

**2020 INTEGRATED REPORT
GUAM EPA**

APPENDIX A

**Marine and Surface Water
Monitoring and Assessment Methodology
2018 and 2019 Data**

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The Guam Environmental Protection Agency (Guam EPA) is responsible for monitoring, assessing and protecting the water quality for the Island of Guam. Guam laws and regulations that support this include the *Guam Environmental Protection Agency Act* (10 GCA¹, Chapter 45) Guam EPA's enabling legislation; the *Water Resources Conservation Act* (10 GCA, Chapter 46) which requires identification of Guam's significant water resources and the necessary planning, regulation and management of these resources for their protection, conservation and rational development; and the *Guam Water Pollution Control Act* (10 GCA, Chapter 47) which authorizes among other powers and duties, the Agency to study, investigate, and determine practical ways and means of eliminating from all ground and surface waters of the territory, all substances and materials which pollute the same; determining practical methods of pollution prevention detrimental to the public health or the health of animals.

The federal Clean Water Act (CWA) Section 305(b) requires that Guam submit a biennial water quality inventory report in April of even numbered years that reports the extent to which state waters provide for water resources that are swimmable, fishable, supports aquatic life and contain organisms that are consumable. CWA Section 303(d) additionally requires that Guam list impaired waters (that are deemed not swimmable, fishable, not supporting of aquatic life and contain organisms that are not suitable for human health) and to provide a priority ranking for the development and implementation of management strategies (e.g. TMDLs) that will reduce pollutant load(s) to the impaired waters. EPA specifies the process for development of both sections². EPA Guidance recommends that Guam submit integrated reports to satisfy 303(d) and 305(b) requirements³.

I. Monitoring Program

Guam EPA implements the *Comprehensive Monitoring Strategy for the Island of Guam* (CMS) (Guam EPA, 2006) in order to meet local and federal requirements. The Strategy is directed at the systematic monitoring and assessment of water resources for comparison to adopted Guam Water Quality Standards in order to determine prevailing conditions and water quality trends of that resource.

Existing Guam EPA Monitoring Projects

The CMS describes ten distinct monitoring projects intended to streamline monitoring and fulfill federal and local reporting requirements. *Table 1* (on the following page) shows the status of these ten proposed projects and describes what activities, if any, have been accomplished over the 2018 and 2019 monitoring period and what is planned for near future monitoring efforts.

¹ Guam Code Annotated

² EPA regulations 40 CFR 130.7 and 40 CFR 130.8).

³ December 22, 2017, Memorandum from John Goodin, EPA Office of Wetlands, Oceans and Watershed, Re: Information concerning 2018 Clean Water Act Sections 303(d), 305(b) and 314 Integrated Reporting and Listing Decisions. <http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/guidance.cfm>.

Table 1. Guam EPA Monitoring Projects

<u>Project</u>	<u>Status (Active or Suspended)</u>	<u>Status type</u>	<u>Information included in this report?</u>
Status and Trends Monitoring Program	Suspended	Field work suspended due to lack of funding.	No
Guam Environmental Monitoring and Assessment Program (GEMAP) (aka US EPA's National Aquatic Resource Survey-NARS)	Active	<ul style="list-style-type: none"> • Data gathering of the 2015 National Coastal Condition Assessment (NCCA) (Data assessment and reporting target completion during next cycle). • Planning for the 2020 NCCA. • Researching for the 2021 National Wetland Condition Assessment (NWCA). 	No
Recreational Beach Monitoring Program	Active	Weekly beach surface water sampling for enterococci.	Yes
Wetlands Monitoring Program	Suspended	Pending implementation of <i>Wetlands Monitoring project plan</i> .	No
Fish and Shellfish Consumption Monitoring Program	Suspended	Pending inclusion to CMS.	No
Groundwater Assessment Monitoring Plan	Active	Pending data submission from WRMP	No
Non-point Source Pollution Monitoring Plan	Active	Pending data submission WPC	No
Underground Injection Control Monitoring Plan	Active	Pending data submission WRMP	No
Man-Made Impoundment Monitoring Plan	Suspended	No lab capacity to sustain program.	No
Marine Preserve Water Quality Assessment Program	Active	Pending reporting data from 2015 <i>Reef flat</i> GEMAP/NCCA.	No

Guam EPA frequently partners with other organizations in monitoring and restoration projects. During this IR cycle, Guam EPA partnered with NOAA in a baseline study of water quality at the Manell Watershed. The study (see Attachment 2.) was conducted by NOAA National Centers for Coastal Ocean Science, Stressor Detection and Impacts Division (*Measurement of Turbidity, Suspended*

Sediments and Nutrients in Three Rivers that Drain to the Achang Preserve from the Manell Watershed, Guam December 2019. <https://doi.org/10.25923/v587-3f08>). This study involved continuous and grab sampling at three rivers in the watershed and one river in the nearby Geus Watershed during 2017 through 2018. Results of the monitoring effort are included in this cycle and allow for an aquatic life use-support determination at four rivers, the Sumay River, As Liyog River, the Ajayan River and the Geus River.

Future Guam EPA Monitoring Efforts

Guam Environmental Monitoring and Assessment Program (GEMAP) Project/NARS

Guam EPA intends to expand the GEMAP to include probability-based surveys on wetlands resources. Furthermore, the Agency intends to streamline efforts to mirror USEPA’s NARS schedule. The table below provides a proposed 4-year timeline that will accomplish implementation goals for each resource while mirroring national efforts.

GEMAP/NARS PROJECT: Timeline & Aquatic Resource Goals

Aquatic Resource	2018	2019	2020	2021
Wadeable Rivers & Streams	Report	Report	Research	Design
Coastal	Report	Design	Field	Lab
Wetlands	Research	Research	Design	Field

GEMAP/NARS surveys will follow the most current nationally adopted design, field, laboratory, data assessment methods and Quality Assurance Project Plans.

Impaired Waters Monitoring (IWM) Project:

303(d) Listed Waters on the current 303(d) impaired waters list will be targeted for assessment starting in the next IR cycle. Table 2 identifies these target waterbodies and the general information considered in new monitoring efforts. The project intends to support decision making relative to an impaired waterbody’s sustained 303(d) listing or delisting.

Table 2. Targets: 303(d) Listed Waters

Watershed / Waterbody	Matrix	Assessment	Rank	Project proposal
Storm drain	Surface water/runoff	Bacteria (E. coli)	Medium	Phase I complete Attached Report Attachment 1 in Sub-Appendices: Guam Stormwater Recommended Best Management Practices Technical Report Island-wide Guam. Phase II TBD during next cycle.
Tumon Bay (Faifai and Gun Beach)	<i>whole fish tissue (reef + pelagic; freshwater)</i>	Dieldrin (<i>found previous in spmds</i>)	High	Title: TMDL Development for the Guam Tumon Bay TMDL (POC Dave Guiliano, USEPA R9, USEPA RFQ No. 68HE0920Q0003
Tumon Bay (Faifai and Gun Beach)	<i>whole fish tissue (reef + pelagic)</i>	Total Chlordane (<i>found previous in spmds</i>)	High	
Tumon Bay	Marine water	Tetrachloroethene (PCE)	High	
Tumon Bay	Marine water	Trichloroethylene (TCE)	High	
Tumon Bay	Marine water	Antimony, Arsenic	High	
Waterbody Assessments on-going by different entity + Impairment being assessed and mitigated by DOD Δ Impairment may be mitigated under Ordot Dump Consent Decree:				
Agana River 1	Surface water	PCBs in fish tissue	Low +	
Agana River 2	<i>whole fish tissue and other orgs (freshwater)</i>	PCBs in fish tissue	Low +	
Agana Swamp	<i>whole fish tissue and other orgs (freshwater)</i>	PCBs in fish tissue	Low +	
Apra Harbor 1, Apra Harbor 2, Gabgab Beach, N Orote, S Orote, Tipalao Bay, Agat Bay 1, Cocos Lagoon 1, Cocos Lagoon 2	whole fish tissue (reef + pelagic)	PCBs in fish tissue	Low +	
Agat Bay 1	whole fish tissue (reef + pelagic)	Chlordane in fish tissue	Low +	

Watershed / Waterbody	Matrix	Assessment	Rank	Project proposal
Agat Bay 1	whole fish tissue (reef + pelagic)	Dioxin in fish tissue	Low +	
Lonfit River 2, Lonfit River 3 (+upper and lower site)	Surface water	Trace Metals in water (Al, Fe, Mn, Cu, Zn, Cr, Ni)	Low Δ	
Lonfit River 2, Lonfit River 3 (+upper and lower site)	Surface water	Salinity, Water Temp, Nitrate, Ammonia, Total Coliform, E. coli, Enterococci	Low Δ	
Waterbody Assessments targeted for future IR cycles				
Storm drain	Surface water/runoff	E. coli, Dissolved Oxygen, Nitrates, TSS, Turbidity, Salinity	Medium	May be addressed during future phases (see BMP project above).
Pago Bay	Surface water	Enterococci, Dissolved oxygen, Nitrate	Medium	
Pago River 1	Surface water	E. coli	Medium	
Pago River 2	Surface water	E. coli, Dissolved Oxygen	Medium	
Agana River 1	Surface water	Enterococcus, Dissolved oxygen	Low	
Tanguisson Beach Area 2; Further studies are needed to ascertain whether or not the toxin is being accumulated and concentrated in the fish.	Gracilaria tsudae + Epiphytic cyanobacteria (blue-green algae)	Toxic seaweed substance (http://guampedia.com/seaweed-gracilaria/)	Low	

Spatial and temporal requirements for sampling and field, laboratory, Quality Assurance/Quality Control, and data assessment methods of proposed monitoring efforts will be presented in upcoming scope of work(s) and in work plans for the 2019-2023 grant cycle. These requirements are critical in making decisions to continue listing or delisting impaired waterbodies.

Impaired Waters with EPA Approved TMDLs - TMDL Implementation Project: Forty-two Guam recreational beach sites and six Ugum River segments, reported as Category 4a marine or surface waters, have an approved or established TMDL to address a specific impairment. These impaired waters will continue to be the target of respective TMDL implementation projects. During the upcoming cycle, microbial source tracking for 'poor' beaches will be conducted.

II. Assessment Methodology

As recommended in the IR Guidance (2006)⁴, this section describes the method Guam uses to determine the water quality attainment status of all waters. Specifically, this section describes the following:

1. Changes in assessment methodology since the last reporting cycle.
2. What data were used to make attainment determinations (e.g. site specific and probability-based survey)
3. How the data and information were used to make attainment determinations and assign the five-part categorization of marine and surface waters.

Guam Marine Waters	Categories of Water (GWQS 2017)		
	Designated-use	M-1 Excellent	M-2 Good
aesthetic enjoyment	X	X	X
aquatic life preservation	X		
aquatic life propagation		X	
aquatic life protection	X		X
aquatic life survival		X	
commercial and industrial use			X
consumption of organisms		X	
contact recreation: limited body			X
contact recreation: whole body	X	X	
industrial cooling waters			X
mariculture activities		X	
marine scientific research	X		
shipping, boating, berthing, marinas			X

Guam Surface Waters	Categories of Water (GWQS 2017)		
	Designated-use	S-1 High	S-2 Medium
aesthetic enjoyment	X	X	X
aquatic life maintenance			X
aquatic life preservation	X	X	
aquatic life propagation	X	X	
consumption as drinking water	X		
consumption as drinking water (after treatment)		X	
contact recreation: limited body			X
contact recreation: whole body	X	X	

⁴ Guidance for 2006 Assessment, Listing and Reporting Requirement Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act: United States Environmental Protection Agency, (July 29, 2005)
<http://water.epa.gov/lawsregs/lawguidance/cwa/tmdl/2006IRG>

Water quality attainment determinations are guided by the 2017 Revised Guam Water Quality Standards (GWQS) which provide narrative and numeric standards applicable to specific “Categories of Waters” (S-1, S-2, S-3, M-1, M-2 and M-3 classification). These categories of waters have defined designated-uses as follows. Generally, these designated-uses are evaluated using the indicators in *Table 3*.

Table 3. Guam Designated-Uses and Indicators (GWQS 2017) for Use Support determination

Designated USE:	Body Contact (primary/whole body, secondary /limited)	Aquatic Life (Preserve, Propagate, Protect, Survival, Maintenance)	Human Health Consumption (Toxics)
GWQS Indicators:	E. coli Enterococci Fecal coliform - shellfish waters	Water Quality: pH Orthophosphates OPO4 Nitrate NO3 Ammonia NH4 Dissolved Oxygen Salinity Chlorides Sulfates SO4 Total Dissolved Solids Total Suspended Solids Turbidity Secchi Disc Visibility Water Temperature Radioactive Materials Conc. of Oil/Petroleum Product Biological/Benthic Assessment Toxicants (Water column and Sediment)	Drinking Water (S-1, S-2) Organisms (and S1 water)

Each indicator listed above is subject to established criteria summarized in the next table (*Table 4*) taken from the 2017 GWQS. Further assessment of Use Support involves determining to what degree these indicators support designated uses.

Table 4. Criteria (GWQS 2017) used to determine Degree of Use Support:

PARAMETERS		GWQS	
Marine Water	Surface Water	M1/S1	M2/S2
Enterococci (applied in 2018)			M3/S3
		MARINE and FRESHWATER: Concentrations of enterococci bacteria shall not exceed 35cfu/100mL based upon the geometric mean of samples taken in a 30-day interval AND the Statistical Threshold Value (STV) of 130cfu/100mL should not be exceeded by more than 10 percent of the samples taken during the same 30-day interval.	
-	E. coli (applied in 2018)		
		FRESHWATER ONLY: Concentrations of E. coli shall be no greater than 126cfu/100mL based upon the geometric mean of samples taken in a 30-day interval AND the Statistical Threshold Value (STV) of 410cfu/100mL should not be exceeded by more than 10 percent of the samples taken during the same 30-day interval.	
Fecal coliform (shellfish harvesting & growing areas)		Median of 14 fecal coliform/100mL and 10% of water samples taken from growing area should not exceed 43 fecal coliform/100mL.	
pH		Marine, Estuarine: 6.5 - 8.5 range (also, in deeper than euphotic zones, not >0.2pH from ambient) Freshwater: 6.5 - 9.0	
Ortho-phosphate (PO4-P)		not > 0.025 mg/L	not > 0.05 mg/L
Nitrate-nitrogen (NO3-N)		not > 0.10 mg/L	not > 0.20 mg/L
Ammonia-nitrogen		MARINE (M-1, M-2, M-3): 0.02 mg/L (table IV GWQS); FRESHWATER (S-1,S-2,S-3): 1hour average conc. not > CMC more than once every 3 years AND 30day average conc. not > CCC more than once every 3 years AND the average conc. over 30days not > CCC AND ambient conc. averaged over 4days not > 2xCCC.	
Dissolved Oxygen		Not decreased to < 75% saturation at any time [OR at 30degC Fresh water not < 5.6 mg/L; Marine and Wetlands Water not < 4.6 mg/L at 26degC Fresh water not < 6.2 mg/L; Marine and Wetlands Water not < 5.0 mg/L]	
Salinity	Salinity/Chlorides/Sulfates	Marine, estuarine, wetlands: not > +10% of ambient Freshwater only: max Cl and SO4 = 250 mg/L; TDS not > 500 mg/L or 133% of ambient; Salinity not > +20% of ambient.	
	Total Dissolved Solids	TSS: not increased from ambient and not > 5 mg/L	TSS: not > +10% ambient and not > 20 mg/L
	Residue (TSS)	TSS: not > +25% ambient and not > 40 mg/L	
	Turbidity	not > 1.0 NTU over ambient (except when due to natural conditions)	
Secchi Visibility (Vertical or Horizontal)		not < 5m from ambient (except when due to natural conditions)	
Water Temperature		not changed more than 1.0°C or 1.8°F from ambient (Thermal effluent not meeting this standard shall be considered as having an adverse effect on aquatic life).	
Radioactive Materials		Discharges at any level into any waters are strictly prohibited.	
Oil or Petroleum Products		1) Shall not detect a visible film, sheen or result in visible discoloration of the surface with a corresponding oil or petroleum product odor, 2) Shall not cause damage to fish, inverts or objectionable degradation of drinking water quality, 3) shall not form an oil deposit on the shores or bottom of the receiving body of water.	
Toxic Substances (water column, sediment, drinking water consumption, organisms consumption)		General: 1) All waters shall be maintained free of toxic substances in concentrations that produce detrimental physiological, acute or chronic responses in human, plant, animal or aquatic life. 2) All waters shall be maintained free of toxic subs in conc. that produce contamination in harvestable aquatic life to the extent that it causes detrimental physiological, acute or chronic responses in humans or protected wildlife, when consumed. 3) The survival of aquatic life in marine and surface waters subjected to a waste discharge, or other controllable water quality factors, shall not be less than that for the same water body in areas unaffected by the waste discharge. Numeric criteria: see Appendix A in 2017 GWQS.	

Data Assembly

Guam reporting relies on data sets from local academia, local government agencies and federal government agencies. This can include data solicitation from the Navy Environmental Office, the National Park Service Water Resources Division, Government of Guam Fisheries and Wildlife Programs, and Guam Environmental Protection Agency projects listed in [Table 1](#).

One Guam EPA project is presented for reporting this assessment period (Table 5 below).

Table 5. Projects with usable data for the Guam 2020 IR assessment:

Organization	Project	Waterbody Type	Use Support	Year of data	Data Quality
Guam EPA	Recreational Beach Monitoring Project (RBMP)	Marine Beaches	Body Contact	Jan – Dec 2018 and 2019	For use support determination
NOAA	<i>Manell Watershed and Geus River</i>	Surface water (fresh water and marine water/brackish)	Aquatic life	Dec 2016 – Dec 2018	For use support determination

The quality of each data set and project was evaluated by reviewing project objectives, quality assurance and quality control requirements, laboratory method compatibility, analysis quality and MDLs. Data was either identified as 'good quality' for direct use in Use Support Determinations. No data has been identified for use as 'evaluation only' (see 'Data Quality' column in table above). 'Evaluation only' data are data sets that do not have specific associated criteria and thus are used as supportive data.

Project: Recreational Beach Monitoring Program (RBMP)

Guam EPA's RBMP is included in this IR assessment and contributes data for use-support determination. Guam's subtropical climate allows for year-round recreation at all marine beaches, and fishing from both along the shoreline and offshore. The majority of this type of recreational activity occurs along stretches of sandy beaches or limestone plateaus easily accessible from shore. These waters are classified as "M-2 waters" or "Good" under the GWQS. To monitor and test for the designated use "Whole Body/Primary (body) Contact", weekly water grab samples are collected and tested for the approved EPA bacterial indicator. Guam EPA under the Recreational Beach Monitoring Program (RBMP) has collected bacteriological data for over 20 years. Data collected weekly from fixed sampling sites along selected stretches of coastline is used to advise the public against swimming in waters exceeding bacterial standards. Weekly press releases identify those beaches (where indicators in weekly water samples exceed water quality standards). Trend analysis (using the weekly data) is used to characterize risks of exposure to contaminated waters. Resulting trends allow for the ranking of beaches, which enable biologists to determine the need for further monitoring or the need to include additional unmonitored beaches to the list.

Table 6. Project Stations

Recreational Beach Monitoring Program (RBMP) [45 stations] Jan 2018 - Dec 2019		
Marine Water Station ID	GWQS class	No. visits
N-01	M-2	99
N-02	M-2	98
N-03	M-2	98
N-04	M-2	98
N-05	M-2	98
N-06	M-2	98
N-07	M-2	99
N-08	M-2	98
N-09	M-2	99
N-10	M-2	99
N-11	M-2	99
N-12 (suspended due to location in boat marina- no swimming zone)	NA	NA
N-13	M-2	99
N-14	M-2	99
N-15	M-2	99
N-16	M-2	98
N-17	M-2	98
N-18	M-3	98
N-19	M-2	98
N-20	M-3	98
N-21	M-2	98
N-22	M-2	98
N-23	M-2	98
N-24	M-2	98
N-25	M-2	98
N-26	M-2	99
N-27	M-2	99
N-28	M-2	99
S-02	M-2	99
S-03	M-2	99
S-04	M-2	99
S-05	M-2	98
S-06	M-2	99
S-07	M-2	99
S-08	M-2	99
S-09	M-2	99
S-10	M-2	99
S-11	M-2	99
S-12	M-2	98
S-13	M-2	97
S-14	M-2	98
S-15	M-2	99
S-17 (suspended on 11/21/2019 due to inaccessible)	M-2	87
S-25 (adopted on 11/21/2019 due to inaccessible S-17)	M-2	6
S-18	M-2	98

Table 7. Project Indicators

RBMP Jan 2018 - Dec 2019	
Laboratory Parameter (WQ)	No. samples used for 2020IR
Enterococci (MPN)	4,177
Field Observations	No. samples used for 2018IR
Weather (past 24 hours)	(No criteria)
Weather at time of station occupation	(No criteria)
Rainfall weekly accumulation	(No criteria)
Average Air Temperature	(No criteria)
Wind type and direction	(No criteria)
Tidal Stage	(No criteria)
Water surface condition	(No criteria)
Water color	(No criteria)
Turbid observation	(No criteria)
Water odor	(No criteria)

Project: NOAA’s Manell Watershed Monitoring

NOAA’s *Manell Watershed and Geus River* baseline study is also included in this IR assessment and contributes data for aquatic life use-support determination. The study occurred at three rivers in the Manell Watershed (Sumay River, As Liyog River and the Ajayan River) that drain into the Achang Reef Flat Marine Preserve Area (MPA) for two years (Dec 2016-Dec 2018), and at the Geus River in the adjacent watershed to the west during the wet season of 2018.

According to the study report, “the project was carried out to provide a baseline of conditions for these parameters, so that local resource managers could better understand the condition of these rivers, and also provide the means for assessing the efficacy of restoration activities being carried out or planned for the Manell watershed”.

Project sample locations, aquatic life indicators and sample sizes included in this assessment are as follows:

	Ajayan Bridge	Liyog Bridge	Sumay Bridge	Geus Fresh water (4 sites)*
Dissolved Oxygen (mg/l)	79	79	71	29
Dissolved Oxygen (%)	79	79	71	29
Suspended Sediment Concentration SSC (mg/l)	65	64	65	29
Nitrate NO3- (mg/l N)	79	79	69	9
Orthophosphate HPO4= (mg/l P)	77	77	64	9

*wet season 2018 samples only

Attainment Determinations

Attainment Determinations identify waterbody segments that meet or do not meet *designated-uses* using monitoring parameters and criteria described in the Guam Water Quality Standards.

Guam Waterbody segments: Guam's Waterbody Segments data set was developed using geospatial processing programs (Esri's ArcGIS) to obtain visual coverage of Guam's natural resources, georeferenced resource segments, and identified associated measurement attributes for data assessments. Guam EPA's River, Marine Waterbodies and Beaches data sets are used to develop monitoring plans and to facilitate reporting on the water quality condition in support of designated uses.

Guam Rivers and Streams (river segments) were assigned based on digitized USGS 7.5-minute, 1:24,000-scale quadrangle series topographic maps for Guam and cross checked with Guam Orthophotos updated by FEMA 2003. The River shapefile attributes include watershed location, Guam River ID number, river or stream name, and channel length (calculated using 'calculate geometry' tool).

