both sucralose and caffeine actually had statistically significant negative relationships with total nitrogen, total phosphorus and nitrate. This could suggest that biological processing (e.g. conversion of urea to ammonium to nitrate (Pajares and Ramos, 2019), or uptake by benthic algae) effectively decouples the concentrations of TN, TP and nitrate in sewage from the tracers. Stream scatter plots may support this hypothesis, as there appear to be relationships between tracers and nutrients (e.g. TN and TP with sucralose, Figure 5) in the stream that are not present in the Bay. Additional tracer and nutrient data from the stream would be useful to better understand this relationship. These correlations demonstrate that human waste is an important, and perhaps the dominant, source of nitrogen to the nutrient budget of Bay.

Because caffeine and sucralose are not ubiquitous in the human diet, it is possible that observed spatial differences in tracer concentrations may be attributable to dietary differences between households of the village, rather than differences in sewage flux. However, because no significant differences among strata for tracers were observed, this is probably unlikely. While natural/plant based sources of caffeine do exist (wild growing coffee or cacao plants), these species are not present in the Vatia watershed (Ian Gurr, horticulturalist, American Samoa Community College, personal communication).

Table 4: Spearman correlation coefficients for tracers (caffeine and sucralose) vs water quality concentrations by stratum. Negative Spearman ρ values indicate negative relationships. Italics indicate statistically significant relationships (α=0.05)

| Strata | Tracer | Nutrient | Spearman ρ | Prob>\|ρ\| |
|---|---|---|---|---|
| Central | *Caffeine* | *Ammonium* | *0.282* | *0.024* |
| Central | *Caffeine* | *Nitrate* | *-0.333* | *0.007* |
| Central | *Caffeine* | *Nitrite* | *0.248* | *0.049* |
| Central | Caffeine | Orthophosphate | 0.115 | 0.367 |
| Central | Caffeine | Silica | -0.078 | 0.540 |
| Central | Caffeine | Total Nitrogen | -0.071 | 0.579 |
| Central | Caffeine | Total Phosphorus | 0.107 | 0.398 |
| Central | *Caffeine* | *Urea* | *0.354* | *0.004* |
| Central | Sucralose | Ammonium | -0.055 | 0.664 |
| Central | Sucralose | NItrate | 0.017 | 0.892 |
| Central | Sucralose | Nitrite | 0.197 | 0.118 |
| Central | *Sucralose* | *Orthophosphate* | *0.382* | *0.002* |
| Central | Sucralose | Silica | 0.207 | 0.100 |
| Central | *Sucralose* | *Total Nitrogen* | *-0.514* | *0.000* |
| Central | Sucralose | Total Phosphorus | 0.153 | 0.228 |
| Central | *Sucralose* | *Urea* | *0.263* | *0.036* |
| Inner | Caffeine | Ammonium | -0.039 | 0.798 |
| Inner | Caffeine | Nitrate | -0.098 | 0.516 |
| Inner | Caffeine | Nitrite | 0.097 | 0.521 |
| Inner | *Caffeine* | *Orthophosphate* | *0.293* | *0.048* |
| Inner | Caffeine | Silica | -0.107 | 0.477 |
| Inner | Caffeine | Total Nitrogen | -0.150 | 0.320 |
| Inner | Caffeine | Total Phosphorus | -0.003 | 0.984 |