The Guam marine waterbodies data set was created in 2010. The shapefile is based on existing information found in USGS quadrangle series topographic maps for Guam (7.5-minute, 1:24,000 scale), the Atlas of the Reefs and Beaches of Guam (Coastal Zone Management Section, and the Bureau of Planning document, R.H. Randall and L.G. Eldredge, 1976), and existing ArcGIS information from the Bureau of Statistics and Plans (i.e. coastal features, ecological reserve areas, Guam seashore reserve areas, Marine Preserve areas). During the design phase of the *2010 and 2015 Guam Reef Flat Condition Assessments* (2015 condition to be reported during the next reporting cycle), marine waterbodies were further delineated to include 21.3 square kilometers of 'Reef flat' zone of the NOAA Benthic Habitat Data shapefile. The Benthic Habitat Data is NOAA's National Ocean Service map production effort to digitally map biotic resources and coordinate a long-term monitoring program that can detect and predict change in U.S. coral reefs, and their associated habitats and biological communities.

The Guam Beaches data set was created in 2003 for use in the USEPA Beach Grant project. Beaches are identified based on public use/access and characteristics that allow for swimming/wading. The dataset was created using existing information in the Atlas of the Reefs and Beaches of Guam (Coastal Zone Management Section, Bureau of Planning, R.H. Randall and L.G. Eldredge, 1976) and existing shapefiles from the Bureau of Planning (i.e. coastal features). Guam Beach location (beach stretch locations and beach monitoring stations), monitoring and notification data is submitted to the U.S. Environmental Protection Agency

(EPA)'s Water Quality Exchange (WQX)⁵ and BEach Advisory and Closing Online Notification (BEACON) system annually. These systems were created by USEPA as mechanisms to publish monitoring and notification data as well as to provide to the public a database of state-specific pollution occurrences for coastal recreation waters. WQX can be viewed on-line at <https://www.epa.gov/waterdata/water-quality-data-wqx>. BEACON can be viewed online at <http://watersgeo.epa.gov/beacon2/about.html>.

Guam Attainment Determination: Attainment determination for this reporting period will be conducted on two projects.

Project: Recreational Beach Monitoring Program (RBMP)

Supporting document for Guam EPA's RBMP (2018-2019) include:

- The Recreational Beach Monitoring Program RBMP Plan: Guam Coastal Waters (GEPAMPQA009 June 2003 Rev. No. 1),
- The STANDARD OPERATING PROCEDURE (SOP): FOR THE COLLECTION OF WADEABLE AND NONWADEABLE SURFACE WATER AND MARINE WATER FOR MICROBIOLOGICAL EXAMINATION (GEPAMP017, April 2001).

Assessment of data from the RBMP begins with identifying beach segments. *Table 8. Guam Beaches* (below) defines Assessment Units included in this IR. The table also shows 102 designated beaches totaling 44.24 beach miles.

⁵ WQX has effectively replaced the STORET (short for STORage and RETrieval) Data Warehouse, which was decommissioned in June 2018.

Table 8. Guam Beaches

Beach Name	Watershed	Bay Name	Size (mi)	USEPA Beach ID	GEPA Beach#	Assessment Unit ID
Aang Beach	Manell	Cocos Lagoon 1	0.12	GU561116	GB79	GU-GB79
Aba Beach	Geus	Cocos Lagoon 2	0.19	GU164107	GB78	GU-GB78
Abong Beach	Cetti	Sella Bay & Cetti Bay	0.62		GB64	GU-GB64
Achang Bay	Manell	Cocos Lagoon 1	0.55		GB80	GU-GB80
Aga Beach	Inarajan	Aga Bay	0.08	GU581113	GB91	GU-GB91
Agfayan River Beach	Inarajan	Agfayan Bay	0.07	GU867073	GB95	GU-GB95
Ajayan River Mouth 1	Manell	Ajayan Bay	0.03	GU344686	GB87	GU-GB87
Ajayan River Mouth 2	Manell	Ajayan Bay	0.06	GU954089	GB89	GU-GB89
Ajmo Beach	Toguan	Toguan Bay & Bile Bay	0.16	GU894296	GB70	GU-GB70
Alupang Island Beach	Northern	Hagatna Bay (East)	0.02		GB21	GU-GB21
Apaca Park Beach	Agat	Agat Bay 2	0.14		GB48	GU-GB48
ASAN MEMORIAL BEACH	Piti & Asan	Asan Bay	0.46	GU732200	GB31	GUN-14
Asanite Point Beach aka First Beach	Togcha	Talofofo Beaches	0.06	GU812957	GB106	GUS-18
Asiga Beach Area (Inarajan)	Asalonso	Asiga Point Beach Area	0.22		GB102	GU-GB102
Atao Beach	Inarajan	Inarajan Reef Flat	0.38	GU303218	GB93	GU-GB93
Beach at Fonte River	Fonte	Hagatna Bay (West)	0.13	GU208533	GB27	GUN-21
Beach at Inarajan Bay	Inarajan	Inarajan Bay	0.56	GU605963	GB97	GUS-10
Beach at Pago Bay	Pago	Pago Bay	0.96	GU875627	GB118	GUS-15
Beach at Pauliluc Bay	Dandan	Pauliluc Bay	0.28	GU208080	GB98	GU-GB98
Beach at Piti Bay	Piti & Asan	Piti Bay	1.16	GU196164	GB32, GB33	GUN-15
Beach north of Acho Point	Inarajan	Inarajan Reef Flat	0.31	GU755429	GB94	GU-GB94
Beach north of Asmafines River	Cetti	Sella Bay	0.12		GB62	GU-GB62
Beach North of Finile River	Agat	Agat Bay 2	0.35	GU324640	GB52	GU-GB52
Beach north of Togcha River	Togcha	Togcha Bay	0.27	GU721999	GB113	GUS-13
Beach North of Ylig Bay	Ylig	Ylig Bay	0.07	GU834889	GB116	GU-GB116
Beach south of Achugao	Cetti	South Facpi Point beaches and rocky shorelines	0.29		GB59	GU-GB59
Beach south of Agaga River	Cetti	South Facpi Point beaches and rocky shorelines	0.25		GB60	GU-GB60
Beach South of Finile River	Agat & Taelayag	Agat Bay 2 & Taleyfac Bay 2	0.88	GU922647	GB53	GUS-04
Beach south of Sella River	Cetti	Sella Bay	0.12		GB63	GU-GB63
Beach to Asgadao Bay	Manell	Asgadao Bay	0.07	GU280102	GB83	GU-GB83

Beach Name	Watershed	Bay Name	Size (mi)	USEPA Beach ID	GEPA Beach#	Assessment Unit ID
Beach to Liyog River Mouth	Manell	Sumay Bay & Asgadao Bay	0.77	GU519084	GB81	GU-GB81
Bile Bay Beach	Toguan	Bile Bay	0.03	GU757210	GB71	GU-GB71
Calvos Beach	Togcha	Talofofu Beaches	0.51	GU304652	GB108	GU-GB108
Cocos Island	Cocos Lagoon	Cocos Lagoon 1	1.16		GB73	GU-GB73
Dadi Beach	Agat	Agat Bay 1	0.61		GB46	GU-GB46
Dungca's Beach, East Hagatna Bay	Northern	Hagatna Bay (East)	0.99	GU203410	GB22	GUN-06, GUN-07
Facpi Point Beaches	Cetti	South Facpi Point beaches and rocky shorelines	0.66		GB58	
Fafai Beach	Tumon Subbasin	Tumon Bay	0.37		GB13	GU-GB13
Falcona Beach	Northern	Falcona Beach Area	0.37		GB6	GU-GB6
Family Beach	Apra	Apra Harbor 2	0.15	GU179655	GB36	GUN-19
Gabgab Beach	Apra	Apra Harbor 2	0.65		GB43	
Gognga Beach, Tumon Bay	Tumon Subbasin	Tumon Bay	0.15	GU610162	GB16	GUN-25
Guijen Rock area	Inarajan	Inarajan Reef Flat	0.44	GU535422	GB92	GU-GB92
Gun Beach, Tumon Bay	Tumon Subbasin	Tumon Bay	0.23	GU446721	GB15	GUN-24
HAGATNA MARINA	Hagatna	Hagatna Bay (West)	0.43	GU176012	GB25	GUN-10, GUN-11
Haputo Beach	Northern	Haputo Beach Area	0.19		GB8	GU-GB8
Head of Fouha Bay	Umatac	Fouha Bay	0.06		GB66	GU-GB66
Head of Paicpouc Cove	Asalonso	Talofofu Bay	0.09		GB103	GU-GB103
Head of Talofofu Bay	Talofofu	Talofofu Bay	0.21	GU367361	GB105	GUS-11
Head of Umatac Bay	Umatac	Umatac Bay 2	0.14	GU654723	GB67	GUS-06
Head of Ylig Bay	Ylig	Ylig Bay	0.18	GU425901	GB115	GU-GB115
Inarajan Pools	Inarajan	Agfayan Bay: Inarajan Pools	0.08	GU057929	GB96	GUS-09
Intermittent beach - Asgadao Bay	Manell	Asgadao Bay	0.12	GU621836	GB84	GU-GB84
Intermittent beach - Shark's Hole	Northern	Tanguisson Beach Area 1	0.19		GB9	GU-GB9
Intermittent beach - Tanguisson Pt.	Northern	Tanguisson Beach Area 2	0.26	GU515461	GB10	GU-GB10
Intermittent beach 1 - Ajayan Bay	Manell	Asgadao Bay	0.12	GU196675	GB85	GU-GB85
Intermittent beach 2 - Ajayan Bay	Manell	Asgadao Bay & Ajayan Bay	0.09	GU461933	GB86	GU-GB86
Intermittent beach 3 - Ajayan	Manell	Ajayan Bay	0.21	GU370300	GB88	GU-GB88

Beach Name	Watershed	Bay Name	Size (mi)	USEPA Beach ID	GEPA Beach#	Assessment Unit ID
Bay						
Intermittent beach 4 - Ajayan Bay	Manell	Ajayan Bay	0.09	GU231281	GB90	GU-GB90
Intermittent beach-North Tanguisson	Northern	Tanguisson Beach Area 2	0.26	GU231281	GB11	GU-GB11
Islet	Cocos Lagoon	Cocos Lagoon 1	0.07		GB74	GU-GB74
Jinapsan Beach	Northern	Jinapsan Beach Area	1.28		GB3	GU-GB3
Jones Beach	Togcha	Talofof Beaches	0.09	GU473279	GB110	GU-GB110
Liyog river Mouth	Manell	Asgadao Bay	0.18	GU463873	GB82	GU-GB82
Marianas Yacht Club Beach	Apra	Sasa Bay	0.18	GU826563	GB41	GU-GB41
Merizo Public Pier Park	Geus	Cocos Lagoon 2	0.46	GU038434	GB75	GU5-08
Mouth of Cetti Bay	Cetti	Cetti Bay	0.5		GB65	GU-GB65
Naton Beach, Tumon Bay	Tumon Subbasin	Tumon Bay	1.1	GU763206	GB17, GB18	GUN-23, GUN-04, GUN-03, GUN-02
NCS Beach/Tanguisson Beach	Northern	Tanguisson Beach Area 2	0.25	GU900850	GB12	GUN-01
Nimitz Beach	Talayag	Taleyfac Bay 2	0.56	GU016244	GB55	GU5-05
North of Togcha Point	Togcha	Togcha Bay & Beach North of Togcha Point	1.1	GU631282	GB114	GU-GB114
North Pago Bay Beach	Pago	Pago Bay	0.24		GB119	GU-GB119
Orote Point Beaches	Apra	Apra Harbor 1 and 2	0.21		GB44	GU-GB44
Outhouse Beach	Apra	Apra Harbor 3	0.46	GU745740	GB35	GUN-18
Perez Beach	Dandan	Nomna Bay	0.26		GB101	GU-GB101
Piga Beach (Talona Beach)	Geus	Cocos Lagoon 2	0.42	GU249673	GB76, GB77	GU-GB76
Pigua River Beach	Toguan	Bile Bay	0.08	GU418215	GB72	GU-GB72
Polaris Beach	Apra	Apra Harbor 2	0.19		GB42	
Port Authority Beach	Apra	Piti Channel and Cabras Island	0.46	GU233053	GB37	GUN-20
Ritidian Beach	Northern	Ritidian Point Beach Area	2.35		GB4	GU-GB4
Rizal Beach	Agat	Agat Bay 1 & 2	0.26	GU031839	GB47	
Sagua Beach	Talayag	Taleyfac Bay 1	0.62		GB57	
Salinas Beach	Agat	Agat Bay 2	0.53	GU959524	GB51	
Ski Beach	Apra	Sasa Bay	0.4	GU427179	GB38	
South of Falcona Beach (Urunao)	Northern	Falcona Beach Area	0.24		GB7	
South of Machadgan Point	Umatac	Umatac Bay 2	0.25		GB68	
SRF Beach	Apra	Apra Harbor 2	0.4		GB40	

Beach Name	Watershed	Bay Name	Size (mi)	USEPA Beach ID	GEPA Beach#	Assessment Unit ID
Talayag Beach	Talayag	Taleyfac Bay 1	0.87	GU524587	GB56	
Tagachan Beach Park	Ylig	Tagachan Beach Park Area	0.18	GU996375	GB117	GU5-14
Tarague Beach (Scout Beach)	Northern	Tarague Beach (Scout Beach Area)	3.42		GB1, GB2	
Tipalao Beach	Apra	Tipalao Bay	0.15		GB45	
Togcha Bay aka Agat Beach	Agat	Agat Bay 2	0.79	GU374433	GB50	GU5-03, GU5-02, GU5-17, GU5-25
TOGUAN BAY	Toguan	Toguan Bay	0.46	GU311098	GB69	GU5-07
Trituhara Beach, East Hagatna Bay	Northern	Hagatna Bay (East)	1.16	GU458635	GB22, GB23, GB24	GUN-08, GUN-26, GUN-09
Ulomai Beach	Dandan	Ulomai Beach Area	0.11		GB99	
Unified Seaman's Service Beach	Piti & Asan	Piti Bay	0.52	GU790189	GB34	GUN-16, GUN-17
Urano Beach	Northern	Urano Beach Area	2.04		GB5	GU-GB5
West Hagatna Beach	Hagatna	Hagatna Bay (West)	1.11	GU468763	GB26	GUN-13, GUN-27, GUN-28
West of Adelup Point, Asan Bay	Fonte	Asan Bay	0.41	GU241731	GB28	GUN-22
West of volcanic headland	Piti & Asan	Asan Bay	0.37	GU845649	GB29	GU-GB29
Ypan Beach Park Beach (Ipan)	Togcha	Togcha Bay	0.3	GU139377	GB111	GU5-12
Ypan Beach, Tumon Bay	Tumon Subbasin	Tumon Bay	0.42	GU425480	GB19	GUN-05

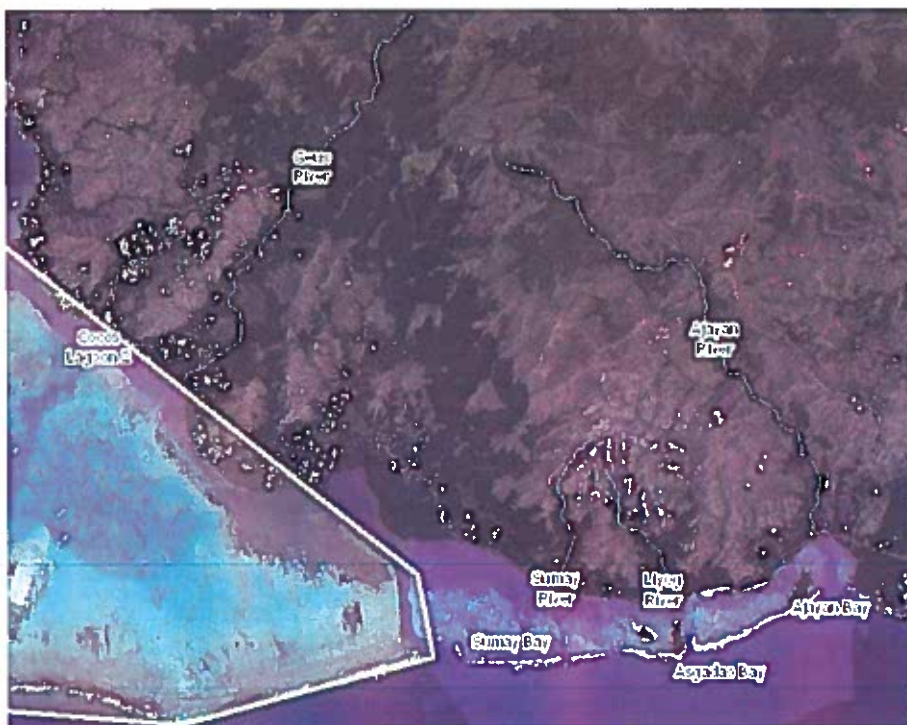
Total: 102 Beaches

Total miles:
44.24

Forty-four (44) monitored stations are listed in *Table 6* and included in *Table 8* (highlighted in green). These stations assess thirty-one (31) beach units (also highlighted in green in *Table 8* above).

Project: NOAA's Manell Watershed Monitoring

The supporting document for NOAA's Manell Watershed project is the *Measurement of Turbidity, Suspended Sediments and Nutrients in Three Rivers that Drain to the Achang Preserve from the Manell Watershed, Guam. Pait, Whitman, et al. NOAA Technical Memorandum NOS NCCOS 268. December 2019.*



Assessment of data from the Manell study begins with identifying river and marine bay segments. As seen in the figure, Geus River, west of the study area, empties into the Cocos Lagoon 2 waterbody and the Achang Reef Flat MPA. The Sumay River empties to the Sunmy Bay, Liyog River empties to Asgado Bay and Ajayan River empties to Ajayan Bay.

The freshwater rivers are classified as "S-2 waters" or "Good" under the GWQS while the marine/brackish waters at the mouths of these rivers (including the Achang Reef Flat MPA) are classified as "M-1" or "Excellent".

Based on described project sample collection locations, the following River Name, Guam EPA Assessment Unit (AU) IDs and size (miles) are to be used in further project data assessment:

- Geus Fresh water: Geus River 1, GUMZRG-1, 0.99 miles; Geus River 2, GUMZRG, 0.52 miles; Geus River 3, GUMZRG-2, 0.78 miles.
- Sumay Bridge: Sumay River, GUMZRSY, 1.06 miles
- Liyog Bridge: Liyog River, GUMZRL, 1.83 miles
- Ajayan Bridge: Ajayan River, GUMZRAJ, 3.95 miles

Degree of Use-Support for each beach and river segment is determined by applying the guidelines shown in Table 9.

Table 9. Guidelines to determine the Degree of Use-Support

Level of Use Support	Criteria	
	Marine Water - Whole Body Contact Recreation M1, M2, M3	Aquatic Life Use Support for Water Quality (Physical/Chemical indicators) S1, S2, S3
Fully Supporting (FS)	<u>Enterococci</u> : A geometric mean of 35 enterococci per 100mL (based on samples taken in a 30-day interval) is not exceeded AND the Statistical Threshold Value (STV) of 130cfu/100mL is not exceeded by more than 10 percent of the samples taken during the same 30-day interval.	For each pollutant, GWQS exceeded in 10% or less of measurements
Partially Supporting (PS)	<u>Enterococci</u> : Geometric mean of 35 enterococci per 100mL (based on samples taken in a 30-day interval) is not exceeded AND the Statistical Threshold Value (STV) of 130cfu/100mL is exceeded by more than 10 percent of the samples taken during the same 30-day interval during the year.	For each pollutant: GWQS exceeded in 11% to 25% of measurements
Not Supporting (NS)	<u>Enterococci</u> : Geometric mean standard of 35 enterococci per 100mL (based on samples taken in a 30-day interval) is exceeded.	For each pollutant: GWQS exceeded in greater than 25% of measurements

III. Assessment Results

This section presents the results of Guam’s recreational water and the NOAA Manell Study data assessments, including the five-part categorization of all water segments.

Five-Part Categorization of Marine and Surface Waters

As recommended in the IR Guidance (2006), the following categories are to be assigned to an assessment unit (AU) depending on the use-support determination of data from the RBMP and the NOAA Manell Study:

Category 1: All designated uses are supported, no use is threatened;

Category 2: Available data and/or information indicate that some, but not all of the designated uses are supported;

Category 3: There is insufficient available data and/or information to make a use support determination;

Category 4: Available data and/or information indicate that at least one designated use is not being supported or threatened, but a TMDL is not needed;

Category 4a: A TMDL to address a specific segment/pollutant combination has been approved or established by EPA;

Category 4b: A use impairment caused by a pollutant is being addressed by the state through other pollution control requirements;

Category 4c: A use is impaired, but the impairment is not caused by a pollutant; and

Category 5: Available data and/or information indicate that at least one designated use is not being supported or is threatened, and a TMDL is needed.

Project: Recreational Beach Monitoring Program (RBMP)

All monitored beaches are assigned to *Category 4a* due to TMDLs that were EPA approved in 2010 and 2015. Nonetheless, individual recreational beach use-support assessments for 31 beaches are presented in *Table 10*. The table shows the degree of use-support for the designated use of Whole Body Contact Recreation using the Enterococci (bacteria) parameter. Thirty-one (31) beaches were assessed in 2018 and 2019 resulting in a categorization of 15.5 miles of Guam's beaches. See [Figure A1. 2018-2019 IR Stations](#) for the location of Beaches and Beach Stations.