| | | | | |
|---|---|---|---|---|
| **Inner** | **Caffeine** | **Urea** | 0.144 | 0.340 |
| **Inner** | **Sucralose** | **Ammonium** | -0.161 | 0.284 |
| **Inner** | **Sucralose** | **Nitrate** | 0.050 | 0.741 |
| **Inner** | **Sucralose** | **Nitrite** | 0.025 | 0.866 |
| **Inner** | ***Sucralose*** | ***Orthophosphate*** | *0.335* | *0.023* |
| **Inner** | **Sucralose** | **Silica** | -0.052 | 0.731 |
| **Inner** | ***Sucralose*** | ***Total Nitrogen*** | *-0.348* | *0.018* |
| **Inner** | **Sucralose** | **Total Phosphorus** | -0.135 | 0.370 |
| **Inner** | ***Sucralose*** | ***Urea*** | *0.327* | *0.027* |
| **North** | **Caffeine** | **Ammonium** | 0.282 | 0.091 |
| **North** | ***Caffeine*** | ***Nitrate*** | *-0.513* | *0.001* |
| **North** | **Caffeine** | **Nitrite** | -0.044 | 0.794 |
| **North** | **Caffeine** | **Orthophosphate** | 0.309 | 0.063 |
| **North** | **Caffeine** | **Silica** | 0.073 | 0.669 |
| **North** | ***Caffeine*** | ***Total Nitrogen*** | *-0.600* | *0.000* |
| **North** | **Caffeine** | **Total Phosphorus** | -0.173 | 0.304 |
| **North** | **Caffeine** | **Urea** | 0.018 | 0.913 |
| **North** | **Sucralose** | **Ammonium** | -0.064 | 0.706 |
| **North** | ***Sucralose*** | ***Nitrate*** | *-0.492* | *0.002* |
| **North** | **Sucralose** | **Nitrite** | 0.076 | 0.655 |
| **North** | **Sucralose** | **Orthophosphate** | 0.153 | 0.366 |
| **North** | ***Sucralose*** | ***Silica*** | *0.393* | *0.016* |
| **North** | ***Sucralose*** | ***Total Nitrogen*** | *-0.403* | *0.013* |
| **North** | **Sucralose** | **Total Phosphorus** | -0.175 | 0.301 |
| **North** | ***Sucralose*** | ***Urea*** | *0.470* | *0.003* |
| **South** | **Sucralose** | **Ammonium** | -0.228 | 0.158 |
| **South** | **Sucralose** | **Nitrate** | 0.005 | 0.976 |
| **South** | **Sucralose** | **Nitrite** | 0.019 | 0.910 |
| **South** | **Sucralose** | **Orthophosphate** | -0.071 | 0.663 |
| **South** | **Sucralose** | **Silica** | -0.181 | 0.265 |
| **South** | ***Sucralose*** | ***Total Nitrogen*** | *-0.399* | *0.011* |
| **South** | ***Sucralose*** | ***Total Phosphorus*** | *-0.429* | *0.006* |
| **South** | ***Sucralose*** | ***Urea*** | *0.549* | *0.000* |

## 4. Conclusions

This study articulated the nutrient status of Vatia Bay. Results showed that while the magnitude of nutrient pollution is similar to the coastal waters around the island, it exceeds the territorial water quality standards for embayments (total nitrogen and total phosphorus). While coral reefs in Vatia are almost certainly exposed to multiple stressors (nutrients, sedimentation, temperature, fishing, etc.), the data in this paper provide strong evidence that nutrient pollution is a problem in Vatia Bay. Furthermore, the use of sucralose and caffeine as tracers have definitively established that human waste is reaching the Bay. This information is critical to coastal managers in making decisions about remediation activities and best management practices.

Local management agencies selected the Vatia watershed as a priority site for conservation efforts (NOAA CRCP 2012). Proposed strategies to reduce pollution include improving on-site sewage disposal systems, and preventing future degradation through watershed and land-use planning. Environmental data, such as the dataset presented here, serve as a baseline of current conditions, which are needed to determine the efficacy of management efforts, i.e. measuring change over time. These data can be utilized by coastal managers to best prioritize management strategies in a way to maximize success in decreasing stressors on coral reef ecosystems.

## 5. Acknowledgments

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Figure 1: Location of Vatia Bay. Inset shows location of American Samoa.

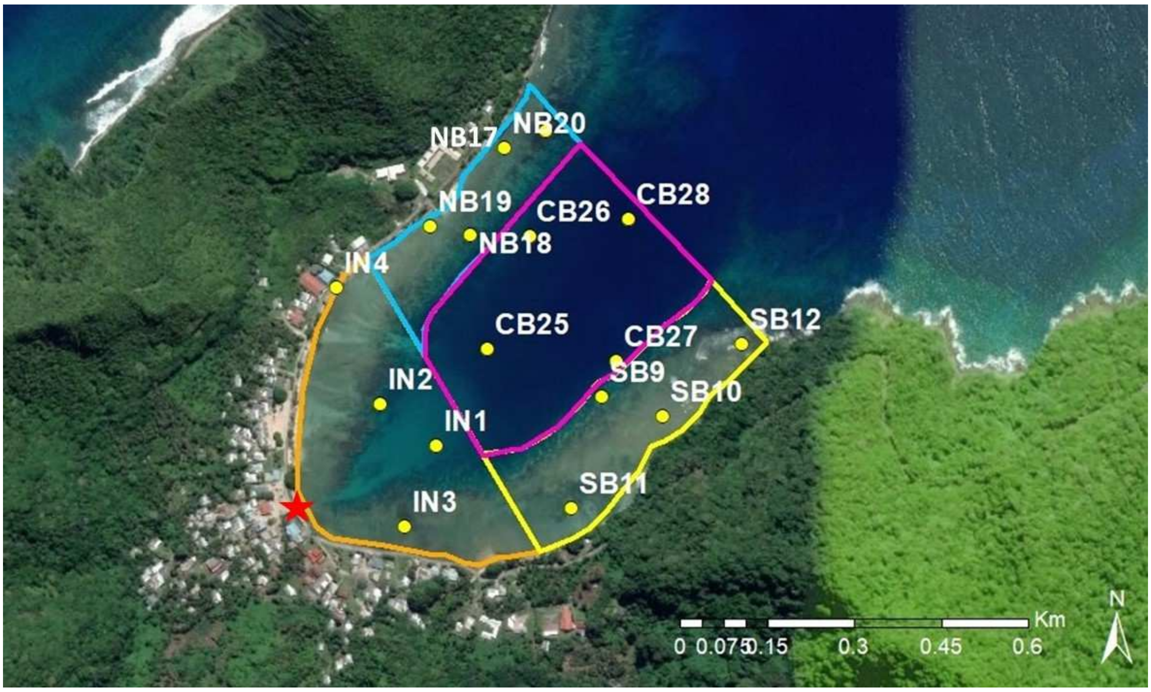

Figure 2: Map of water quality sites (yellow dots) selected via stratified random sampling design. Red star shows location of targeted stream site. Colored lines depict strata (orange=inner; yellow=south; blue=north; purple=central).