Table 10. Individual Recreational Beach Use-Support Assessment

Beach Name	Size (mi)	USEPA Beach ID	GEPA Beach#	Assessment Unit ID	2018 Use Determination	2019 Use Determination
NCS Beach/Languisson Beach	0.25	GU900850	GB12	GUJN-01	NS	NS
Naton Beach, Tumon Bay	1.1	GU763206	GB17, GB18	GUJN-02	NS	NS
				GUJN-03	PS	PS
				GUJN-04	NS	PS
				GUJN-23	NS	NS
Ypao Beach, Tumon Bay	0.42	GU425480	GB19	GUJN-05	NS	NS
Dungca's Beach, East Hagatna Bay	0.99	GU203410	GB22	GUJN-06	NS	NS
				GUJN-07	NS	NS
Trinchera Beach, East Hagatna Bay	1.16	GU458635	GB22, GB23, GB24	GUJN-08	NS	NS
				GUJN-09	NS	NS
				GUJN-26	NS	NS
HAGATNA MARINA	0.43	GU176012	GB25	GUJN-10	NS	NS
West Hagatna Beach	1.11	GU468763	GB26	GUJN-11	NS	NS
				GUJN-13	NS	NS
ASAN MEMORIAL BEACH	0.46	GU732200	GB31	GUJN-14	NS	NS
Beach at Piti Bay	1.16	GU196164	GB32, GB33	GUJN-15	NS	NS
				GUJN-16	NS	NS
United Seamen's Service Beach	0.52	GU790189	GB34	GUJN-17	NS	PS
Outhouse Beach	0.46	GU745740	GB35	GUJN-18	PS	PS
Family Beach	0.15	GU179655	GB36	GUJN-19	PS	PS
Port Authority Beach	0.46	GU233053	GB37	GUJN-20	PS	NS
				GUJN-21	NS	NS
Beach at Fonte River	0.13	GU208533	GB27	GUJN-22	NS	NS
West of Adelup Point, Asan Bay	0.41	GU241731	GB28	GUJN-23	NS	NS
Cun Beach, Tumon Bay	0.23	GU446721	GB15	GUJN-24	NS	NS
Cogonga Beach, Tumon Bay	0.15	GU610162	GB16	GUJN-25	NS	NS
Fogcha Bay aka Aggat Beach	0.79	GU374433	GB50	GUJN-26	NS	NS
				GUJN-27	NS	NS
				CRUB-02	NS	NS

Beach Name	Size (mi)	USEPA Beach ID	GEPA Beach#	Assessment Unit ID	2018 Use Determination	2019 Use Determination
				GUS-03	NS	NS
				GUS-17	NS	NS
				S-25	--	NS
Beach South of Finile River	19 0.88	GU922647	GB53	GUS-04	NS	NS
Nimitz Beach	20 0.56	GU016244	GB55	GUS-05	NS	NS
Head of Umatac Bay	21 0.14	GU654723	GB67	GUS-06	NS	NS
TOGUAH BAY	22 0.46	GU311098	GB69	GUS-07	NS	NS
Merizo Public Pier Park	23 0.46	GU038434	GB75	GUS-08	NS	NS
Inarajan Pools	24 0.08	GU057929	GB96	GUS-09	NS	NS
Beach at Inarajan Bay	25 0.56	GU605963	GB97	GUS-10	NS	NS
Head of Talofofo Bay	26 0.21	GU367361	GB105	GUS-11	NS	NS
Ypan Beach Park Beach (Ipan)	27 0.3	GU139377	GB111	GUS-12	NS	FS
Beach north of Togcha River	28 0.27	GU721999	GB113	GUS-13	NS	NS
Togachan Beach Park	29 0.18	GU996375	GB117	GUS-14	NS	PS
Beach at Pagg Bay	30 0.96	GU875627	GB118	GUS-15	NS	NS
Asanite Point Beach aka First Beach	31 0.06	GU812957	GB106	GUS-18	FS	PS

Total Assessed Benches:31

Total Assessed Size: 15.5 miles

Project: NOAA's Manell Watershed Monitoring

Individual aquatic life *use-support* determination of the Manell Watershed rivers show that Dissolved Oxygen, Orthophosphate, and Suspended Solids are not supporting (NS) for all three rivers. Nitrates (NO₃-) are not supporting (NS) at Sumay River but fully supporting (FS) for Ajayan River and Liyog River. Dissolved Oxygen, Suspended Solids, Nitrates and Orthophosphate are fully supporting (FS) at the Geus River segments. See the following table.

Table 11. Individual Aquatic Life *Use-Support* Assessment of NOAA's Manell Study

	Dissolved Oxygen (mg/l)	Dissolved Oxygen (%)	SSC (mg/l)	NO ₃ - (mg/l N)	HPO ₄ = (mg/l P)
Ajayan River, GUMZRAJ, 3.95 miles					
exceedances	51	61	40	0	68
number of samples	79	79	65	79	77
exceedance freq (%)	65	77	62	0	88
<i>use-support determination</i>	NS	NS	NS	FS	NS
MIN	2.01	31	1.40	0.000	0.010
MAX	8.47	100	82.17	0.090	0.310
AVG	4.30	63	7.52	0.012	0.084
Liyog River, GUMZRL, 1.83 miles					
exceedances	40	44	60	0	71
number of samples	79	79	64	79	77
exceedance freq (%)	51	56	94	0	92
<i>use-support determination</i>	NS	NS	NS	FS	NS
MIN	0.77	12	3.32	0.000	0.010
MAX	78.70	100	129.06	0.090	0.320
AVG	5.35	67	23.37	0.009	0.104
Sumay River, GUMZRSY, 1.06 miles					
exceedances	66	67	15	45	31
number of samples	71	71	65	69	64
exceedance freq (%)	93	94	23	65	48
<i>use-support determination</i>	NS	NS	NS	NS	NS
MIN	0.29	4	1.45	0.020	0.010
MAX	6.70	88	71.57	0.340	0.460
AVG	3.54	47	7.51	0.188	0.049
Geus River 1, GUMZRG-1, 0.99 miles. Geus River 2, GUMZRG, 0.52 miles. Geus River 3, GUMZRG-2, 0.78 miles. (total miles: 2.29 miles)					
exceedances	0	0	1	0	0
number of samples	29	29	29	9	9
exceedance freq (%)	0	0	3	0	0
<i>use-support determination</i>	FS	FS	FS	FS	FS
MIN	6.55	84	0.19	0.030	0.010
MAX	11.57	100	24.49	0.110	0.020
AVG	7.50	92	2.62	0.073	0.013

During this reporting cycle, *use-support* assessments were not conducted at these rivers for the other designated uses (i.e. body contact use [DU1] and human health consumption use [DU3]). Therefore, these four rivers are categorized (Five-Part Categorization of Marine and Surface Waters) as follows:

Guam River ID #	River Name	AU ID	303d listed?	DU1: Body Contact	DU2 Aquatic Life Phys/Chem Water Quality	DU2 Aquatic Life Bioassessment	DU3 Human Health Consumption	Single Category Assignment (5part)
G-32	Geus River	GUMZRG-1	NO	none	FS	none	none	C3
		GUMZRG-2	NO	none	FS	none	none	C3
		GUMZRG	NO	none	FS	none	none	C3
G-37	Sumay River	GUMZRSY	NO*	none	NS	none	none	C5
G-38	Liyog River	GUMZRL	NO*	none	NS	none	none	C5
5G-41	Ajayan River	GUMZRAJ	NO*	none	NS	none	none	C5

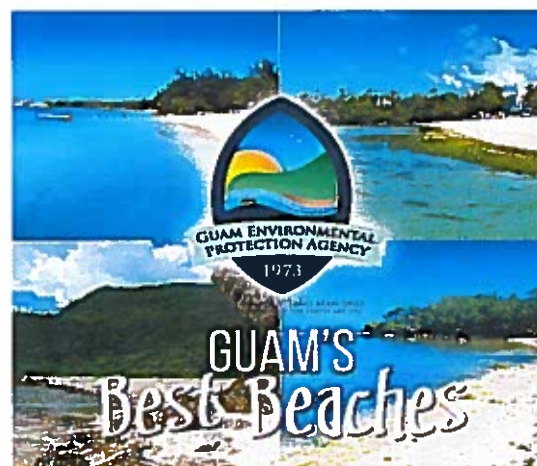
*these waterbodies will be further assessed for inclusion in Guam's 2020 303(d) List of Impaired Waters next reporting cycle

Geus River segments are assigned a category 3 (C3) indicating that there is insufficient information/data to make a single category assignment. The three Manell Watershed rivers are assigned a category 5 (C5) indicating that one designated use (DU2) is not supported and that a TMDL is needed. Guam EPA however notes that current active and future restoration efforts in the Manell Watershed may be sufficient to begin mitigating water quality impairments. (See Part II. Manell Watershed narrative pages 35-38). Sampling and analysis in the Manell Watershed rivers should be continued to determine Single Category Assignments for the remaining designated uses.

IV. Trend Analysis for Marine and Surface Waters

Recreational Beach Monitoring Program

A story map showing the best beaches based on frequency of advisory for the 2019 calendar year was created. The story map can be viewed at <https://www.arcgis.com/apps/MapSeries/index.html?appid=e827565f65f1439fb62a709c3788fe76>.



Status and Trends Monitoring Program

The Monitoring Program is continuing efforts to develop a water quality inventory document (entitled “STMP Marine and Surface Water Quality Assessment”) based on data collected via its Status and Trends Monitoring Program and recorded in USEPA’s STORET. A preview of the STMP 2013 assessment document featuring the Hagatna Watershed was included in Guam’s 2014 IR.

The document presents information on:

- Guam’s delineated watersheds and specific watershed links to river and marine waterbody segment information (e.g. known point and non-point pollution sources, advisory areas, water quality monitoring sites and biological monitoring sites);
- Watershed monitoring site information (e.g. site description, assigned Guam water quality classification, years sampled, total number of sampling events); and
- Watershed specific raw monitoring data and descriptive statistics of the data (e.g. number of samples, average concentration, minimum/maximum values, number of GWQS exceedances, percentage exceedance and box and whiskers charts showing yearly data distribution, Observed Mobile Invertebrates, Percent Cover of major benthic Group Categories, and Species Observed).

Agency managers may use the document for planning purposes. Also, segments of the document may be provided online to the public.

Table 12 summarizes where exceedances occurred at 75% or more of samples taken. Continued investigation should be conducted at these sites in order to determine sources of these exceedances. Inclusion of these sites to the IWM program can be considered for further investigation.

Table 12. STMP Sites with 75% or greater Exceedances

Bacteria Concentration (MPN)		Ortho-phosphate (mg/L)		Nitrate (mg/L)		Oxygen (% Saturation)		Total Suspended Solids (mg/L)	
SITE	WATER SHED	SITE	WATER SHED	SITE	WATER SHED	SITE	WATER SHED	SITE	WATER SHED
AGMX	Hagatna	MZRAC	Manell	AGRA-1	Hagatna	A5	Hagatna	DRM	Northern
AGRA-1	Hagatna	MZRL	Manell	INRI1	Inarajan	AGRA-1	Hagatna	DRMI	Northern
INRAGB-3	Inarajan	TURTG-1B	Togcha	AGRD	Northern	AGRA-2	Hagatna	P2	Pago
MZRAC	Manell			P2	Pago	AGRA-3	Hagatna	ATMA	Taelayag
MZRAJ	Manell			PGRLO	Pago	AGRD	Northern		
MZRL	Manell			ASRI-2	Piti / Asan	P2	Pago		
TANG	Northern			T3	Togcha	P8	Pago		
ASRI-1	Piti / Asan			TURTG-1B	Togcha	P9	Pago		
ASRI-3	Piti / Asan					PGEP	Pago		
ASRM	Piti / Asan					PGRLO	Pago		
MA1	Piti / Asan					MZRP-2	Toguan		
TUM11	Talofofo					FW site	Apra		
TURTG-C	Togcha								
MZRP-2	Toguan								
MZRT-1	Toguan								
FW site	Apra								

Manell Watershed, Guam Water Quality Monitoring Project 2017-2018

NOAA's *Manell Watershed and Geus River* results indicated some important trends and conclusions that should be considered in on-going and future restoration activities in the Manell Watershed. The following statements were taken directly out of the report:

- There is a significant (Spearman's $Rho = 0.0880$, $p < 0.0001$) positive correlation between rainfall and turbidity (collected using a sonde). Ajayan and the As Liyog rivers respond rapidly to rainfall in terms of stream height and as was shown for the Ajayan, also for turbidity. This highlights the need for restoring and maintaining good soil cover in the watershed, in order to reduce the amount of water and sediment transported into the rivers and then out onto the Achang Preserve and the rest of Cocos Lagoon.
- The effects of wildfires leaving the soil bare, followed by revegetation either naturally or as a part of restoration efforts, could lead to a decrease in the amount of base soil

present, and perhaps to less erosion. Additional monitoring, and surveys of ground cover would help to determine any trends.

- It's possible there is a link between revegetation of the hillsides either through restoration efforts (e.g., replanting of native species of plants) or natural regrowth of grasses after wildfires, to reduced inputs of SSC and orthophosphate into the rivers.

V. Other Issues

Reported spills

The Guam EPA receives reports of spill occurrences that reach or have the potential to reach surface water and/or marine water. The National Response Center, NPDES permittees, concerned citizens and local government officials submit these reports to the Agency. *Table 13* on the following page summarizes the number of spill occurrences, by location and type, reported to the Monitoring Program at Guam EPA.

The volume/quantity of these spills is not always reported. Wastewater/sewage and oil spills are the most numerous type of spill reported.

Table 13. Spills - number of Occurrences (2018 - 2019)

Location (Village)	Wastewater Spill	Oil (Diesel Lube, Hydraulic, motor, Fuel: No. 1-D, Non-PCB insulating, other, unknown, waste)	Diesel	Wastewater Bypass	Automotive (Unleaded) and Petroleum	Urea	Ethylene Glycol (engine coolant)	White Paint	Unknown Material	Air Cooler Water	Bilge Water	TOTAL
Asan	1	-	-	-	-	-	-	-	-	-	-	1
Maina	-	-	1	-	-	-	-	-	-	-	-	1
Mongmong	1	-	-	-	-	-	-	-	-	-	-	1
Ordot	1	-	-	-	-	-	-	-	-	-	-	1
Sinajana	1	-	-	-	-	-	-	-	-	-	-	1
Talofofo	1	-	-	-	-	-	-	-	-	-	-	1
Toto	1	-	-	-	-	-	-	-	-	-	-	1
Yona	1	-	-	-	-	-	-	-	-	-	-	1
Agana Heights	2	-	-	-	-	-	-	-	-	-	-	2
Inarajan	1	1	-	-	-	-	-	-	-	-	-	2
Tumon (Tamuning)	1	1	-	-	-	-	-	-	-	-	-	2
Yigo	2	-	-	-	-	-	-	-	-	-	-	2
Agana	1	-	1	-	-	-	-	1	-	-	-	3
Agat	2	1	-	-	-	-	-	-	-	-	-	3
Harmon	3	-	-	-	-	-	-	-	-	-	-	3
Umatac	3	-	-	-	-	-	-	-	-	-	-	3
Mangilao	6	-	-	-	-	-	-	-	-	-	-	6
Umatac/Merizo	-	-	-	8	-	-	-	-	-	-	-	8
Barrigada	9	-	-	-	-	-	-	-	-	-	-	9
Dededo	11	-	-	-	-	-	-	-	-	-	-	11
Merizo	12	-	-	-	-	-	-	-	-	-	-	12
Tamuning	18	-	-	-	-	-	-	-	-	-	-	18
Santa Rita	13	4	3	-	1	-	-	-	-	-	-	21
Apra	-	20	3	-	1	1	-	-	-	1	1	27
Piti	1	49	8	-	1	1	2	1	2	-	-	65
TOTAL	92	76	16	8	3	2	2	2	2	1	1	205

VI. Sub-Appendices

- Attachment 1. Guam Stormwater Recommended Best Management Practices Technical Report Island-wide Guam
- Figure A1. 2018-2019 IR Stations
- Attachment 2. Measurement of Turbidity, Suspended Sediments and Nutrients in Three Rivers that Drain to the Achang Preserve from the Manell Watershed

Attachment 1.

**Guam Stormwater Recommended Best Management Practices
Technical Report Island-wide Guam**



Guam Stormwater Recommended Best Management Practices Technical Report

Island Wide Guam

Prepared for:

**Guam Environmental Protection Agency
17-3304 Mariner Avenue
Tiyán, Guam 96913**

Prepared by:

**EA Engineering, Science, Technology, Inc., PBC
1001 Army Drive, Suite 103
Barrigada, Guam 96913-1402
(671) 646-5231**

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2	BMP Ranking

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ACRONYMS AND ABBREVIATIONS

BMP	best management practice
CNMI	Commonwealth of the Northern Mariana Islands
CWA	Clean Water Act
EA	EA Engineering, Science, and Technology, Inc., PBC
EPA	U.S. Environmental Protection Agency
Guam EPA	Guam Environmental Protection Agency
mL	milliliter(s)
MPN	most probable number
MS4	municipal separate storm sewer systems
TMDL	total maximum daily load
UV	ultraviolet

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EXECUTIVE SUMMARY

The Guam Environmental Protection Agency (Guam EPA) has initiated an effort to achieve compliance with the bacteria Total Maximum Daily Load requirements for 42 waterbodies impaired due to Enterococci exceedances of the Guam Water Quality Standards. The focus of this effort is to reduce Enterococci loads to Guam beaches through stormwater best management practices (BMPs), including structural methods, pollution prevention and source control. While implementation throughout the island of Guam was evaluated, this project focused on Agana Bay and the Duncga's stream.

The initial efforts of this project identified and ranked potential structural BMPs for use on Guam to reduce bacterial loads in stormwater. The results of the ranking system indicate that the most effective BMPs with respect to bacteria removal are infiltration basins, wetland structures and bioretention structures. Local conditions are an important factor for the implementation of any structural stormwater BMP and as a result the project ranking system should be used to select the most appropriate structural BMP.

With respect to the Duncga's stream location, a constructed extended detention wetland is recommended to maximize bacterial removal. The BMP design and maintenance must maximize exposure to sunlight to provide optimal bacterial removal.

Of the evaluated non-structural stormwater BMPs, sanitary sewer source control measures can have the greatest impact in the project study area. An effective source control program will require the cooperation of Guam EPA, Department of Public Works and the Guam Waterworks Authority.

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1.0 INTRODUCTION

The Clean Water Act (CWA) is administered by the U.S. Environmental Protection Agency (EPA) and requires the establishment of Total Maximum Daily Loads (TMDLs) for stormwater entering impaired waterbodies. A TMDL is based on a calculation of the maximum load of a pollutant that a water body can receive and still meet water quality standards (EPA 2018a). Because TMDL implementation is relatively new, there is a need for information on the efficacy of stormwater best management practices (BMPs) for pollutant reduction (Abbasi and Antti 2013). Stormwater BMPs encompass structural methods, such as retention basins, infiltration strips, bio-swales, and detention basins, or nonstructural methods, such as source control, stream restoration, tree planting, vegetation management, and public education and awareness. The general goal of stormwater BMPs is to regulate flow and capture pollutants in stormwater runoff.

1.1 Purpose

This report has been prepared by EA Engineering, Science, and Technology, Inc., PBC (EA) for the Guam Environmental Protection Agency (Guam EPA) to summarize the Project work and recommend effective stormwater BMPs for bacterial removal on Guam.

1.2 Document Organization

This report will identify and recommend BMPs based on their ability to reduce bacteria and feasibility of implementation on Guam.

This report is organized as follows:

- Section 1 – Introduction
- Section 2 – Background
- Section 3 – Best Management Practices on Guam
- Section 4 – Structural Best Management Practice Ranking
- Section 5 – Sampling Events
- Section 6 – Results and Discussion
- Section 7 – Conclusions and Recommendations
- Section 8 – References

It must be clearly noted that the conclusions reached by EA during this assessment are based on the review of existing available information and specific studies.

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2.0 BACKGROUND

The Guam EPA Recreational Beach Monitoring Program tests for bacteria and notifies the public of the swimming and wading quality of Guam's recreational beaches. Guam EPA has performed this community service weekly since 1974. The program is based on EPA guidance for beach monitoring and notification, as well as required performance criteria for national beach grants.

The beach monitoring effort has resulted in 42 Guam "waterbodies" being assigned high priority status on the CWA Section 303(d) Impaired Waters List. To identify the discharge parameters necessary for CWA compliance, bacteria TMDLs for the 42 waterbodies were developed and approved by the EPA in December 2010 and February 2015. The TMDL development removed Guam's 42 beaches from the CWA Section 303(d) Impaired Waters List; however, the 42 waterbodies remain impaired due to Enterococci exceedances of the Guam Water Quality Standards. In response, Guam EPA has initiated efforts to reduce Enterococci loads to Guam beaches. Appropriate BMPs, such as structural stormwater BMPs, pollution prevention and source control, will be investigated for their efficacy in reducing Enterococci concentrations in marine waters.

The current structural BMP practices acceptable on Guam have been identified in the Guam Transportation Stormwater Drainage Manual (Parsons Transportation Group 2010) and the Commonwealth of the Northern Mariana Islands (CNMI) and Guam Stormwater Management Manual (2006). This report will provide recommendation for the use of stormwater BMPs to reduce bacteria and help meet the TMDLs.

2.1 Stormwater Impacts

Stormwater runoff is generated by precipitation not infiltrated into the ground, stormwater flows are increased by land development and the construction of impervious infrastructure. A typical city block can generate up to five times more runoff than a woodland area of the same size (EPA 2003). To prevent flooding and manage increased stormwater runoff, stormwater is collected and conveyed in stormwater sewer systems. High velocities and contact with a multitude of surfaces often results in contamination such as; bacteria, viruses, chemicals, sediment, and trash. Direct discharge into waterbodies can impair those waters and pose a risk to human health, wildlife, and the environment.

In response to potential hazards, the EPA currently regulates stormwater pollutants in programs such as the National Pollutant Discharge and Elimination System Program and the CWA Section 303(d) Impaired Waters List (EPA 2017 and 2018a). As described above in Section 2.0, Guam EPA is responsible for meeting bacteria TMDLs for 42 Guam waterbodies.

2.2 Stormwater Mitigation

Stormwater BMPs are structural or nonstructural methods used to mitigate both quantity and quality of stormwater runoff. Benefits of this include protection of wetlands and aquatic ecosystems, improved quality of receiving waterbodies, conservation of water resources,

protection of human health, and flood control (EPA 2017). BMPs include public education and awareness, public involvement, illicit discharge detection and elimination, construction site stormwater runoff and control, post-construction stormwater management in new development and redevelopment, and pollution prevention (EPA 2018b).

Various stormwater BMP criteria are available, and each is based on a holistic system management approach. As recommended by the EPA, the approach should take into account the effectiveness of each BMP and its costs (EPA 2018b). The stormwater BMP criteria developed with Guam EPA and used to develop the BMP ranking included in this report consists of the following:

- Effectiveness at removing Enterococci bacteria
- Implementation cost
- Environmental impacts
- Long-term effectiveness
- Maintenance requirements.

3.0 BEST MANAGEMENT PRACTICES ON GUAM

3.1 Structural Best Management Practices

EA requested BMP information from local agencies and performed visual field assessments to determine the type of BMP structures in use on Guam. The following BMPs have been confirmed in use on Guam:

- Retention ponds
- Detention ponds
- Infiltration ponds/chambers
- Injection wells
- Oil/water separators.

The CNMI and Guam Stormwater Management Manual (2006) includes bacteria removal assessments for coastal waters in regionally recommended BMPs. The relevant data can be found in Table 1 and coincides with the general findings of documents in the literature review. Based on these findings the following BMPs categories were evaluated:

- Ponds
- Wetlands
- Infiltration
- Filtering Systems.

Table 1. Select Data from “BMP Selection Matrix 5-Pollutant Removal”

BMP Group	Critical Resource Area Specific Criteria
	Coastal Waters
Ponds	Moderate bacteria removal.
Wetlands	Provide long extended detention (>48 hours) for maximum bacteria die-off.
Infiltration	OK, but maintain 3 feet separation distance from seasonal high groundwater.
Filtering Systems	OK, moderate to high bacteria and nitrogen removal.
Open Channels	Poor bacteria removal.

3.2 Non-Structural Best Management Practices

At the time of this report, Guam is beginning adherence to the requirements stipulated by the EPA under the municipal separate storm sewer systems (MS4) permit program. The MS4 compliance will result in the development of source control measures, such as an illicit discharge survey.

At the time of this report, no documentation of institutional measures aimed at improving stormwater quality or other non-structural stormwater BMPs have been made available.

3.3 Bacteria Removal Factors

The removal or inactivation of bacteria within structural stormwater BMPs is accomplished primarily through the following mechanisms:

- Ultraviolet (UV) light
- Sedimentation
- Filtration
- Predation and biological processes.