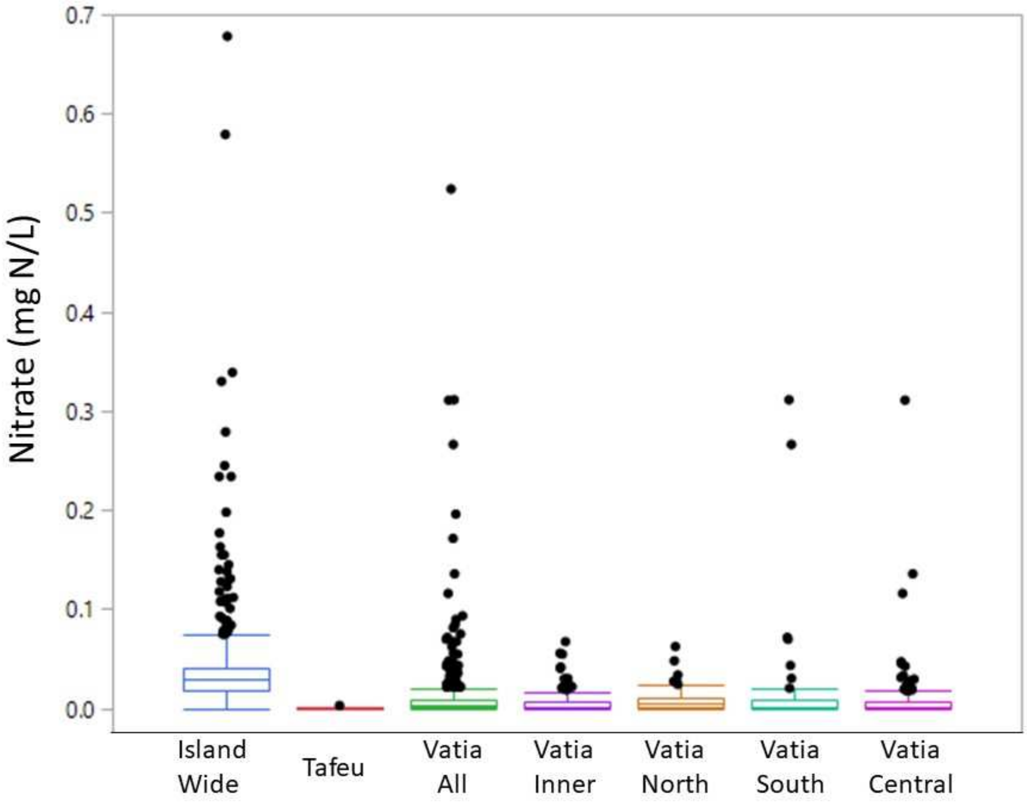

Figure 3: Comparison of ammonium data from this study (by strata, all months and depths, “All Vatia” includes stream data), limited NOAA data collected in a pristine Bay (Tafeu) on the north side of the island, and an island wide assessment of water quality (reef flat data from Comeros-Raynall et al. 2017; Island Wide Mean).

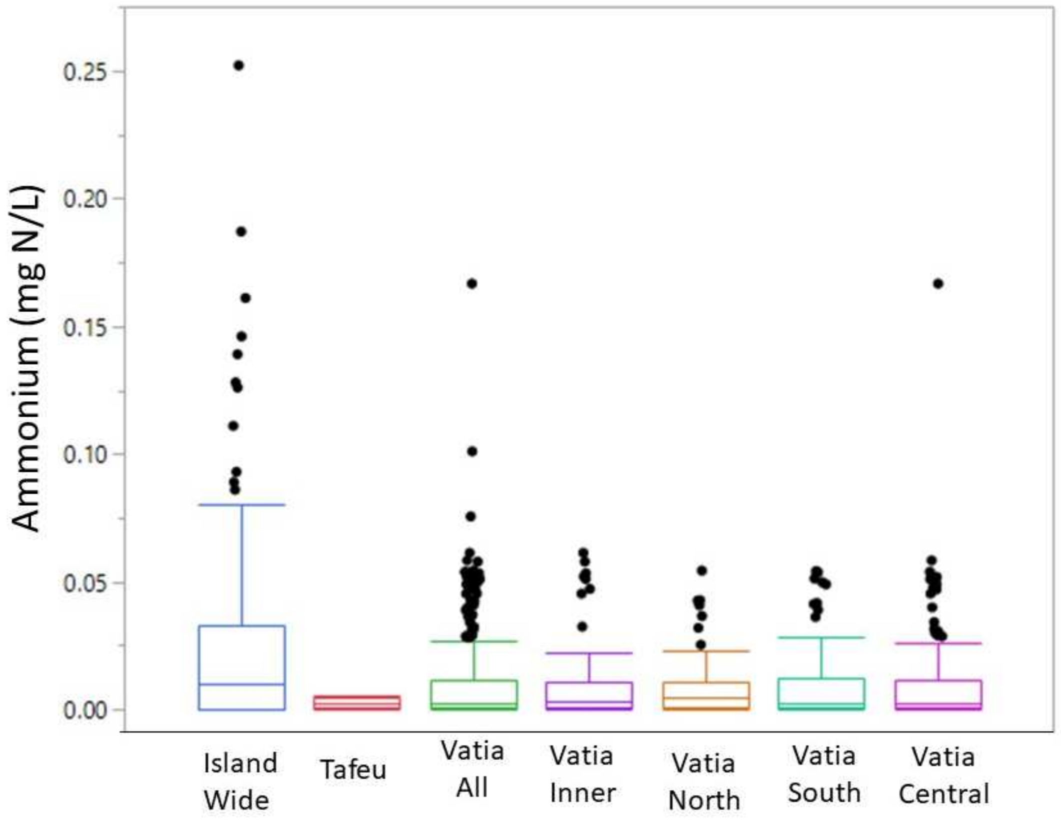

Figure 4: Comparison of ammonium data from this study (by strata, all months and depths, "All Vatia" includes stream data), limited NOAA data collected in a pristine Bay (Tafeu) on the north side of the island, and an island wide assessment of water quality (reef flat data from Comeros-Raynall et al. 2017; Island Wide Mean).

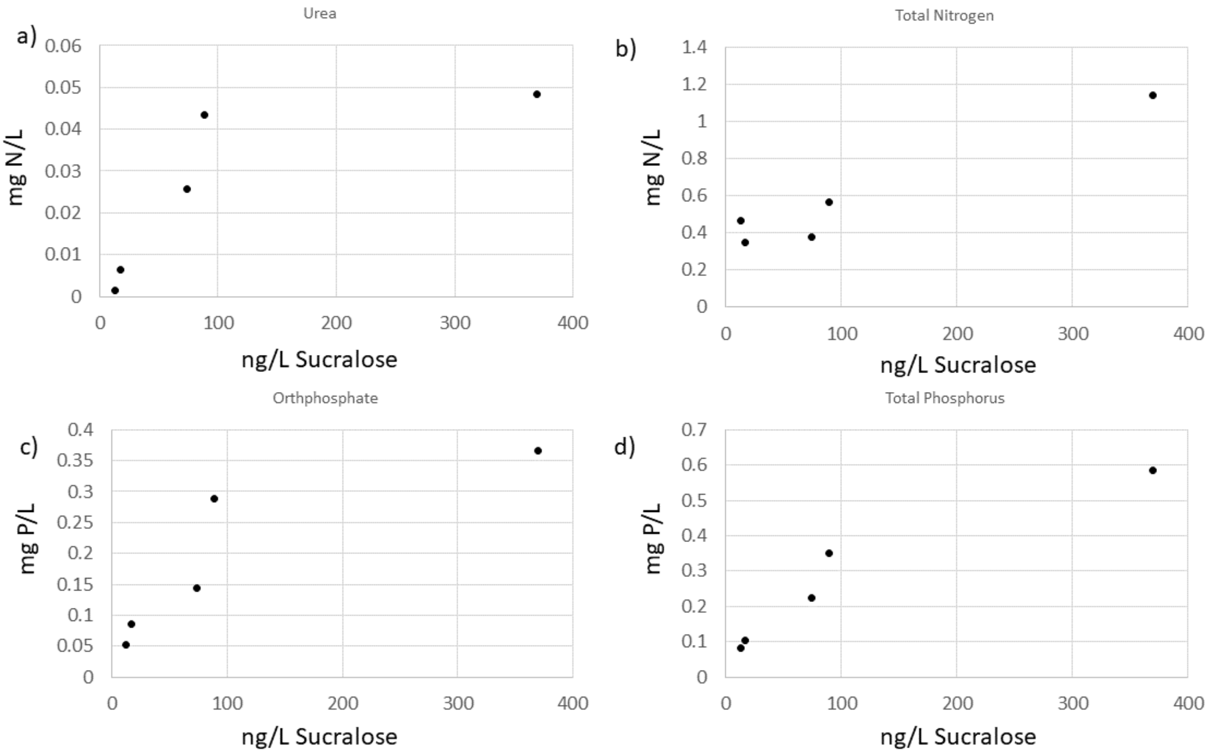

Figure 5: Scatter plots of sucralose concentrations vs urea (panel a), total nitrogen (panel b), orthophosphate (panel c) and total phosphorus (panel d) in the stream in Vatia.