The prevalent mechanism will vary dependent upon the individual BMP and the local conditions. The results of existing studies indicate that UV light can be a key factor in the reduction of bacteria (J.M. Hathaway et. al. 2008). The design, construction and maintenance of each BMP must be done in a manner to facilitate the factors that inhibit or remove bacteria in stormwater.

4.0 STRUCTURAL BEST MANAGEMENT PRACTICE RANKING

The Guam Stormwater Best Management Practices Performance Ranking Technical Report (EA 2018) developed a ranking scheme to aid in the selection of BMPs in Guam. A ranking scheme was chosen over the recommendation of a single BMP due to variability of conditions at potential sites.

4.1 Best Management Practice Ranking Summary

Table 2 is a summary of the five ranking categories combined in this document. The following parameters were incorporated into the ranking scheme:

- Bacteria removal
- Cost
- Environmental impact
- Lifespan
- Maintenance.

The three highest ranking scores are shown in **bold**.

Table 2. BMP Ranking

BMP Group	BMP Design	Total Rank
Pond	Micropool Extended Detention	63
	Wet Pond	67
Wetland	Shallow Marsh	68
	Extended Detention Wetland	79
	Pocket Wetland	63
Infiltration	Infiltration Chamber/Trench	66
	Infiltration Basin	93
Filters	Sand Filter	52
	Media Filter	52
	Bioretention	69
Open Channels	Dry Swale	56
	Wet Swale	51

Infiltration basins scored the highest rank in the bacteria removal category, but are not suited for locations near the shoreline where ground water levels are high. Bioretention structures also require separation from groundwater, but are typically constructed at the stormwater collection point. Coastal settings favor wetland structures which can depend on high groundwater to maintain a constant water level. The varying suitability of each BMP allows for a unique approach to mitigation which incorporates multiple strategies.

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5.0 SAMPLING EVENTS

The Project work summarized in this document included four sampling events. The sampling objectives were to determine the source of bacterial contamination in Agana Bay (Figure 1) and to evaluate areas of high concentration. The four sampling events are listed below:

- Wet weather sampling event at six EPA designate sites along Agana Bay (Figure 2).
 - Analysis:
 - Six samples for Bio Markers [*B. dorei* (human), *EPA Developed Assay* (human), *C. marimammalium* (gull)],
 - One sample for Bio Markers [*B. Bacteroides* and *Prevotella* (dog), *H. pametensis* (bird)],
 - Six samples for *Enterococcus*
- Dry weather sampling event at six EPA designate sites along Agana Bay and three locations with Dungca's stream (Figure 3).
 - Analysis:
 - Six samples for Bio Markers [*B. dorei* (human), *B. Bacteroides* and *Prevotella* (dog), *H. pametensis* (bird)],
 - Nine samples for *Enterococcus*
- Wet weather stormwater inlet sampling event (Figure 4).
 - Analysis:
 - Four samples for *Enterococcus*
- Docomo Pacific basement groundwater sampling event (Figure 4).
 - Analysis:
 - One sample for *Enterococcus*.

A wet weather sampling event was performed to identify the impact of active run-off and potential sanitary overflows have on the bacteria concentration when compared to dry weather conditions. Typical dry weather sampling events outlined by the EPA require 72 hours of dry weather, but due to Guam's wet tropical climate this requirement was reduced to prevent delays. The dry weather parameters for this study were established as 48 hours with less than 0.1 inches of rain, and at least 24 hours with no rain prior to sampling. The weather station at the Guam international airport was used as a basis for monitoring due to its proximity to the Agana Bay.

The wet weather sampling event was conducted first due to weather conditions. Based on the wet weather sample results, three additional *Enterococcus* samples were added to the dry weather sampling event in order to further evaluate Dungca's stream.

To further evaluate contaminated source streams and potential illicit discharges, samples were collected in stormwater inlets and an open groundwater source in the basement of the Docomo Pacific Headquarters Building, located at 890 South Marine Corps Drive, Tamuning (Figure 4) The samples were analyzed for *Enterococcus* only. The stormwater inlet and Docomo sampling

points were chosen for their proximity to Dungca's Stream. Each inlet sample location was selected to capture flow from distinct areas and the Docomo sample to provide a background groundwater concentration of *Enterococcus*.

6.0 RESULTS AND DISCUSSION

6.1 Analytical Results

Wet Weather Sampling Event – 6 April 2018

Human marker *B. dorei* was detected in all wet weather samples analyzed for bio markers. Concentrations were detected between 227 and 8,450 copies/milliliter (mL), with the two highest concentration detected in the two western most samples. Enterococci were detected in four of the six wet sampling locations, 10 to 414 Most Probable Number (MPN)/100 mL. The higher concentration of human markers detected in the western portion of the Bay does not coincide with the detection of *Enterococcus* which was low (10 MPN/100 mL) and non-detect in the two western samples. The higher detection of human markers in the western portion of the Bay may be a result of inactive human biomarkers origination from the wastewater treatment plant in that area.

The Gull marker, *C. marimammalium* was not detected in any of the wet weather samples. One sample, collected from the stormwater outfall Dungca's Outlet was tested for both dog markers *B. Bacteroides* and *Prevotella*, and bird marker *H. pametensis*. Both the dog and bird markers were detected in low concentrations in the sample.

The *Enterococcus* sample within Dungca's stream, 414 MPN/100 mL, was an order of magnitude greater than the other samples, which indicates that fecal pollution is being conveyed through stormwater via the stream to the Agana Bay. The dominant microbial source tracking markers were human, indicating that the source of the fecal pollution is mainly sewage. Due to the elevated results and the intended project focus on Dungca's stream, consecutive stormwater sampling focused on identifying physical sources within the watershed by sampling from wastewater and stormwater infrastructure (e.g., manholes and culverts). The laboratory reports can be found in Appendix A.

Dry Weather Sampling Event – 1 November 2018

Of all the samples collected and analyzed for biomarkers, human marker *B. dorei* was detected in low levels in samples collected from the three Western most locations. The results for the samples collected from sites N-08, N-09, and N-26 (Figure 3) show detection for human markers. However, the concentrations were not quantifiable. Dog markers *B. Bacteroides* and *Prevotella* were detected at sites N-08 and N-09. The presence of the bird marker *H. pametensis* was detected in all the samples collected during the dry weather event. In the samples that dog and bird markers were detected, it is noted that the concentrations were not quantifiable. Enterococcus was detected in five of the nine dry weather sampling locations, 10 to 211 MPN/100 mL. The concentrations of cultured Enterococci are significantly lower in the dry weather samples than the wet weather samples. The same is true for the quantification of human fecal markers indicating that stormwater is a source of human fecal bacteria pollution.

When compared to the corresponding wet weather *Enterococcus* sample, the dry weather results were lower, with the exception of the western most sample which was non-detect (<10MPN/100 mL) for the wet weather sample and 10 MPN/100 mL for the dry. These results demonstrate an increase in *Enterococcus* loading from human origin (i.e., sewage pollution) to East Agana Bay during rain events. The Dungca's stream sample was again the highest among the original sampling sites (119 MPN/100 mL) indicating that the stream conveys fecal pollution to the Bay during both wet and dry weather conditions.

To further evaluate the stream, three additional samples were collected during the dry weather event; one at each of the two culvert outfall points and another taken from a manhole just upstream of the main culvert. Each culvert sample result was comparable to the Dungca's stream sample at 95 and 74 MPN/100 mL, however; the manhole hole sample was almost twice as high at 211 MPN/100 mL. This suggests that the manhole is closer to the contamination source.

Stormwater Inlet Sampling Event – 16 October 2018

Four stormwater inlets, upstream of the subject manhole sampled during the dry weather event, were sampled during wet weather. *Enterococcus* was detected in all four of the inlet samples collected at respectively high concentrations ranging from 3,300 to >24,196 MPN/100 mL. Two of the samples exceeded the method detection limit, these samples were taken from stormwater inlets conveying water along each side of Marine Corps Drive.

Docomo Pacific Headquarters Building Sampling Event - 31 October 2018

The Docomo Pacific Headquarters Building is located approximately 165 meters from the Dungca's stream primary outfall. The building was constructed with a basement requiring a large sump to pump local ground water from its foundation. This location was selected as a sampling location to determine if shallow groundwater was contaminated with enterococci from potential upgradient sources.

The sample result was non-detect for *Enterococcus* and eliminates local shallow groundwater as a potential source. During an initial site visit and the sample collection event it was noted by separate parties that a chlorine odor was present in the sump chamber.

6.2 Literature Review and Best Management Practice Ranking

The available documentation reviewed in developing this project ranking system indicate that bioretention, wetlands and infiltration structures result in statistically significant removal of bacteria, primarily when good UV exposure is present. Infiltration type BMPs are the predominant type of BMP currently used on Guam, but their performance is dependent on local site conditions such as geology and groundwater levels.

The developed ranking system should be used to evaluate new stormwater BMP location on a case-by-case basis. Taking into consideration the local conditions at Dungca's stream, most

importantly the shallow groundwater table, an extended detention wetland ranks highest and is recommended to reduce bacterial loading to Agana Bay.

6.3 Visual Evidence

During Project field activities visual evidence of a sanitary overflow into Dungca's stream was identified and photographed. Photo #1 depicts a sanitary manhole adjacent to Dungca's streams primary outfall. A trail of sanitary debris is clearly seen around the manhole and accumulated on the fence bordering the stream.



Photo #1 – Sanitary manhole adjacent to Dungca's stream and Guam Waterworks Authority Bayside Pump Station showing visual evidence of overflow into the stream.

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7.0 CONCLUSIONS AND RECOMMENDATIONS

Stormwater may be impacted by multiple pollutant sources which can be difficult to identify and mitigate. While active stormwater treatment options exist and can significantly reduce pollutants, they are cost prohibitive and not practical for Guam. Passive structural and non-structural BMPs can reduce stormwater bacterial pollutants, however; one individual practice is unlikely to provide enough reduction to meet regulatory limits. Therefore, a successful stormwater bacteria reduction program should incorporate multiple strategies of which the cumulative effect can have a significant impact.

Local conditions are an important factor for the implementation of any structural stormwater BMP and as a result the project ranking system should be used to select the most appropriate BMP. Of the non-structural stormwater BMPs, source control measures focused on sanitary sewers can have the greatest impact in the project study area.

7.1 Best Management Practices Recommendations

Infiltration, wetland, and bioretention structural BMPs ranked highest based on the evaluation parameters selected in this project. Each structure is different with respect to the site suitability and will provide options as local project determinations are made. UV exposure is an important factor and should be maximized in all designs when feasible. The aforementioned BMP discussion is focused on end of stream treatment, but all stormwater basins can benefit through the implementation of treatment of the collection point. Systems such as bio-retention basins, tree boxes begin the stormwater treatment process before it enters the system and can have a cumulative effect when paired with endpoint BMPs. These systems should be implemented with a focus on bacteria removal by using an adequate amount of high-quality soil with organics to support a good bacteria population.

With respect to the Dungca's stream location, a constructed extended detention wetland is recommended to maximize bacterial removal. The BMP design and maintenance must maximize exposure to sunlight as UV exposure is imperative for the inactivation of bacteria. The Dungca's stream is located in a popular public recreation area, a constructed wetland is an opportunity for beach beautification and public education. The wetland design should incorporate public foot traffic with constructed paths throughout. These paths can be fitted with informative signs discussing the functionality of the wetland and the types of plants and animals which will inevitably use the wetland as habitat.

Based on agency reporting and visual evidence, sanitary sewer source control measures can have significant impact on the mitigation of bacteria in stormwater. While bacteria contributions to stormwater can come from wildlife, pets, and naturally occurring systems, these are difficult to assess and to control. The bacteria removal efficiency of structural stormwater BMPs of all types varies widely within the literature. There is no single type of BMP that consistently demonstrates the ability to reduce bacteria to Guam Water Quality Standards, and so it is unlikely that structural BMPs alone will reduce bacteria loads to within the TMDL limits. Therefore, it is recommended that source control be the primary focus to reduce bacteria

loadings. The total *Enterococcus* monitoring and microbial source tracking samples collected as part of this study show that human contributions through sanitary sewer overflows are the likely dominant source of fecal bacteria loading to the East Agana Bay. Based on the results of the monitoring data, it is recommended that improvements to the sanitary sewer infrastructure be the given the top priority to restoring the Bay.

7.2 Agency Responsibilities

An effective source control program will require the cooperation of Guam EPA, Department of Public Works, and the Guam Waterworks Authority. Guam EPA serves as the regulatory body not only to monitor Guam waters, but to enforce regulations and resolve issues which contribute bacterial loading to Guam's stormwaters.

Guam is in the process of incorporating an MS4 permit for which Guam Department of Public Works is responsible. The MS4 requirements include stormwater illicit discharge surveys which are a valuable part of source control and can identify illicit discharges.

The identification and resolution of sanitary sewer leaks and overflows is the responsibility of Guam Waterworks Authority. Resolving these issues requires a long-term approach and large amounts of funding to repair and upgrade infrastructure.

7.3 Additional Sampling

It is recommended that further sampling for bio-markers and upstream flows at the two storm sewers located at the intersection of Marine Corps Drive and Governor Camacho Road be performed. This may provide important information in identifying sources of Enterococci.

It is also recommended that the water underneath the Docomo Headquarters Building be analyzed for chlorine concentrations. Observations during sampling indicated that flow from the sump under the building and surrounding drainage area could be diluting the bacteria concentrations in the stormwater system. The chlorine concentration in the groundwater may also indicate a leak in a potable water distribution line near the building.

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Figures

LOCATION MAP



VICINITY MAP



Legend

 Duncas's Outlet Stream

 Project Area - Agaña Bay



EA Engineering, Science, and Technology, Inc.
 1001 Army Drive, Suite 101,
 Bangor, ME 04913-1402
 Telephone: (207) 646-5231
 Facsimile: (207) 646-5230

Guam Stormwater Recommended
 Best Management Practices
Figure 1
 Site Vicinity Map

Drawing No.
 Fig. 1, Site Map

Date: 11/09/18
 Drawn By: TCC

EA Project No. 1590001



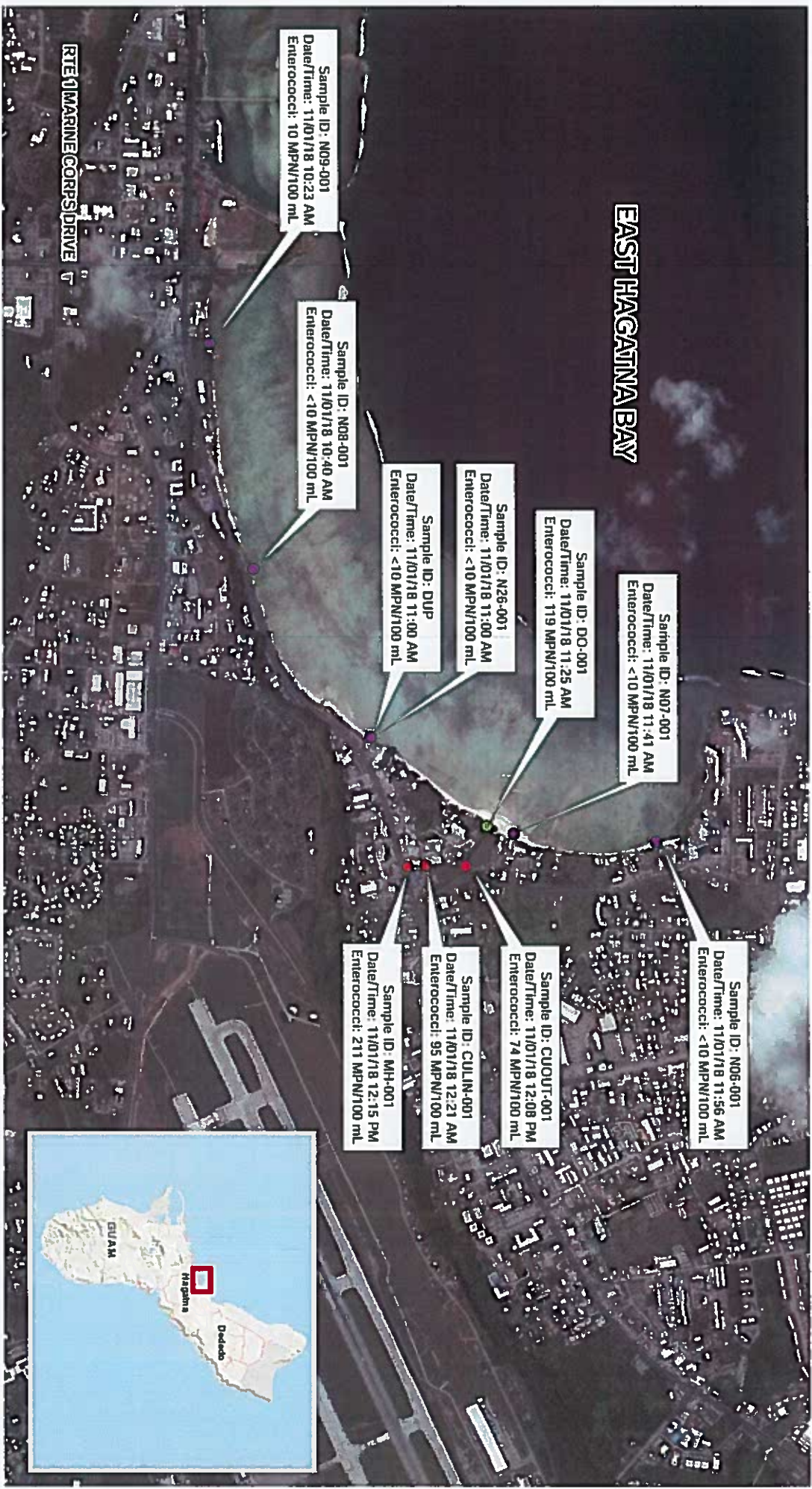
- Legend**
- Stormwater Outlet
 - Beach Sampling Sites



<p>EA Engineering, Science, and Technology, Inc. 1001 Army Drive, Suite 102, Barrigada, 96913-1402 Telephone: (671) 646-5231 Facsimile: (671) 646-5230</p>	<p>Guam Stormwater Recommended Best Management Practices</p>	
	<p>Figure 2 Wet Weather Sampling Sites</p>	<p>Date: 04/23/18 Drawn By: TCC EA Project No. 1590001</p>



EAST HAGATINA BAY



Legend

- Beach Sampling Sites
- Stormwater Outlet
- Dry Weather Sampling (Addl. Sites)



1993 Guam Geologic Network Transverse Section

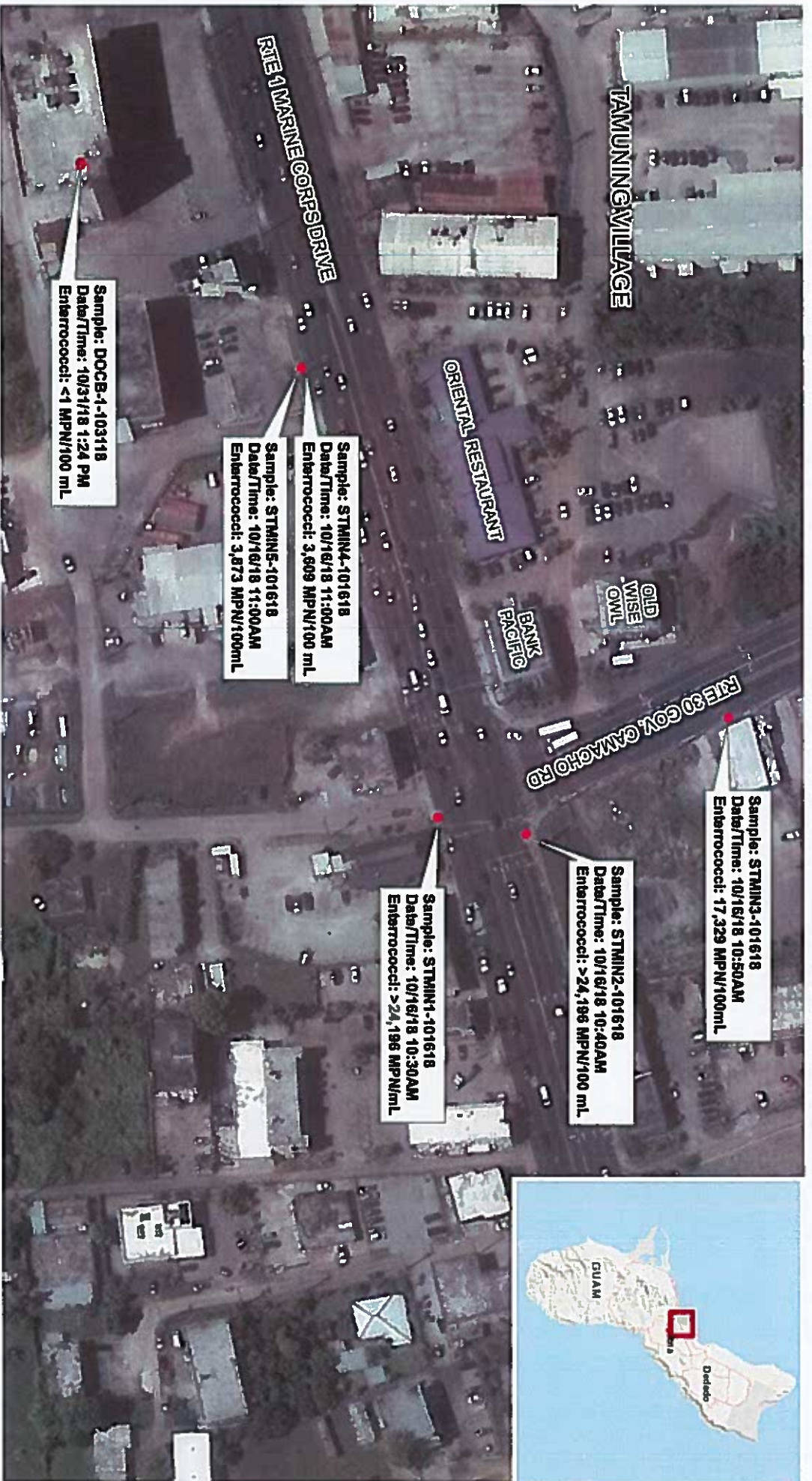


EA Engineering, Science, and Technology, Inc.
 1001 Army Drive, Suite 102,
 Barrigada, 96913, 1402
 Telephone: (671) 646-5231
 Facsimile: (671) 646-5230

Guam Stormwater Recommended
 Best Management Practices

Figure 3
 Dry Weather Sampling Locations

Drawing No. Fig. 3, Dry Weather Sampling Sites Date: 11/09/18 Drawn By: TCC EA Project No. 1560001



EA Engineering, Science, and Technology, Inc.
1001 Army Drive, Suite 100,
Bangor, 96913-1402
Telephone: (871) 646-5231
Facsimile: (871) 646-5230

Guam Stormwater Recommended Best Management Practices
Figure 4
Stormwater Inlet and Ducto Sampling Sites

Drawing No. **1028/18** Date: 10/28/18
Drawn By: TCC
EA Project No. 1560001

Fig. 1. Agaña Bay Stormwater fields

EAST HAGATNA BAY



Sample ID: N09-001
 Date/Time: 04/06/18 2:10 PM
 B. Dorei (Human): $8.45 \times 10^{\wedge}03$ cfu/100 mL
 EPA Developed Assay : $7.53 \times 10^{\wedge}02$ cfu/100 mL
 C. maritimmallum (gull): ND

Sample ID: N08-001
 Date/Time: 04/06/18 1:50 PM
 B. Dorei (Human): $8.35 \times 10^{\wedge}02$ cfu/100 mL
 EPA Developed Assay : DND
 C. maritimmallum (gull): ND

Sample ID: N26-001
 Date/Time: 04/06/18 1:30 PM
 B. Dorei (Human): $1.03 \times 10^{\wedge}03$ cfu/100 mL
 EPA Developed Assay : DND
 C. maritimmallum (gull): ND

Sample ID: DC-002
 Date/Time: 04/06/18 12:50PM
 B. Dorei (Human): $4.01 \times 10^{\wedge}02$ cfu/100 mL
 EPA Developed Assay : ND
 C. maritimmallum (gull): ND
 B. Bacteroides and Prevotella (dog): DND
 H. pamestensis (bird): DND

Sample ID: DC-001
 Date/Time: 04/06/18 12:50PM
 B. Dorei (Human): $4.13 \times 10^{\wedge}02$ cfu/100 mL
 EPA Developed Assay : DND
 C. maritimmallum (gull): ND


Sample ID: N07-001
 Date/Time: 04/06/18 11:55AM
 B. Dorei (Human): $3.66 \times 10^{\wedge}02$ cfu/100 mL
 EPA Developed Assay : DND
 C. maritimmallum (gull): ND

Sample ID: N06-001
 Date/Time: 04/06/18 11:05AM
 B. Dorei (Human): $2.27 \times 10^{\wedge}02$ cfu/100 mL
 EPA Developed Assay : DND
 C. maritimmallum (gull): ND



- Legend**
- Beach Sampling Sites
 - Stormwater Outlet
 - Sewer Pump Station
 - SewerMain
 - Sewer Lateral
- ND: Not Detected
 DND: Detected Not Quantified



 EA Engineering, Science and Technology, Inc. 1001 Army Drive, Suite 103, Bangor, 96913-1402 Telephone: (871) 648-5231 Facsimile: (871) 648-5230	Drawing No. Fig. 5. Wet Weather gPCR	Date: 08/10/19
	Guam Stormwater Recommended Best Management Practices	Figure 5 Wet Weather Sampling Locations Results for Genetic Markers

EAST HAGATINA BAY



Sample ID: N09-001
 Date/Time: 11/01/18 10:23 AM
 B. Dorei (Human): DNO
 B. Bacteroides and Prevotella (dog): DNO
 H. parvitenis (bird): DNO

Sample ID: N08-001
 Date/Time: 11/01/18 11:40 AM
 B. Dorei (Human): DNO
 B. Bacteroides and Prevotella (dog): DNO
 H. parvitenis (bird): DNO

Sample ID: N26-001
 Date/Time: 11/01/18 11:00 AM
 B. Dorei (Human): DNO
 B. Bacteroides and Prevotella (dog): ND
 H. parvitenis (bird): DNO

Sample ID: D0-001
 Date/Time: 11/01/18 11:25 AM
 B. Dorei (Human): ND
 B. Bacteroides and Prevotella (dog): ND
 H. parvitenis (bird): DNO

Sample ID: N07-001
 Date/Time: 11/01/18 11:41 AM
 B. Dorei (Human): ND
 B. Bacteroides and Prevotella (dog): ND
 H. parvitenis (bird): DNO

Sample ID: N06-001
 Date/Time: 11/01/18 11:56 AM
 B. Dorei (Human): ND
 B. Bacteroides and Prevotella (dog): ND
 H. parvitenis (bird): DNO



- Legend**
- Beach Sampling Sites
 - Stormwater Outlet
 - Sewer Pump Station
 - Sewer Main
 - Sewer Lateral
- ND: Not Detected
 DNO: Detected Not Quantified



 EA Engineering, Science, and Technology, Inc. 1001 Army Drive, Suite 103, Bangagade, 96913-1402 Telephone: (877) 448-5231 Facsimile: (877) 448-5230	Drawing No. Fig. 6_Dry Weather gPCR	Date: 09/10/18	Drawn By: TCC	EA Project No. 1560001
	Guam Stormwater Recommended Best Management Practices Figure 6 Dry Weather Sampling Locations Results for Genetic Markers			



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**Appendix A
Laboratory Analysis Reports**

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Water and Environmental Research Institute
of the Western Pacific

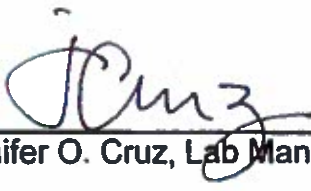
April 6, 2018

EA Engineering
1001 Army Drive, Suite 103
Barrigada, Guam 96913-1402

Sample(s) collected on: 6-Apr-18 10:00 AM
Sample(s) received on: 6-Apr-18 3:00 PM
Sample Site: East Hagatna Bay

Sample ID/Description	Nitrate	Phosphate	Enterococci	WERI Lab ID
	NO ₃ /NO ₂	PO ₄ -P		
	mg/L as N	mg/L as P	MPN / 100 mL	
N06-001			20	W2018-0438.1
N08-001			10	W2018-0438.2
N07-001			20	W2018-0438.3
D0-001			414	W2018-0438.4
D0-002			505	W2018-0438.5
N09-001			<10	W2018-0438.6
FB-001			<10	W2018-0438.7
N26-001			<10	W2018-0438.8


Mallery Duenas, Chemist II


Jennifer O. Cruz, Lab Manager



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Water and Environmental Research Institute
of the Western Pacific

April 6, 2018

EA Engineering
1001 Army Drive, Suite 103
Barrigada, Guam 96913-1402

Sample(s) collected on: 6-Apr-18 10:00 AM
Sample(s) received on: 6-Apr-18 3:00 PM
Sample Site: East Hagatna Bay

Sample ID/Description	Nitrate	Phosphate	Enterococci	WERI Lab ID
	NO3/NO2	PO4-P		
	mg/L as N	mg/L as P	MPN / 100 mL	
N06-001			20	W2018-0438.1
N08-001			10	W2018-0438.2
N07-001			20	W2018-0438.3
D0-001			414	W2018-0438.4
D0-002			505	W2018-0438.5
N09-001			<10	W2018-0438.6
FB-001			<10	W2018-0438.7
N26-001			<10	W2018-0438.8

Mallery Duenas, Chemist II

Jennifer O. Cruz, Lab Manager

October 19, 2018

EA Engineering, Science, & Technology Inc. PBC
1001 Army Drive, Suite 103
Barrigada, Guam 96913

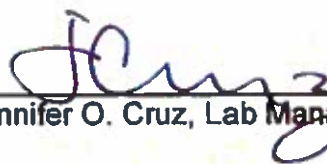
Sample(s) collected on: 16-Oct-18 10:00 AM
Sample(s) received on: 16-Oct-18 1:39 PM
Sampling Site: Tamuning

Sample ID/ Description	Enterococcus		WERI Lab ID
	MPN per 100 mL		
	Method	SM9230D	
Analysis Date	10/16/2018		
STMIN1-101618	>24196		W2019-0043.1
STMIN2-101618	>24196		W2019-0043.2
STMIN3-101618	17329		W2019-0043.3
STMIN4-101618	3609		W2019-0043.4
STMIN5-101618	3873		W2019-0043.5

P(+) Present A(-) Absent



Cris H. Tagudin, Lab Technician



Jennifer O. Cruz, Lab Manager

The University of Guam-WERI Laboratory is certified by Guam Environmental Protection Agency (GEPA).
Certificate No. 18-003 valid through 9 December 2020.

WATER AND ENVIRONMENTAL RESEARCH INSTITUTE OF THE WESTERN PACIFIC

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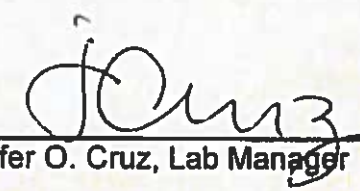
November 1, 2018

EA Engineering, Science, & Technology Inc. PBC
1001 Army Drive, Suite 103
Barrigada, Guam 96913

Sample(s) collected on: 31-Oct-18 1:24 PM
Sample(s) received on: 31-Oct-18 3:15 PM
Sampling Site: Docomo Basement

Sample ID/ Description	Enterococcus		WERI Lab ID
	MPN per 100 mL		
	Method	SM9230D	
	Analysis Date	10/31/2018	
DOCB-1-103118	<1.0		W2019-0080


Mallery Buenas, Chemist II


Jennifer O. Cruz, Lab Manager

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November 5, 2018


EA Engineering, Science, & Technology Inc. PBC
1001 Army Drive, Suite 103
Barrigada, Guam 96913

Sample(s) collected on: 1-Nov-18 10:23 AM
Sample(s) received on: 1-Nov-18 2:16 PM
Sampling Site: East Hagatna Bay

Sample ID/ Description	Enterococcus		WERI Lab ID
	MPN per 100 mL		
	Method	SM9230D	
	Analysis Date	11/1/2018	
DUP	< 10		W2019-0085.1
N06-001	< 10		W2019-0085.2
N07-001	< 10		W2019-0085.3
DO-001	119		W2019-0085.4
N26-001	< 10		W2019-0085.5
N08-001	< 10		W2019-0085.6
N09-001	10		W2019-0085.7
MH-001	211		W2019-0085.8
CULIN-001	95		W2019-0085.9
CUOUT-001	74		W2019-0085.10

P(+) Present A(-) Absent


Mallery Duenas, Chemist II


Jennifer O. Cruz, Lab Manager

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 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733



Dog Fecal Quantification ID

Detection and quantification of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR)
 DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: April 30, 2018
 Report Generated: August 10, 2018

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8H08015	DO-002	Dog Bacteroidetes ID: Target 1	DNQ	Detected

Limitation of Damages – Repayment of Service Price

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Revision 1.2
 Effective Date 11/2/17



4985 SW 74th Court, Miami, FL 33155 USA
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733



**ISO 17025 Accredited
 Testing Laboratory**

Preliminary Interpretation of Dog Fecal “Quantification” ID Results

Detection and quantification of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: April 30, 2018
 Report Generated: August 10, 2018

Sample ID	INTERPRETATION	
	Concentration of Dog Fecal Pollution in Sample	Comment
DO-002	Low Concentration	Low levels of Dog fecal biomarker(s)

The opinions/interpretations identified/expressed in this report are outside the scope of this organization’s A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: August 10, 2018

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Dog Bacteroidetes “Quantification” ID™

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.¹ Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Dog Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.^{2,3,4,5,6} Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in dog. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in dog.^{2,3,5,6} As such, these bacterial strains can be used as indicators of dog fecal contamination.

One of the advantages of the Dog Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the dog *Bacteroidetes* gene biomarker is not present.

References

- ¹ Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions**. Appl. Environ. Microbiol. (2002) 68: 5796-5803.
- ² Bernhard, A.E., and K.G. Field (2000a). Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Applied and Environmental Microbiology, 66: 1,587-1,594.
- ³ Bernhard, A.E., and K.G. Field (2000b). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. Applied and Environmental Microbiology, 66: 4,571-4,574.
- ⁴ Kreader, C.A. (1995). Design and evaluation of *Bacteroides* DNA probes for the specific detection of human fecal pollution. Applied and Environmental Microbiology, 61: 1,171-1,179.
- ⁵ Fogarty, Lisa R., Voytek, Mary A. **Comparison of *Bacteroides-Prevotella* 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.
- ⁶ Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Santo Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification** Appl. Environ. Microbiol. 2005 71: 3184-3191.



4985 SW 74th Court, Miami, FL 33155 USA
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733



Bird Fecal Quantification ID

Detection and quantification of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR)
 DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: April 30, 2018
 Report Generated: August 10, 2018

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8H08014	DO-002	Bird Fecal ID	DNQ	Detected

Limitation of Damages – Repayment of Service Price

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Revision 1.2
 Effective Date 11/2/17



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 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733



**ISO 17025 Accredited
 Testing Laboratory**

Preliminary Interpretation of Bird Fecal “Quantification” ID Results

Detection and quantification of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
Date Received: April 30, 2018
Report Generated: August 10, 2018

Sample ID	INTERPRETATION	
	Concentration of Bird Fecal Pollution in Sample	Comment
DO-002	Low Concentration	Low levels of Bird fecal biomarker(s)

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: August 10, 2018

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Bird Fecal ID™ Quantification

The genus *Helicobacter* is a group of gram-negative, microaerophilic bacteria that were initially classified under the *Campylobacter* genus prior to 1989. Since then, they have been reclassified into the genus *Helicobacter* after 16S rRNA sequencing differentiated them from other *Campylobacter* species. This group of bacteria typically have a spiral, curved or fusiform morphology with multiple flagella allowing them to rapidly maneuver in the intestinal mucous lining of their hosts. *Helicobacter* species colonize the gastrointestinal tract of mammals and birds and are shed in feces. There are approximately 20 strains of *Helicobacter*¹. Certain strains, such as *Helicobacter pylori*, are pathogenic to humans causing chronic gastritis, peptic ulcers and stomach cancer.

The Bird Fecal Quantification ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Helicobacter* genus are specific to wild birds. These *Helicobacter* sequences can be used as indicators of bird fecal contamination. Several species have been isolated from specific animal hosts such as *H. fennelliae* from humans, *H. hepaticus* from mice and *H. felis* from cats and dogs.¹ The Bird Fecal Quantification ID™ service targets a bird-associated gene biomarker in *Helicobacter pametensis*.² The biomarker is present at different degrees in the feces of various birds including but not limited to gull, goose, chicken, pigeon and duck.

One of the advantages of the Bird Fecal Quantification ID™ service is that the entire population of *Helicobacter* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish.

Accuracy of the results is possible because the method uses qPCR DNA technology. qPCR simultaneously confirms and quantifies the bird-associated gene biomarker. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genome to be detected. This qPCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve indicates that the bird-associated *Helicobacter* gene biomarker is not present.

References

¹ Goldman, E. and Green, L. H. (2009). *Practical Handbook of Microbiology* (2nd ed) . Boca Raton, FL: CRC Press.

² Seymour, C., Lewis, R.G., Kim, M., Gagnon, D.F., Fox, J.G., Dewhirst, F.E., and Paster, B.J. Isolation of *Helicobacter* Strains from Wild Bird and Swine Feces. *Appl. Environ. Microbiol.* (1994) 60:3, 1025-1028.



4985 SW 74th Court, Miami, FL 33155 USA
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



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Human Fecal Quantification ID

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: April 30, 2018 ND: Not Detected
 Report Generated: May 18, 2018 DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8E07001	DO-002	Human Bacteroidetes ID: Dorei	4.01E+02	Detected
SM-8E07002	FB-001	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8E07003	N06-001	Human Bacteroidetes ID: Dorei	2.27E+02	Detected
SM-8E07004	N07-001	Human Bacteroidetes ID: Dorei	3.66E+02	Detected
SM-8E07005	DO-001	Human Bacteroidetes ID: Dorei	4.13E+02	Detected
SM-8E07006	N26-001	Human Bacteroidetes ID: Dorei	1.03E+03	Detected
SM-8E07007	N08-001	Human Bacteroidetes ID: Dorei	8.35E+02	Detected
SM-8E07008	N09-001	Human Bacteroidetes ID: Dorei	8.45E+03	Detected
SM-8E07009	DO-002	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8E07010	FB-001	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8E07011	N06-001	Human Bacteroidetes ID: EPA	DNQ	Detected
SM-8E07012	N07-001	Human Bacteroidetes ID: EPA	DNQ	Detected
SM-8E07013	DO-001	Human Bacteroidetes ID: EPA	DNQ	Detected
SM-8E07014	N26-001	Human Bacteroidetes ID: EPA	DNQ	Detected
SM-8E07015	N08-001	Human Bacteroidetes ID: EPA	DNQ	Detected
SM-8E07016	N09-001	Human Bacteroidetes ID: EPA	7.53E+02	Detected

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Revision 1.2
 Effective Date 11/2/17



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 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



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Preliminary Interpretation of Human Fecal “Quantification” ID Results

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
Date Received: April 30, 2018
Report Generated: May 18, 2018

Sample ID	INTERPRETATION	
	Concentration of Human Fecal Pollution in Sample	Comment
DO-002	Low Concentration	Low levels of Human fecal biomarker(s)
FB-001	Not Detected	Human fecal biomarker not detected
N06-001	Low Concentration	Low levels of Human fecal biomarker(s)
N07-001	Low Concentration	Low levels of Human fecal biomarker(s)
DO-001	Low Concentration	Low levels of Human fecal biomarker(s)
N26-001	Low Concentration	Low levels of Human fecal biomarker(s)
N08-001	Low Concentration	Low levels of Human fecal biomarker(s)
N09-001	Low Concentration	Low levels of Human fecal biomarker(s)

The opinions/interpretations identified/expressed in this report are outside the scope of this organization’s A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: May 18, 2018

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non -detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species: *B. dorei*

The Human Bacteroidetes ID™ Species: *B. dorei* service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.^{1,2} The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.³ The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world^{1,3,4} makes the Human Bacteroidetes ID™ Species: *B. dorei* service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.⁵ Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.^{3,5,6,7,8} Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.^{3,6} As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species: *B. stercoris*,
Human Bacteroidetes ID™ Species: *B. fragilis*, and
Human Bacteroidetes ID™ Species: *B. thetaiotaomicron*.

¹Boehm, A., Fuhman, J., Mrse, R., Grant, S. Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ Sci Technol.* 2003 37: 673-680

²Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. *Bacteroides dorei* sp. nov., isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 2006 56: 1639-1641.

³Bernhard, A., Field, K. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 2000b 66: 4571-4574.

⁴Ahmed, w., Masters, N., Toze, S. Consistency in the host specificity and host sensitivity of the *Bacteroides* HF183 marker for sewage pollution tracking. *Lett. Appl. Microbiol.* 2012 55: 283-289.

⁵Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. Microbial Source Tracking: Current Methodology and Future Directions. *Appl. Environ. Microbiol.* 2002 68: 5796-5803.

⁶Bernhard, A., Field, K. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Appl. Environ. Microbiol.* 2000a 66: 1587-1594.

⁷Fogarty, L., Voytek, M. A Comparison of *Bacteroides-Prevotella* 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species. *Appl. Environ. Microbiol.* 2005 71: 5999-6007.

⁸Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., et al. Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification. *Appl. Environ. Microbiol.* 2005 71: 3184-3191.

Human Bacteroidetes ID™: EPA Developed Assay

The Human Bacteroidetes ID™: EPA Developed Assay service targets a functional gene biomarker in *Bacteroidales*-like anaerobic bacteria that is present in high concentrations in the human gut. The U.S. Environmental Protection Agency (U.S. EPA) was the first to target the biomarker using Polymerase Chain Reaction (PCR) technology in order to detect ground and surface waters impacted by human fecal pollution.¹ Since its development, the assay has been used successfully around the U.S to identify fecal pollution originating from human sources, such as sewage and septage wastewaters.

The U.S. EPA Developed assay has been shown to be highly associated with human fecal pollution. It has successfully been validated in multiple nationwide studies using at least 300 individual reference fecal material from 22 different animal species known to commonly contaminate environmental waters.^{1,2} A reported 99.2% specificity to human fecal material makes this one of the leading assays to confirm the presence of fecal contamination that is of human origin.¹ The *Bacteroidales*-like bacteria is widely distributed. It was detected in 100% of hundreds of sewage and human reference fecal samples collected from more than 20 human populations, making it highly sensitive. Internal validations have also been conducted on hundreds of wastewater, human and animal host fecal samples archived in the Source Molecular fecal bank.

Fecal anaerobic bacteria are considered for several reasons an interesting alternative to more traditional fecal indicator organisms such as *E. coli* and *Enterococci*.³ Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems.³ This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™: EPA Developed Assay service is designed around the principle that fecal *Bacteroidales*-like bacteria are found in large quantities in feces of warm-blooded animals.^{4,5} Furthermore, certain strains have been shown to be associated with humans.^{4,5} As such, these bacterial strains can be used as indicators of human fecal contamination. An advantage of the Human Bacteroidetes ID™ service is that the entire portion of water sampled is filtered to concentrate bacteria. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates. This is an advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available, detected and quantified.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species: *B. dorei*,
Human Bacteroidetes ID™ Species: *B. fragilis*, and
Human Bacteroidetes ID™ Species: *B. stercoris*

¹ Shanks, O., Kelly, C., Sivaganesan, M., Varma, M. and Haugland, R. Quantitative PCR for Genetic Markers of Human Fecal Pollution. *Appl. Environ. Microbiol.* 2009 75: 5507-5513.

² Layton, B., Cao, Y., Ebentier, D., Hanley, K., Ballesté, E., Brandão, J., *et al.* Performance of Human Fecal Anaerobe-Associated PCR-Based Assays in a Multi-Laboratory Method Evaluation Study. *Water Research.* 2013 In Press.

³ Scott, T., Rose, J., Jenkins, T., Farrah, S. and Lukasik, J. Microbial Source Tracking: Current Methodology and Future Directions. *Appl. Environ. Microbiol.* 2002 68: 5796-5803.

⁴ Bernhard, A., Field, K. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Appl. Environ. Microbiol.* 2000a 66: 1587-1594.

⁵ Bernhard, A., Field, K. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides*-*Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 2000b 66: 4571-4574.



4985 SW 74th Court, Miami, FL 33155 USA
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: Info@sourcemolecular.com



Gull Fecal Quantification ID

Detection and quantification of the fecal associated Gull gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: April 30, 2018 ND: Not Detected
 Report Generated: May 18, 2018

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8E07017	DO-002	Gull Fecal ID	ND	Not Detected
SM-8E07018	FB-001	Gull Fecal ID	ND	Not Detected
SM-8E07019	N06-001	Gull Fecal ID	ND	Not Detected
SM-8E07020	N07-001	Gull Fecal ID	ND	Not Detected
SM-8E07021	DO-001	Gull Fecal ID	ND	Not Detected
SM-8E07022	N26-001	Gull Fecal ID	ND	Not Detected
SM-8E07023	N08-001	Gull Fecal ID	ND	Not Detected
SM-8E07024	N09-001	Gull Fecal ID	ND	Not Detected

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Revision 1.2
 Effective Date 11/2/17



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 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



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Preliminary Interpretation of Gull Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Gull gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: April 30, 2018
 Report Generated: May 18, 2018

Sample ID	INTERPRETATION	
	Concentration of Gull Fecal Pollution in Sample	Comment
DO-002	Not Detected	Gull fecal biomarker not detected
FB-001	Not Detected	Gull fecal biomarker not detected
N06-001	Not Detected	Gull fecal biomarker not detected
N07-001	Not Detected	Gull fecal biomarker not detected
DO-001	Not Detected	Gull fecal biomarker not detected
N26-001	Not Detected	Gull fecal biomarker not detected
N08-001	Not Detected	Gull fecal biomarker not detected
N09-001	Not Detected	Gull fecal biomarker not detected

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: May 18, 2018

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non -detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

C. marimammalium Gull Fecal "Quantification" ID™

C. marimammalium are shown to be ubiquitous in the gull gastrointestinal tract for multiple species of the gull genus *Larus* found throughout North America.¹

Classified as a novel genus and species in 2006, *C. marimammalium* is a Gram-positive, catalase-negative, facultatively anaerobic, coccus-shaped bacterium, related to, although distinct from, other catalase-negative genera which include *Enterococcus*, *Melissococcus*, *Tetragenococcus* and *Vagococcus*².

As a novel bacterium species, the pathogenesis of *C. marimammalium* is relatively unknown. However, there are increasing public health concerns regarding avian fecal contamination in the environment due to the potential spread of microbial avian pathogens to humans, domesticated animals, and human food sources¹. Studies have shown also that waterfowl carry human pathogens such as *Campylobacter spp*³, *Salmonella spp*⁴, and *E. coli*⁵, as well as being reservoirs of influenza viruses⁶.

The Gull Fecal ID™ service is designed around the principle that *C. marimammalium* is highly specific and sensitive to numerous gulls of the genus *Larus*¹. This *C. marimammalium* bacterium can be used as an indicator of gull fecal contamination. Use of real-time (quantitative) Polymerase Chain Reaction (qPCR) allows for the rapid amplification of the gene biomarker to demonstrate the presence of gull feces and allow for the real-time visualization of the target.

Accuracy of the results is possible because the method uses real-time (quantitative) PCR DNA technology. Real-time (quantitative) PCR allows small DNA sequences to be amplified exponentially and detected in real-time via fluorescent probes.

DNA amplification is accomplished with small pieces of DNA called primers that are specific to the genomes of interest. Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers. The DNA is replicated to create exact copies of the desired DNA fragment (i.e. the gene biomarker). This process is repeated rapidly many times ensuring an exponential growth in the number of copied DNA.

If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection. With real-time (quantitative) PCR, the desired DNA fragments are also bound by fluorescent reporter probes. Consequently, the more copies of the desired DNA fragments that are made, the stronger the fluorescent signal, thus allowing for a straightforward detection and quantification of the targeted gene in real-time via the real-time PCR associated software. Nonetheless, as with all analytical tests, in order to strengthen the validity of the results, the Gull Fecal ID™ service should be combined with other DNA analytical services such as the *E. coli* ID™ service.

References

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³Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in Different Ecological Guilds and Taxa of Migrating Birds Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R.P., Wagenaar, J.A., Olsen, B.; *Appl. Environ. Microbiol.*, 2002, 68: 5911-5917.

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⁵Detection and Characterization of Shiga-toxin Producing *E. coli* from Seagulls Makino, S., Korbi, H., Asakura, H., Watarai, M., Shirahata, T., Ikeda, T., Takeshi, K., Tsukamoto, T.; *Epidemiol. Infect.*, 2000, 125: 55-61.

⁶Influenza in Migratory Birds and Evidence of Limited Intercontinental Virus Exchange Krauss, S., Obert, C.A., Franks, J., Walker, D., Jones, K., Seiler, P., Niles, L., Pryor, S.P., Obenauer, J.C., Naeve, C.W., Widjaja, L., Webby, R.J., Webster, R.G.; *PLoS Pathog.*; 2007, 3: 167.



15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



Dog Fecal Quantification ID

Detection and quantification of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR)
 DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: March 15, 2019
 Report Generated: April 10, 2019

ND: Not Detected
 DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9D01015	LB	Dog Bacteroidetes ID: Target 1	ND	Not Detected
SM-9D01016	N06-001	Dog Bacteroidetes ID: Target 1	ND	Not Detected
SM-9D01017	N07-001	Dog Bacteroidetes ID: Target 1	ND	Not Detected
SM-9D01018	DO-001	Dog Bacteroidetes ID: Target 1	ND	Not Detected
SM-9D01019	N26-001	Dog Bacteroidetes ID: Target 1	ND	Not Detected
SM-9D01020	N08-001	Dog Bacteroidetes ID: Target 1	DNQ	Detected
SM-9D01021	N09-001(A)	Dog Bacteroidetes ID: Target 1	DNQ	Detected

Limitation of Damages – Repayment of Service Price

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Revision 1.3
 Effective Date 9/25/18



15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



**ISO 17025 Accredited
 Testing Laboratory**

Preliminary Interpretation of Dog Fecal “Quantification” ID Results

Detection and quantification of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: March 15, 2019
 Report Generated: April 10, 2019

Sample ID	INTERPRETATION	
	Concentration of Dog Fecal Pollution in Sample	Comment
LB	Not Detected	Dog fecal biomarker not detected
N06-001	Not Detected	Dog fecal biomarker not detected
N07-001	Not Detected	Dog fecal biomarker not detected
DO-001	Not Detected	Dog fecal biomarker not detected
N26-001	Not Detected	Dog fecal biomarker not detected
N08-001	Low Concentration	Low levels of Dog fecal biomarker(s)
N09-001(A)	Low Concentration	Low levels of Dog fecal biomarker(s)

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: April 10, 2019

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Dog Bacteroidetes "Quantification" ID™

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.¹ Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Dog Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.^{2,3,4,5,6} Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in dog. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in dog.^{2,3,5,6} As such, these bacterial strains can be used as indicators of dog fecal contamination.

One of the advantages of the Dog Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the dog *Bacteroidetes* gene biomarker is not present.

References

¹ Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions**. Appl. Environ. Microbiol. (2002) 68: 5796-5803.

² Bernhard, A.E., and K.G. Field (2000a). Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Applied and Environmental Microbiology, 66: 1,587-1,594.

³ Bernhard, A.E., and K.G. Field (2000b). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. Applied and Environmental Microbiology, 66: 4,571-4,574.

⁴ Kreader, C.A. (1995). Design and evaluation of *Bacteroides* DNA probes for the specific detection of human fecal pollution. Applied and Environmental Microbiology, 61: 1,171-1,179.

⁵ Fogarty, Lisa R., Voytek, Mary A. **Comparison of *Bacteroides-Prevotella* 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

⁶ Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Santo Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification** Appl. Environ. Microbiol. 2005 71: 3184-3191.



15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: Info@sourcemolecular.com



Bird Fecal Quantification ID

Detection and quantification of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: March 15, 2019
 Report Generated: April 10, 2019

ND: Not Detected
 DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9D01008	LB	Bird Fecal ID	ND	Not Detected
SM-9D01009	N06-001	Bird Fecal ID	DNQ	Detected
SM-9D01010	N07-001	Bird Fecal ID	DNQ	Detected
SM-9D01011	DO-001	Bird Fecal ID	DNQ	Detected
SM-9D01012	N26-001	Bird Fecal ID	DNQ	Detected
SM-9D01013	N08-001	Bird Fecal ID	DNQ	Detected
SM-9D01014	N09-001(A)	Bird Fecal ID	DNQ	Detected

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 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



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Preliminary Interpretation of Bird Fecal “Quantification” ID Results

Detection and quantification of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: March 15, 2019
 Report Generated: April 10, 2019

Sample ID	INTERPRETATION	
	Concentration of Bird Fecal Pollution in Sample	Comment
LB	Not Detected	Bird fecal biomarker not detected
N06-001	Low Concentration	Low levels of Bird fecal biomarker(s)
N07-001	Low Concentration	Low levels of Bird fecal biomarker(s)
DO-001	Low Concentration	Low levels of Bird fecal biomarker(s)
N26-001	Low Concentration	Low levels of Bird fecal biomarker(s)
N08-001	Low Concentration	Low levels of Bird fecal biomarker(s)
N09-001(A)	Low Concentration	Low levels of Bird fecal biomarker(s)

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: April 10, 2019

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Bird Fecal ID™ Quantification

The genus *Helicobacter* is a group of gram-negative, microaerophilic bacteria that were initially classified under the *Campylobacter* genus prior to 1989. Since then, they have been reclassified into the genus *Helicobacter* after 16S rRNA sequencing differentiated them from other *Campylobacter* species. This group of bacteria typically have a spiral, curved or fusiform morphology with multiple flagella allowing them to rapidly maneuver in the intestinal mucous lining of their hosts. *Helicobacter* species colonize the gastrointestinal tract of mammals and birds and are shed in feces. There are approximately 20 strains of *Helicobacter*¹. Certain strains, such as *Helicobacter pylori*, are pathogenic to humans causing chronic gastritis, peptic ulcers and stomach cancer.

The Bird Fecal Quantification ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Helicobacter* genus are specific to wild birds. These *Helicobacter* sequences can be used as indicators of bird fecal contamination. Several species have been isolated from specific animal hosts such as *H. fennelliae* from humans, *H. hepaticus* from mice and *H. felis* from cats and dogs.¹ The Bird Fecal Quantification ID™ service targets a bird-associated gene biomarker in *Helicobacter pametensis*.² The biomarker is present at different degrees in the feces of various birds including but not limited to gull, goose, chicken, pigeon and duck.

One of the advantages of the Bird Fecal Quantification ID™ service is that the entire population of *Helicobacter* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish.

Accuracy of the results is possible because the method uses qPCR DNA technology. qPCR simultaneously confirms and quantifies the bird-associated gene biomarker. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genome to be detected. This qPCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve indicates that the bird-associated *Helicobacter* gene biomarker is not present.

References

¹ Goldman, E. and Green, L. H. (2009). *Practical Handbook of Microbiology* (2nd ed) . Boca Raton, FL: CRC Press.

² Seymour, C., Lewis, R.G., Kim, M., Gagnon, D.F., Fox, J.G., Dewhirst, F.E., and Paster, B.J. Isolation of *Helicobacter* Strains from Wild Bird and Swine Feces. *Appl. Environ. Microbiol.* (1994) 60:3, 1025-1028.



15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



Human Fecal Quantification ID

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
Date Received: March 15, 2019 *ND: Not Detected*
Report Generated: April 10, 2019 *DNQ: Detected Not Quantified*

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9D01001	LB	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-9D01002	N06-001	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-9D01003	N07-001	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-9D01004	DO-001	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-9D01005	N26-001	Human Bacteroidetes ID: Dorei	DNQ	Detected
SM-9D01006	N08-001	Human Bacteroidetes ID: Dorei	DNQ	Detected
SM-9D01007	N09-001(A)	Human Bacteroidetes ID: Dorei	DNQ	Detected

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of Source Molecular Corporation, as well as its agents or representatives, the liability of the company shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to Source Molecular Corp. The company shall not be liable for any damages, either direct or consequential. Source Molecular Corp. provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request. The sample(s) cited in this report may be used for research purposes after an archiving period of 3 months from the date of this report. Research includes, but is not limited to internal validation studies and peer-reviewed research publications. Anonymity of the sample(s), including the exact geographic location will be maintained by assigning an arbitrary internal reference. These anonymous samples will only be grouped by state / province of origin for research purposes. The client must contact Source Molecular in writing within 10 days from the date of this report if he/she does not wish for their submitted sample(s) to be used for any type of future research.

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15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016
 Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
 Email: info@sourcemolecular.com



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Preliminary Interpretation of Human Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: EA Engineering, Science, and Technology, Inc. PBC
 Date Received: March 15, 2019
 Report Generated: April 10, 2019

Sample ID	INTERPRETATION	
	Concentration of Human Fecal Pollution in Sample	Comment
LB	Not Detected	Human fecal biomarker not detected
N06-001	Not Detected	Human fecal biomarker not detected
N07-001	Not Detected	Human fecal biomarker not detected
DO-001	Not Detected	Human fecal biomarker not detected
N26-001	Low Concentration	Low levels of Human fecal biomarker(s)
N08-001	Low Concentration	Low levels of Human fecal biomarker(s)
N09-001(A)	Low Concentration	Low levels of Human fecal biomarker(s)

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: EA Engineering, Science, and Technology, Inc. PBC

Report Generated: April 10, 2019

Non-Detect Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results

In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at sourcemolecular.com/tests

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species: *B. dorei*

The Human Bacteroidetes ID™ Species: *B. dorei* service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.^{1,2} The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.³ The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world^{1,3,4} makes the Human Bacteroidetes ID™ Species: *B. dorei* service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.⁵ Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.^{3,5,6,7,8} Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.^{3,6} As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as Human Bacteroidetes ID™ Species: *B. stercoris*, Human Bacteroidetes ID™ Species: *B. fragilis*, and Human Bacteroidetes ID™ Species: *B. thetaiotaomicron*.

¹Boehm, A., Fuhman, J., Mrse, R., Grant, S. Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ Sci Technol.* 2003 37: 673-680.

²Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. *Bacteroides dorei* sp. nov., isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 2006 56: 1639-1641.

³Bernhard, A., Field, K. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 2000b 66: 4571-4574

⁴Ahmed, w., Masters, N., Toze, S. Consistency in the host specificity and host sensitivity of the *Bacteroides* HF183 marker for sewage pollution tracking. *Lett. Appl. Microbiol.* 2012 55: 283-289.

⁵Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. Microbial Source Tracking: Current Methodology and Future Directions. *Appl. Environ. Microbiol.* 2002 68: 5796-5803.

⁶Bernhard, A., Field, K. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Appl. Environ. Microbiol.* 2000a 66: 1587-1594.

⁷Fogarty, L., Voytek, M. A Comparison of *Bacteroides-Prevotella* 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species. *Appl. Environ. Microbiol.* 2005 71: 5999-6007.

⁸Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., et al. Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification. *Appl. Environ. Microbiol.* 2005 71: 3184-3191.

Figure A1. 2018-2019 IR Stations

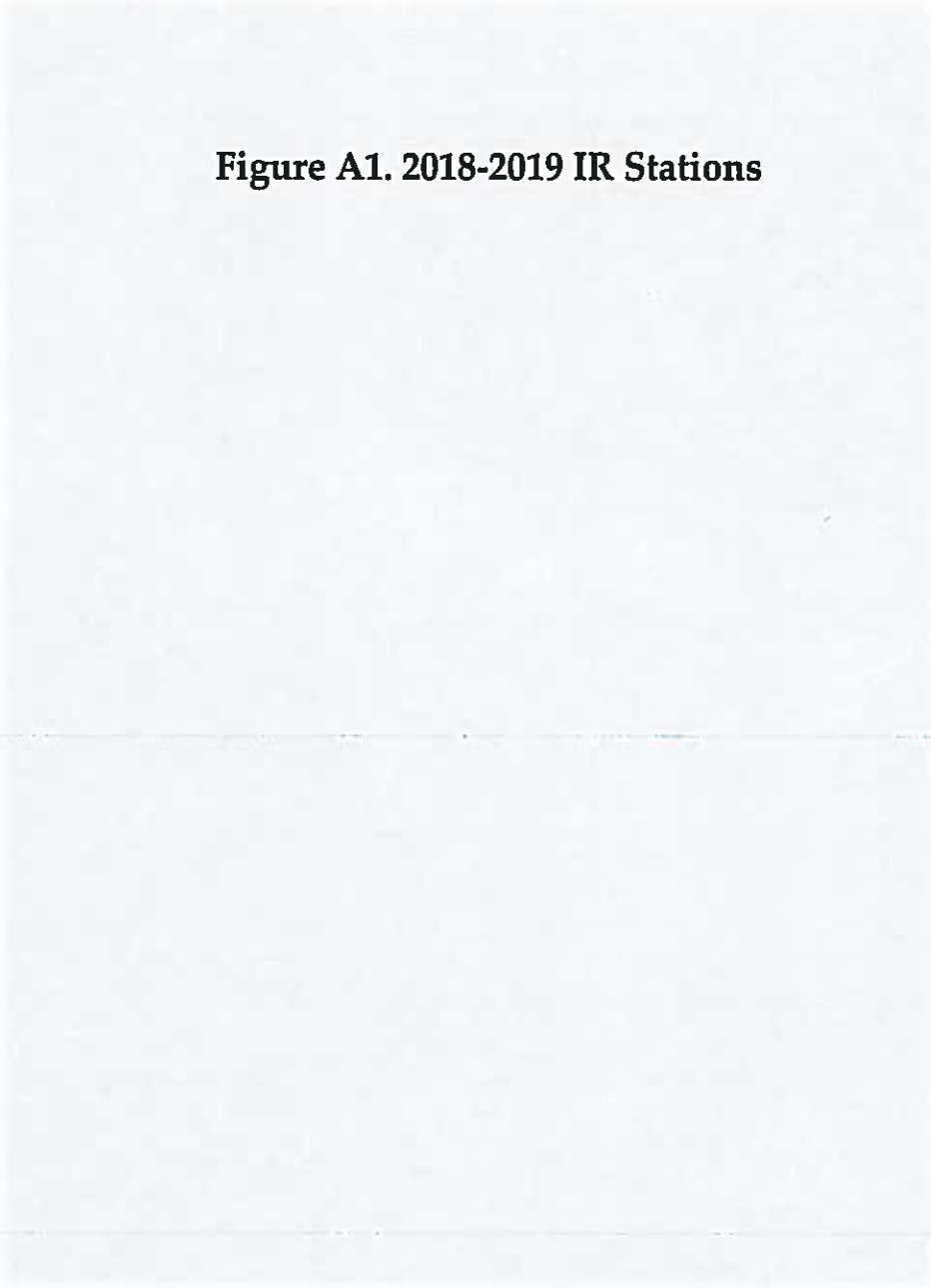


Figure A1. 2018-2019 IR Stations



Attachment 2.
Measurement of Turbidity, Suspended Sediments and Nutrients
in Three Rivers that Drain to the Achang Preserve
from the Manell Watershed

Measurement of Turbidity, Suspended Sediments and Nutrients in Three Rivers that Drain to the Achang Preserve from the Manell Watershed, Guam

NOAA National Centers for Coastal Ocean Science
Stressor Detection and Impacts Division

Anthony S. Pait

William M.C. Whitman

S. Ian Hartwell

David R. Whittall

Dennis A. Apeti



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Measurement of Turbidity, Suspended Sediments and Nutrients in Three Rivers that Drain to the Achang Preserve from the Manell Watershed, Guam

Anthony S. Pait¹, William M.C. Whitman, S. Ian Hartwell¹, David R. Whitall¹,
and Dennis A. Apeti¹

¹National Oceanic and Atmospheric Administration
National Ocean Service (NOS)
National Centers for Coastal Ocean Science (NCCOS)
Stressor Detection and Impacts Division (SDI)
Monitoring and Assessment Branch
1305 East/West Highway (SSMC-4, N/SCI-1)
Silver Spring, MD 20910
USA

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United States Department
of Commerce

Wilbur Ross
Secretary

National Oceanic and Atmospheric
Administration

Neil Jacobs
Acting Administrator

National Ocean
Service

Nicole LeBoeuf
Acting Assistant
Administrator

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ABSTRACT

The goal of this project, funded by NOAA's Coral Reef Conservation Program and requested by local partners, was to monitor water quality in three rivers that drain to the Achang Reef Flat Marine Preserve at the southern tip of Guam, in order to provide a baseline of conditions for environmental managers. The spatial and temporal variation of turbidity, suspended sediment concentration (SSC), and nutrients were determined at sites on the Ajayan, As Liyog, and Sumay rivers.

Using Guam EPA water quality standards, SSC and turbidity in the rivers were generally classified as excellent to good, although occasionally the waters were ranked as fair, particularly on the As Liyog River during higher rainfall. Overall, nitrate was found to be in the excellent range, and orthophosphate generally in the good to fair range.

There was some evidence that a number of the parameters showed decreasing trends in concentration during the project. Further monitoring would help determine if these decreases are real, which could be an indication of the benefits of the ongoing restoration activities in the watershed, evidence of natural revegetation subsequent to wildfires, or a combination of both. In any case, additional restoration efforts along with public education and outreach would be helpful to further reduce runoff to the rivers that drain to the Achang Reef Flat Marine Preserve.

INTRODUCTION

Guam is the most southerly and largest (both in area and population) member of the Mariana Islands, a crescent-shaped archipelago in the western North Pacific Ocean. The Mariana Island chain extends from Guam towards Japan, and is part of the larger island group referred to as Micronesia. The island of Guam has a land area of approximately 550 square kilometers, and a maximum altitude of 406 meters (Emery, 1962). The capitol of Guam is Hagåtña, located towards the middle of the island (Figure 1).

The northern half of Guam is a broad limestone plateau bordered by steep cliffs, while the southern half of the island is a dissected volcanic upland fringed with limestone, primarily along the east coast (Tracey et al., 1964). At the southern tip of Guam is Cocos Lagoon (Figure 1), an atoll-like coral reef lagoon. Geologically,

Cocos Lagoon is thought to have grown on the basement of the Umatac formation, a thick sequence of volcanic rock which occupies the southern central portion of Guam, and named after the town of Umatac on the west coast of the island (Tracey et al., 1964).

Manell Watershed

The Manell and Geus watersheds on the northeastern border of Cocos Lagoon were designated as a Habitat Focus Area for NOAA's Habitat Blueprint, and the watersheds and lagoon are a NOAA Coral Reef Conservation Program



Figure 1. The island of Guam.

(CRCP) priority for Guam. A map of the Manell and Geus watersheds can be seen in Figure 2. In 1997, a network of marine preserves was established in Guam in response to decreasing nearshore fish stocks (NOAA, 2007). The Achang Reef Flat Marine Preserve (Figure 2), located on the eastern end of Cocos Lagoon, was established by the Division of Aquatic and Wildlife Resources of the Guam Department of Agriculture.

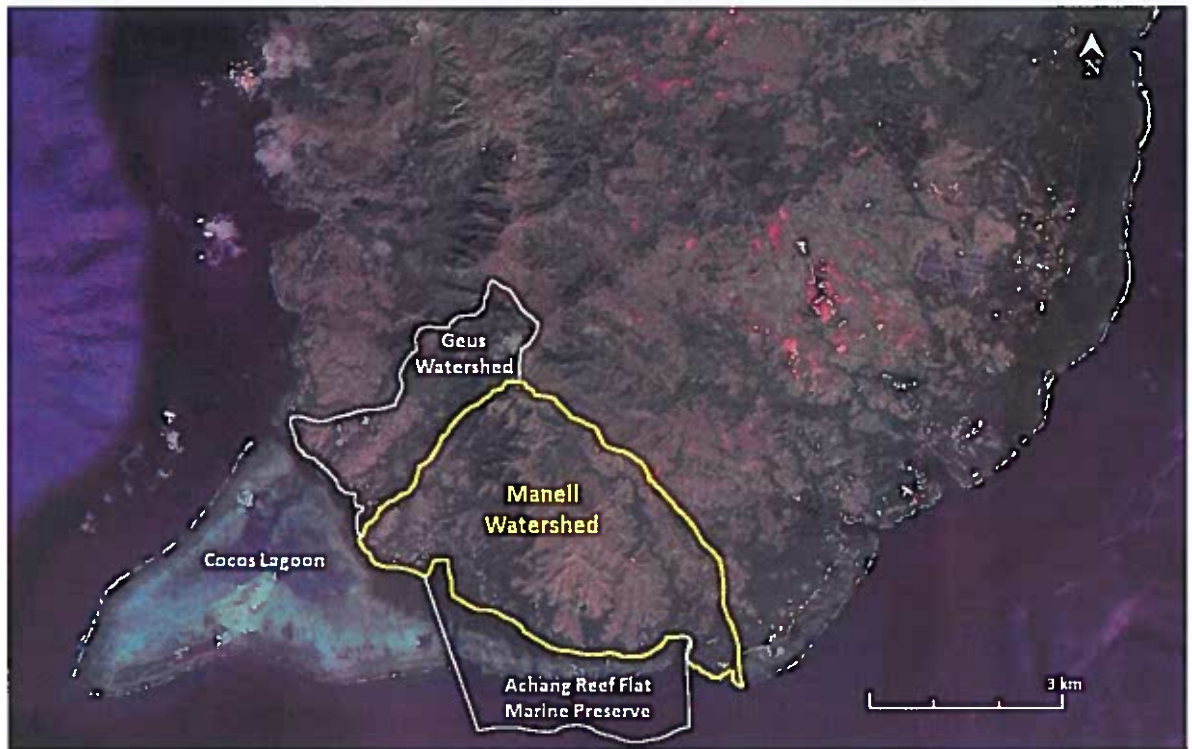


Figure 2. Boundaries of the Manell watershed, along with the Geus watershed and the Achang Reef Flat Marine Preserve.

There are extensive patch reefs and seagrass beds within Cocos Lagoon, and seagrass beds are abundant in the shallow waters of the Achang Preserve. The seagrass beds in the Preserve provide valuable nursery habitat for a variety of fish and are an important habitat for green and hawksbill sea turtles. Since the establishment of the Preserve, fish stocks have increased there by 115%, while surveys at comparison sites outside the Achang Preserve in Cocos Lagoon have shown a further 4% decrease (Porter *et al.*, 2005).

The watersheds incorporate some interesting landscapes, resulting from the geological formation of the island. The geomorphology of southern Guam with a narrow limestone fringe along the coast, has a volcanic origin that has resulted in steep hills within the watersheds (Tracey *et al.* 1964). Behind the coastal plain, elevations rise rapidly to over 400 meters. The tallest peak in Guam is Mount Lamlam at 406 meters, which is within 2 km of the coastline in the southwest part of Guam, near the town of Agat (Figure 1).

The Manell watershed (Figure 2) has an area of approximately 11.8 square kilometers. The adjacent Geus watershed has an area of approximately 4.5 square kilometers. In the Manell watershed, Mount Sasalaguan, is within 2.5 km

of the coast, and has an elevation of 337 meters.

The Manell watershed contains a variety of land uses including grassland, forest, and urban areas (Table 1). Grassland savanna with steep slopes comprise the highest land use, while forested areas are limited to ravines (EA Engineering, 2014). An image of the Manell watershed taken from the Achang Reef Flat Marine Preserve can be seen in Figure 3.

From the watershed, a series of rivers flow into the Achang Preserve including the Ajayan, As Liyog, and Sumay rivers, which are the focus of this report. Urban areas comprise approximately 23 percent of the land use in the watershed. The municipality and town of Merizo (Figure 1) is located within the Manell and Geus watersheds. The population of Merizo was 1,850 in 2010 (Guam Bureau of Statistics and Plans, 2011). Tourism accounts for roughly 60% of the economy in Guam (NOAA, 2007), and tourism is also important in Merizo.

Within the watershed, grazing by feral animals, off-roading vehicles, and in particular wildfires, promote erosion of soils from the steep hillsides and along stream banks.

Table 1. Land use in the Manell watershed.

Land Use	%	km ²
Urban	23.4	2.76
Forest	33.2	3.91
Burned	4.22	0.50
Barren	0.77	0.09
Grassland	37.6	4.43

From: Wen *et al.*, 2009

Wildfires are a constant threat in the Manell watershed, and burning the grasslands leaves the soil bare, facilitating erosion. The fires also inhibit the growth of trees and shrubs that would hold the soil and absorb rainfall. Many of the fires are reportedly caused by arson, set by poachers for the purpose of encouraging the emergence of new grasses for grazing by Philippine deer and feral pigs which are hunted (EA Associates, 2014). Winds exacerbate the destruction caused by the wildfires. The dry season on Guam occurs from December to April, which also has some of the higher average wind speeds (~14 km/hour).

Within the watershed, high velocity runoff events of relatively short duration are frequent in the mountainous areas of southern Guam. Increased runoff can also lead to the flooding of downstream areas. It has been estimated that in the last five years, nearly 60 percent of the residents in the town of Merizo (Figure 1) have been affected by flooding.

Eroded soils from the hillsides are transported as suspended sediments to the freshwater streams and rivers in the watershed, impacting the aquatic organisms there. The sediments which are then transported out into the Achang Preserve in Cocos Lagoon, can impact water quality in the seagrass areas and patch reefs (Khosrowpanah *et al.*, 2015; NOAA, 2007). Sedimentation on coral reefs can have serious impacts (Fabricius, 2005; Burke *et al.*, 2011; Waddell *et al.*, 2005). The deposition of sediments in reef areas can act to smother corals and physically abrade coral tissues. At the very least, sediments deposited on corals results in the organisms having to expend more energy to remove sediment particles, meaning there is less energy available for other functions including growth and reproduction. Elevated sedimentation has been linked to less coral cover, lower diversity and recruitment, along with lower growth and calcification rates (ISRS, 2004; Rogers, 1990).

Sediments associated with runoff have been identified as one of the most significant threats to coral reef ecosystems in Guam (Burdick, *et al.*, 2008). Fires, erosion and sedimentation were all identified as high threats in the Manell and Geus watersheds, and sedimentation was also identified as a high threat to coral reef ecosystems during the Manell/

Geus Conservation Action Planning (CAP) Workshop (GCMP, 2013).

Nutrients originating from wildlife, septic systems and agricultural activities (crops and livestock) can also make their way into streams and rivers and out onto the Achang



Figure 3. Photograph of the steep hillsides in the Manell watershed that border the Achang Reef Flat Marine Preserve.

Preserve and the rest of Cocos Lagoon. While dissolved nutrients are essential to productivity in aquatic systems, an overabundance of nutrients can trigger macroalgae and phytoplankton blooms, resulting in degradation of water quality and habitat (Bricker *et al.*, 2007). In coral reef systems, algal blooms can lead to the algae out-competing and then smothering juvenile and adult corals, ultimately resulting in the loss of those corals (D'Angelo *et al.*, 2014; Box and Mumby, 2007). It has also been shown that nutrients can have direct effects on corals. Ammonium and phosphate in parts per billion (ug/l or ppb) concentrations can impact fertilization success (Harrison and Ward, 2001), while elevated nitrate has been shown to decrease calcium deposition in corals (Marubini and Davis, 1996).

A variety of watershed restoration activities have been undertaken to try and restore vegetation and reduce erosion in the upland areas in the Manell and adjacent watersheds. These include public education and outreach, planting of native species to help reduce erosion in upland areas as well as along streambanks, installation of riparian buffer strips, and removal of non-native bamboo (GCMP, 2013; EA Associates, 2014). Bamboo is specifically targeted and removed because it can block drainage culverts during storms, leading to flooding of roads and populated areas.

NOAA/NCCOS Involvement

In 2016, local resource managers reached out to NOAA’s National Centers for Coastal Ocean Science (NCCOS) for help with monitoring water quality in three rivers that flow into the Achang Preserve. With funding from NOAA’s CRCP, NCCOS scientists met with local resource managers

and scientists from the Guam Environmental Protection Agency (Guam EPA), CRCP and NOAA’s National Marine Fisheries Service Pacific Islands Regional Office, to develop a project to monitor turbidity, suspended sediments, and nutrients (e.g., nitrogen and phosphorus), for a period of two years. The goal was to assess the condition of these rivers, and establish a baseline which could

then be used to assess the efficacy of planned restoration efforts within the Manell watershed.

MATERIALS AND METHODS

Field sampling events occurred several times a month beginning in December 2016 through December 2018.

The purpose of these field visits was to take water quality measurements, and to collect water samples for the analysis of the suspended sediments concentration (SSC) and nutrients at locations on the Ajayan, As Liyog, and Sumay rivers in the Manell watershed. Observations were also made to document weather and stream conditions. During these visits, data logger downloads and maintenance of the automated turbidity logger

placed in the Ajayan River and the two instream level loggers placed in the Ajayan and the As Liyog rivers were also conducted.

In total, 76 rounds of sampling were completed over the two-year data collection period. At times, the collection



Figure 4. Location of the monitoring sites in the Manell watershed, along with the location of the rain gauge station.

of water samples could not occur because of storm events that led to flooding of the roads, making it too dangerous to travel to the sampling sites.

A map of the sample locations can be seen in Figure 4, along with the site coordinates in Table 2. The location of the rain gauge station, used to monitor rainfall for this project, is also shown in the figure and table. Towards the end of the project, a site was added on the Geus River and sampled between July 2018 and December 2018, for comparison.

Below is a more detailed description of each of the sample collection and analysis techniques.

Table 2. Latitude and longitude coordinates of the sampling sites for this project.

Site Name	Latitude (DD)	Longitude (DD)
Ajayan River	13.25132	144.71881
As Liyog River	13.24716	144.70738
Sumay River	13.24800	144.70005
Geus River	13.27067	144.67933
Rain Gauge Station	13.26278	144.71761

Water Quality Field Measurements

In situ field measurements were conducted prior to each sample collection for the following water quality parameters: temperature, dissolved oxygen, specific conductance, salinity, and turbidity. To limit the influence of seawater mixing from the rising tide as much as possible, samples were collected during low tide or during a falling tide.

All water quality measurements, with the exception of turbidity, were measured using a YSI Professional Plus (Pro Plus) hand-held field instrument. Turbidity (Hach turbidity) was measured using a Hach 2100Q Portable Turbidimeter. All water quality instruments were calibrated and maintained according to the manufacturer recommendations. Water quality measurements were recorded at each sample location along with any other environmental conditions of potential significance (i.e., stream debris, weather, wildfires, etc.).

Data Logging Instrumentation

Three data logging instruments were set-up at the sites within the Manell watershed; two stream level loggers (Ajayan and As Liyog sites) and one turbidity logger (Ajayan site). In addition, a data logging rain gauge was installed in the mountain ridge upland of the Ajayan River (Figure 4).

The stream level loggers were HoboWare® U20 water level data loggers with a range of 0 to 30 ft and an accuracy of 0.015 ft. These loggers use pressure (in psi) to determine the height of the water column above. The level loggers were suspended at the bottom of PVC housings secured to the bridge abutments at Ajayan River and As Liyog River. In addition, one pressure logger was placed at a secure location outside of the water, to monitor changes in atmospheric pressure. Stream level was calculated by subtracting the atmospheric pressure from the streambed pressure readings.

A turbidity logger was deployed in the Ajayan River, within a separate dedicated PVC housing secured to the Ajayan Bridge (Figure 5). The turbidity logger was suspended in the water column approximately 0.46 m above the streambed. The turbidity logger was a Manta 2 water

quality logging sonde with a turbidity sensor equipped with a self-cleaning wiper. The As Liyog River was too shallow for a turbidity logger.



Figure 5. Photograph of turbidity logger (right) and level logger (left) housings attached to the Ajayan River bridge abutment.

Rainfall data for the Manell watershed was collected using a HoboWare® rain gauge installed along the eastern mountain ridge of the watershed, in an area known as Ija. The rain gauge uses two tipping buckets that collect water as it falls, recording each time the tipping buckets are activated representing a specific quantity of rainfall (0.01 in. per tip).

All data loggers (turbidity logger, level loggers, and rain gauge) were programmed to collect readings every 15-minutes. The data loggers were deployed from December 2016 through December 2018. Periodically, the data loggers were briefly taken out of service for calibration and maintenance. Calibration and maintenance were completed in accordance with the manufacturer's recommendations. The turbidity logger was calibrated monthly or as needed. HoboWare® data loggers were factory calibrated. All the data logging instruments required periodic cleaning and the time and date were calibrated on a regular basis.

Nutrient Samples

Nutrient samples (surface) were collected at the designated sample locations during each field visit. Disposable nitrile gloves were worn during the collection of samples. Water samples were collected by hand into 125 ml HDPE bottles from the shore, labeled with the collection time, date, and location. Prior to sample collection, each bottle was pre-rinsed three times with sample water. Samples were then placed on ice and transferred to the sample freezer on the same day, at the Guam EPA laboratory.

Frozen samples for nutrient analysis were sent off in batches for analysis by the Geochemical and Environmental Research Group (GERG) at the Texas A&M University. GERG was subcontracted to TDI-Brooks, Inc, a NOAA analytical contract laboratory. All nutrient samples were analyzed for nitrate, nitrite, ammonium, total nitrogen, orthophosphate, total phosphate, and silica.



Figure 6. Locations of the three monitoring sites in the Manell watershed.

The following is a brief summary of methods used for the analysis of nutrients in the water samples collected. Nitrate and nitrite analyses were based on the methodology of Armstrong *et al.* (1967) and use a ground cadmium column for reduction of nitrate to nitrite. Orthophosphate was measured using the methodology of Bernhardt and Wilhelms (1967), with the modification of hydrazine as a 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 (Harwood and Kuhn, 1970) using dichloroisocyanurate as an oxidizer. Urea was measured using diacetyl-monoximine and themicarbozide. Total nitrogen and total phosphorus concentrations were determined after an initial decomposition step. This method involves persulfate oxidation while heating the sample in an autoclave (Hansen and Koroleff 1999). After oxidation of the samples, nutrient determinations were conducted on a Technicon® II analyzer.

Suspended Sediment Concentration (SSC)

Water samples for SSC analysis were collected at the three sites (Figure 6) during each field visit starting in April 2017 through December 2018. Sample water was collected in 500 ml plastic amber bottles, labeled with the collection time, date, and location. Samples were immediately placed in a cooler on ice.

Upon return to the laboratory, samples were kept chilled in a refrigerator awaiting laboratory analysis. All SSC samples were analyzed at the Guam Environmental Protection Agency (GEPA) laboratory within one week from the date of sample collection. SSC sample analyses were completed in accordance with the ALS Environmental (2011) methods and guidance. Briefly, each sample was filtered through a pre-weighed glass fiber filter mounted in a suction flask apparatus. The filtered sample was rinsed several times with deionized water in order to remove salts, then dried at 105°

C, and weighed to the nearest 0.001 g. SSC was calculated as follows:

$$\text{SSC (mg/L)} = ([A-B]*1000)/C$$

Where A = End weight of the filter in grams (g)

B = Initial weight of the filter in grams (g)

C = Volume of water filtered

Shipping

There was no direct overnight shipping available from Guam to TDI-Brooks in College Station, Texas. As a result, the samples were first shipped overnight to the NOAA Pacific Islands Fisheries Science Center in Honolulu, Hawaii. The water samples were then placed in a walk-in freezer at the facility for a few days, and then shipped out to TDI-Brooks.

Statistical Analyses

The data were analyzed using JMP® statistical software Version 12.1.0. To assess differences in turbidity, suspended sediments and nutrients between sites, a Shapiro-Wilk test was first run on the data to see if it was normally distributed. None of the data were normally distributed, and transformations were not effective. As a result, the Wilcoxon nonparametric test was used for two groups, and the Kruskal-Wallis nonparametric test was used when there were more than two groups. Pairwise comparisons were carried out using the Wilcoxon nonparametric test on each pair within JMP, which is the nonparametric version of the Student's t-test. Finally, Spearman's correlations were also calculated.

RESULTS AND DISCUSSION

Water Quality Parameters

A summary of results from the water quality monitoring by river can be seen in Table 3. More detailed results can be found in Appendix A. In general, the Sumay River site was different from the Ajayan and As Liyog sites. Temperature varied significantly at some of the sites (Chi-Square =

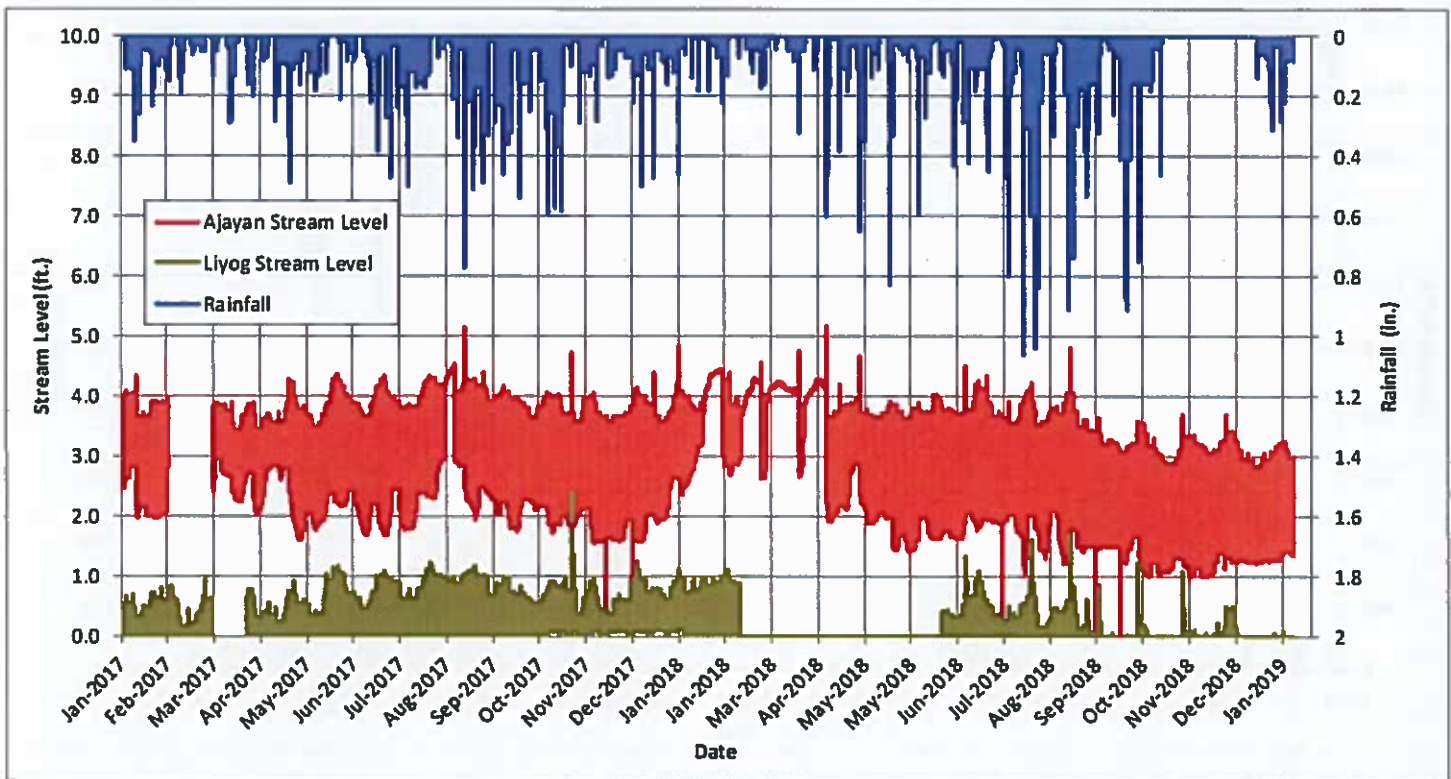


Figure 7. Stream level and rainfall at the Ajayan and As Liyog river sites.

69.7613, $p < 0.0001$). The Kruskal-Wallis nonparametric test indicated that temperature in the Sumay River site was significantly different (lower) than the As Liyog and Ajayan River sites. However, temperature in the As Liyog and Ajayan river sites were not significantly different from each other. Dissolved oxygen was also significantly different among the sites (Chi-Square = 10.4423, $p = 0.0054$). As with temperature, the Sumay River site had significantly lower dissolved oxygen than the Ajayan and As Liyog sites.

Specific conductivity (Chi-Square = 124.9015, $p < 0.0001$) and salinity (Chi-Square = 123.5802, $p < 0.0001$) were lower at the Sumay River site compared to the As Liyog and Ajayan sites (Table 3). The three sites for this project were established

at the bridges that go over the rivers along Route 4 in southern Guam. Both the Ajayan and As Liyog

sites (Figure 6) are more open to the Achang Reef Flat Marine Preserve waters than the Sumay River site, and have significant deltas which may facilitate the mixing of tidal seawater with the freshwater flowing down these two

rivers. The Sumay River, on the other hand, does not have a well developed delta, and the sampling site for the Sumay River appears more separated and upstream from where it flows into the Achang Preserve (Figure 6). At the beginning of this project, attempts were made to locate sampling sites further upstream on the As Liyog and Ajayan rivers, however, the dense jungle along these rivers did not allow sites further upstream to be established, even when using a kayak. In addition, there were uncertainties related to land ownership in the areas further upstream.

Results from Automated Data Logging

The results from the automated data logging for stream level at the Ajayan and the As Liyog river sites can be seen in

Table 3. Water quality parameters of sites in the Manell watershed.

Site	Temperature (°C)	Dissolved Oxygen (mg/l)	Specific Conductivity (µs/cm)	Salinity (ppt)
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Ajayan River	30.8 ±0.2	4.10 ±0.13	32042 ±20	20.2 ±1.1
As Liyog River	30.5 ±0.2	5.24 ±1.05	41439 ±26	26.3 ±0.9
Sumay River	28.7 ±0.1	3.54 ±0.13	5138 ±3	2.9 ±0.6

Figure 7. Note that the units in Figure 7 are in feet (stream level) and inches (rainfall). Approximately 73,000 observations were made using the automated stream and rainfall loggers in the Ajayan and As Liyog rivers over the two year period. Water or stream level was used as a proxy for

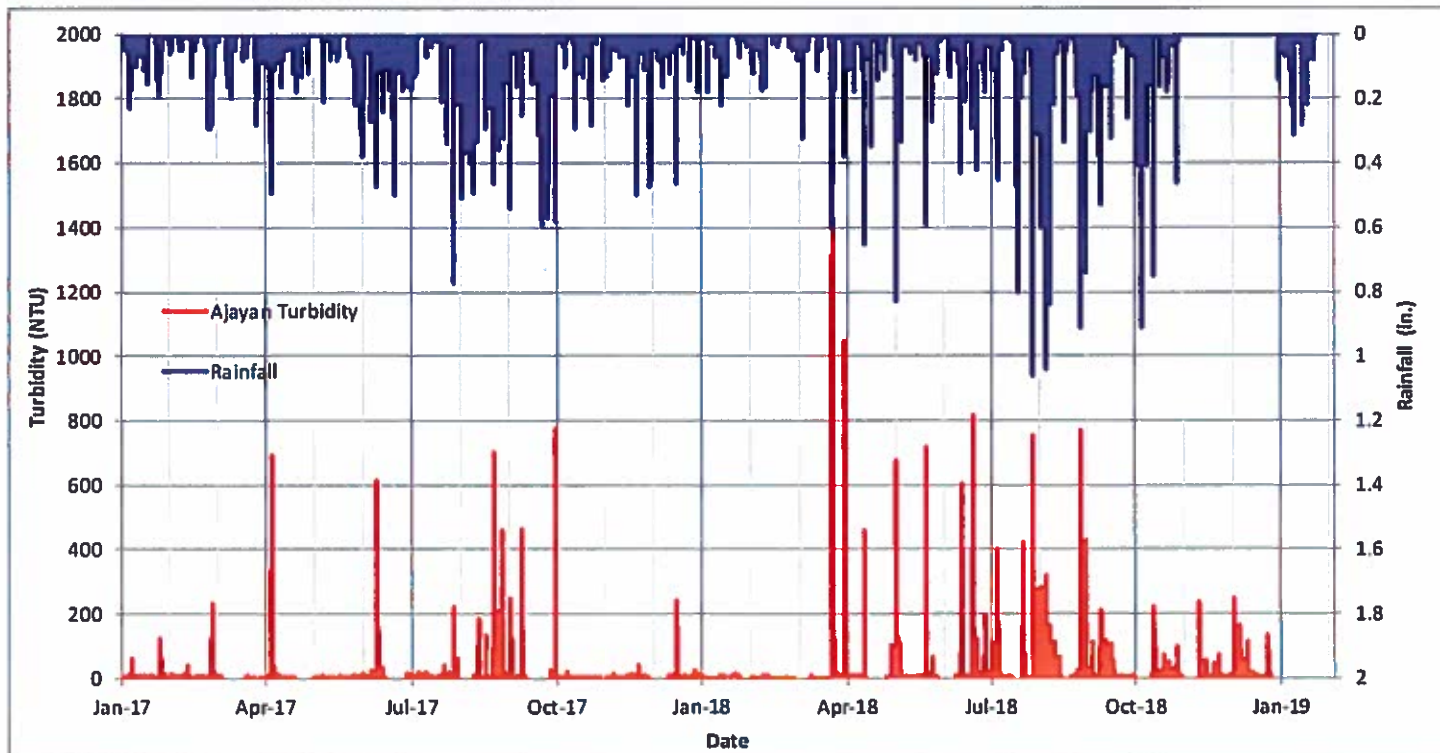


Figure 8. Stream level and turbidity at the Ajayan River site.

stream flow. Automated data logging could not be carried out in the Sumay River, as the water level at the site was too low to deploy the equipment.

From Figure 7, it can be seen that in general, the water level at the As Liyog River site was substantially lower than at the Ajayan River site. The mean water level during the monitoring in the Ajayan River was 0.91 meters (Table 4), or 3 ft. In the As Liyog, the mean water level was 0.03 meters. Nonparametric analysis of data from the automated monitoring revealed a significant and positive correlation (Spearman’s Rho = 0.0147, $p = 0.0001$) between Ajayan stream level and rainfall (Appendix B). Likewise, there was a significant positive correlation (Spearman’s Rho = 0.0335, $p < 0.0001$) between rainfall and stream level in the As Liyog River. This indicates that rainfall had a significant effect on the water levels in these two rivers, rising with increasing rainfall.

Table 4. Mean stream level (meters) at the Ajayan and As Liyog river sites.

	Mean ±SE	Minimum	Maximum
Ajayan River	0.91 ±0.00	0	1.58
As Liyog River	0.03 ±0.00	0	0.73

Notes: SE, standard error

From Figure 7, it can be seen that rainfall varied through the course of the year. In Guam, the wet season is roughly from May through November, and the dry season from December through April. Data from the stream level measurements for both the Ajayan and As Liyog rivers were classified as occurring either in the dry or the wet season. The data were then analyzed using Wilcoxon’s nonparametric

test. Results indicated that the stream level in the Ajayan River varied significantly (Chi-Square = 915.6 $p < 0.0001$), with higher stream levels occurring in the wet season. The same was true for the As Liyog, that is the months classified as part of the wet season had higher (Chi-Square = 591.8 $p < 0.0001$) water levels than during the dry season.

For both the Ajayan and As Liyog river sites, there was a short span of time, typically between 15 and 30 minutes, between when a significant rain event occurred and the water level in these rivers rose, highlighting the role that the steep slopes in the watershed play in quickly delivering runoff to downstream areas. In the adjacent Geus watershed, Khosrowpanah *et al.* (2015), found that the Geus River returned to normal flow within an hour and a half after a rainfall event. The same appeared to be true for the Ajayan and As Liyog rivers.

At the Ajayan River site, a turbidity logger (Manta 2 water quality sonde) was installed. As with the level loggers, the turbidity logger was programmed to take readings every 15 minutes. A graph of turbidity versus rainfall can be seen in Figure 8. There was a significant (Spearman’s Rho = 0.0880, $p < 0.0001$) positive correlation between rainfall and turbidity as measured by the Manta 2 automated turbidity sonde, indicating that turbidity was more likely to increase with rainfall, result-

ing from surface water runoff carrying sediments from the watershed into the Ajayan River, and ultimately out into the Achang Preserve.

There were a number of events during the monitoring when increased rainfall led to spikes in the turbidity readings from the Manta automated turbidity sonde. Between 6 and 8 April 2018, approximately 10.4 cm (4.09 inches) of rain fell, resulting in high turbidity levels, with readings over 1,400 NTUs (nephelometric turbidity units) (Figure 8). In the Geus River, Khosrowpanah *et al.* (2015) recorded turbidity readings as high as high 1,000 NTUs. Around 17 October 2017, approximately 8.8 cm (3.46 in) of rain fell in the Manell watershed over a 24 hour period, producing turbidity readings as high as 774 NTU. However, in July 2018, a rainfall total of only 3.7 cm (1.46 in) produced a turbidity reading of over 800 NTUs, perhaps demonstrating the variability of the system.

From these results, it appears that both the Ajayan and the As Liyog rivers respond rapidly to rainfall in terms of stream height and as was shown for the Ajayan, also for turbidity. This is perhaps not surprising given the slope of the watershed, which results in the rapid delivery of water and eroded soils to the lower elevations of the watershed. This rapid response highlights the need for restoring and maintaining good soil cover in the watershed, in order reduce the amount of water and sediment transported into the rivers and then out onto the Achang Preserve and the rest of Cocos Lagoon.

Guam Water Quality Standards

The marine and surface waters in Guam have been classified (Guam EPA, 2001) based on their use and desired water quality. Those classifications are shown in Table 5. Marine and surface waters have been divided into three categories. M1 (marine) and S1 (surface waters) are the designations given to those waters that should exhibit excellent or the highest water quality, either for recreational activities and the preservation of marine life (M1), or for drinking

water and preservation of aquatic life (S1). The next categories M2 and S2, are classified as good or medium water quality either for the propagation of marine life (M2), or

for recreational activities or as drinking water after suitable treatment (S2). The third category (M3 and S3) is a designation given to those waters suitable for commercial or industrial use.

Guam EPA has developed a water classification

map with areas designated as to their desired water quality (Guam EPA, 2001). Cocos Lagoon is designated as M1. The Manell watershed appears to be designated as S2.

Water quality standards for Guam were adopted in accordance with the US Water Pollution Control Act (Clean Water Act), Public Law 92-500, and the Guam Water Pollution Control Act (Guam EPA/USEPA, 2018). For the current project, four standards, including those for the suspended sediment concentration, turbidity, nitrate and orthophosphate, were most relevant. By necessity, the sites monitored in the Ajayan, As Liyog and Sumay rivers were located where they were accessible, that is adjacent to the highway bridges that cross these rivers. For the Ajayan and the As Liyog rivers, the locations represent the interface between marine waters and surface waters. Because of this, the figures presenting the results for the four parameters include both the marine and the surface water standards (e.g., M1 and S1).

Table 6 contains a summary of the monitoring of the suspended sediment concentration (SSC), Hach turbidity (also measured in NTUs), and nutrients. The Ajayan and As Liyog rivers were sampled and analyzed a total of 76 times. The site in the Sumay River was added a little later in the project, and was sampled 71 times. Table 7 lists the Guam water quality standards for SSC, turbidity, nitrate, and orthophosphate.

Suspended Sediment Concentration (SSC)

The mean SSC across all sites was 12.7 ±1.20 mg/l SSC (Table 6). More detailed data from the analysis of SSC can

Table 5. Classification of Guam marine and surface waters.

Marine Waters		
Category	Ranking	Description
M1	Excellent	Suitable to protect whole body contact recreation, and also to ensure preservation of marine life
M2	Good	Allows for propagation and survival of marine organisms
M3	Fair	Suitable for general, commercial and industrial use
Surface Waters		
Category	Ranking	Description
S1	High	Suitable for drinking water, wilderness areas, propagation and preservation of aquatic life, whole body contact and recreational enjoyment
S2	Medium	Suitable for recreational purposes, including whole body contact recreation, and as use as potable water after adequate treatment is provided.
S3	Low	Primarily used for commercial, agricultural, and industrial activities

Table 6. Summary of results for the monitoring of suspended sediments, Hach turbidity, and nutrients from the three rivers in the Manell watershed.

Parameter	Ajayan River (n = 76)			As Liyog River (n = 76)			Sumay River (n = 71)			Overall		
	Mean ±SE	Minimum	Maximum	Mean ±SE	Minimum	Maximum	Mean ±SE	Minimum	Maximum	Mean ±SE	Minimum	Maximum
Suspended Sediment Concentration (SSC) (mg/l)	7.52 ±1.21	1.4	82.2	23.4 ±2.67	3.23	129.1	7.51 ±1.43	1.45	71.6	12.7 ±1.20	1.4	129
Hach Turbidity (NTU)	10.6 ±2.19	1.13	135.5	17 ±2.11	3.38	146	7.53 ±1.43	1.43	75.6	11.8 ±1.16	1.13	146
Nitrate (NO ₃) (mg/l N)	0.01 ±0.00	0.0	0.09	0.01 ±0.00	0.0	0.09	0.19 ±0.01	0.02	0.34	0.07 ±0.01	0.00	0.34
Nitrite (NO ₂) (mg/l N)	0.01 ±0.00	0.0	0.01	0.01 ±0.00	0.0	0.01	0.0 ±0.00	0.0	0.01	0.01 ±0.00	0.00	0.01
Ammonium (NH ₄ ⁺) (mg/l N)	0.07 ±0.00	0.01	0.22	0.09 ±0.01	0.03	0.25	0.04 ±0.00	0.02	0.16	0.07 ±0.00	0.01	0.25
Urea (NH ₂ CONH ₂) (mg/l N)	0.02 ±0.00	0.0	0.05	0.02 ±0.00	0.1	0.06	0.01 ±0.00	0.0	0.08	0.02 ±0.00	0.00	0.08
Orthophosphate (HPO ₄ ⁻) (mg/l P)	0.08 ±0.01	0.01	0.31	0.1 ±0.01	0.01	0.32	0.05 ±0.01	0.01	0.46	0.08 ±0.00	0.00	0.46
Silica (HSiO ₃) (mg/l)	10.32 ±0.78	1.74	29.01	7.77 ±0.62	0.81	23.99	23.07 ±1.21	3.43	40.0	13.5 ±0.68	0.81	40.0
Total Nitrogen (mg/l N)	0.5 ±0.04	0.27	2.95	0.54 ±0.02	0.31	0.99	0.57 ±0.02	0.4	1.27	0.53 ±0.01	0.27	2.95
Total Phosphorus (mg/l P)	0.12 ±0.01	0.03	0.35	0.14 ±0.01	0.05	0.37	0.10 ±0.01	0.03	0.83	0.12 ±0.01	0.03	0.83

Abbreviation: SE, standard error

be found in Appendix A. With a mean of 23.4 ±2.67 mg/l, SSC at the As Liyog site was the highest. The maximum SSC recorded during this project was also at the As Liyog site, at 129 mg/l, and occurred in October 2017. SSC was significantly different in the three rivers (Chi-Square = 82.7, p < 0.0001). The correlation between SSC and water level was highly significant for both the Ajayan (Spearman’s Rho = 0.3670, p < 0.0001) and the As Liyog sites (Spearman’s Rho = 0.2944, p < 0.0001), indicating that increased water level (proxy for flow and runoff from the watershed) was correlated with increasing sediment in the water column.

The SSC mean for the Ajayan and Sumay rivers (Table 6) can be compared to the water quality standard for SSC (Table 7). The means for both the Ajayan and the Sumay river sites were below the M2/S2 (good) standard of 20 mg/l, but slightly higher than the M1/S1 (excellent) standard of 5 mg/l. The mean for SSC at the As Liyog River site (23.4 ±2.67 mg/l) was between the M2/S2 and the M3/S3 (good to fair) standard.

Table 7. Guam water quality standards for suspended sediments, turbidity and nutrients.

	Category		
	M1	M2	M3
Marine Waters			
Suspended sediment concentration (SSC)	5 mg/l	20 mg/l	40 mg/l
Turbidity*	0.5 NTU	1.0 NTU	1.0 NTU
Nitrate nitrogen (NO ₃ -N)	0.10 mg/l	0.20 mg/l	0.50 mg/l
Orthophosphate (PO ₄ -P)	0.025 mg/l	0.05 mg/l	0.10 mg/l
	Category		
	S1	S2	S3
Surface Waters			
Suspended sediment concentration (SSC)	5 mg/l	20 mg/l	40 mg/l
Turbidity*	0.5 NTU	1.0 NTU	1.0 NTU
Nitrate nitrogen (NO ₃ -N)	0.10 mg/l	0.20 mg/l	0.50 mg/l
Orthophosphate (PO ₄ -P)	0.025 mg/l	0.05 mg/l	0.10 mg/l

* Above ambient concentration

Figure 9a and 9b show results of the SSC analysis graphically. The sampling for SSC did not begin until April 2017. The SSC graphs were divided in two in order to make it easier to view the results over the course of the project. Also superimposed on the graphs are the Guam water quality standards for SSC. The collection of water samples on 14 October 2017, indicated substantially elevated SSC in all three rivers, with SSC values higher than the 40 mg/l

SSC water quality standards for M3 and S3 (fair) waters (Figure 9a). The As Liyog River recorded an SSC value of 129.1 mg/l, substantially higher than the fair SSC standard. During this period, nearly 25 cm (9.82 in) of rain fell, which likely lead to increased amounts of SSC. As will be seen, there was an increase in turbidity during this time as well.

In Figure 9b, it appears that SSC could have been lower in the second half of 2018. A variety of factors could impact the SSC found

in the rivers. Lower rainfall would reduce the amount of runoff, however, rainfall during this period (Figure 8) was

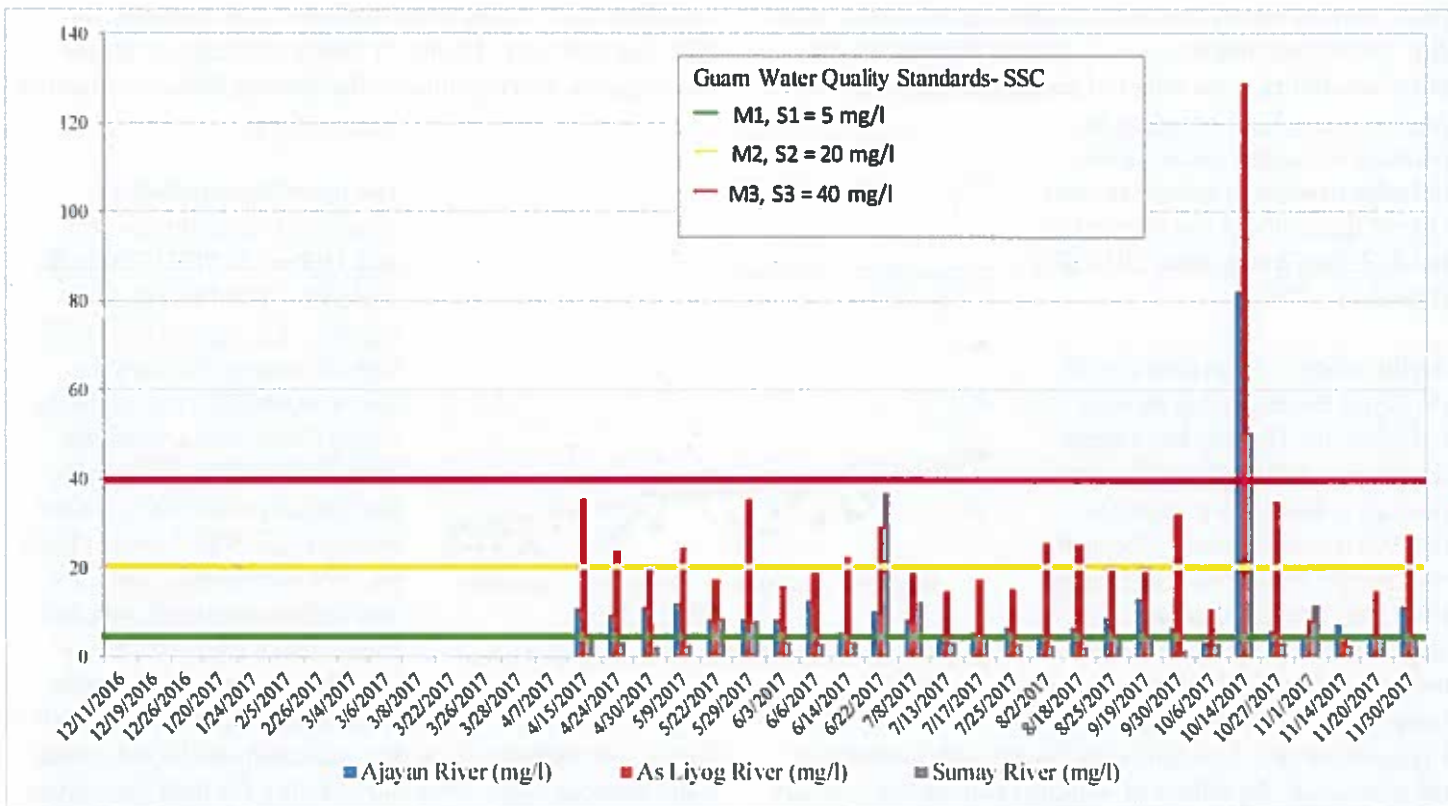


Figure 9a. Suspended sediment concentration (SSC) (mg/l) in the three rivers for the period 12/11/2016 - 11/30/17 compared to Guam water quality standards.

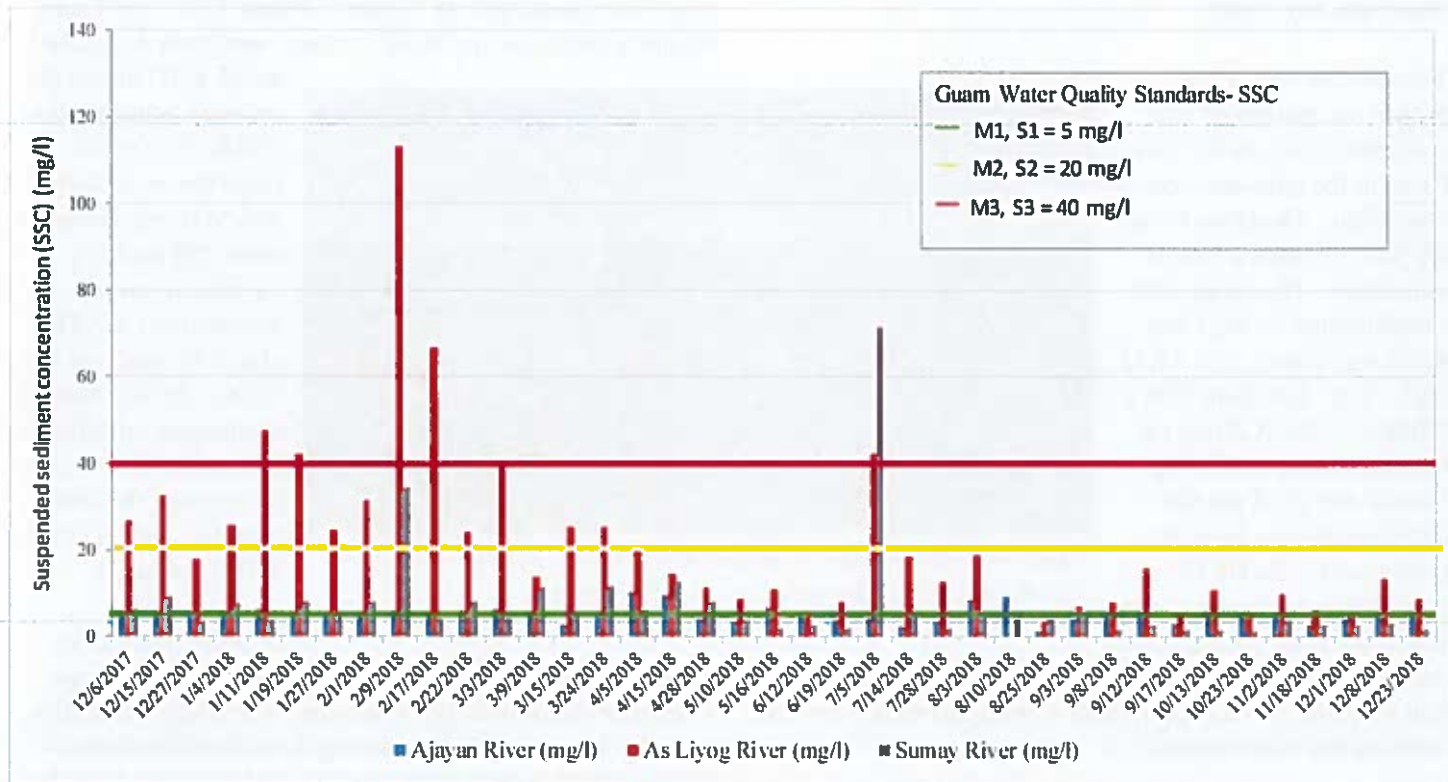


Figure 9b. Suspended sediment concentrations (SSC) (mg/l) in the three rivers for the period 12/6/2017 - 12/23/2018 compared to Guam water quality standards.

fairly normal, with some of the higher rainfall totals in the July to October timeframe, as is typical for Guam. Another possibility is the effect of restoration activities in the Manell watershed, including the planting of vegetation along the hillsides in order to reduce erosion. One of these efforts has focused on the As Liyog River, since 2014 (EA Associates, 2014).

A plot of SSC versus time for the As Liyog River site can be seen in Figure 10. The line has a negative slope. Although the R^2 value is small (0.066), the p value ($p = 0.0402$) was significant. The same plot for the Ajayan and the Sumay river sites both showed negative slopes, but with smaller R^2 values. It is not clear if there was any temporal trend in SSC at the As Liyog River site. In addition to the restoration efforts in the watershed, the effects of wildfires leaving the soil bare, followed by revegetation either naturally or as a part of restoration efforts, could lead to a decrease in the amount of bare soil present, and perhaps to less erosion. Additional monitoring, and surveys of ground cover would help to determine any trends.

Towards the end of the project, an additional site was established on the Geus River in the adjacent Geus watershed. The Geus River site was sampled a total of nine times. The mean SSC concentration on the Geus River was lower, 2.55 ± 1.53 mg/l. SSC data from EPA's STORET (WQP, 2019) for Guam was also reviewed. Most if not all of the SSC data appeared to have been generated by the US Geological Survey, for the La Sa Fua River near Umatac, and the Ugum River near Talofof. The mean SSC concentration was 676.5 ± 48.1 mg/l, quite a bit higher than what was found in the current study.

Hach Turbidity

Turbidity measurements using a Hach turbidimeter (Hach turbidity) were also made in the three rivers during each

sampling event, when water samples were collected for SSC and nutrients. Figure 11 shows an image of the turbidity plume moving out into the Achang Preserve from the Ajayan River.

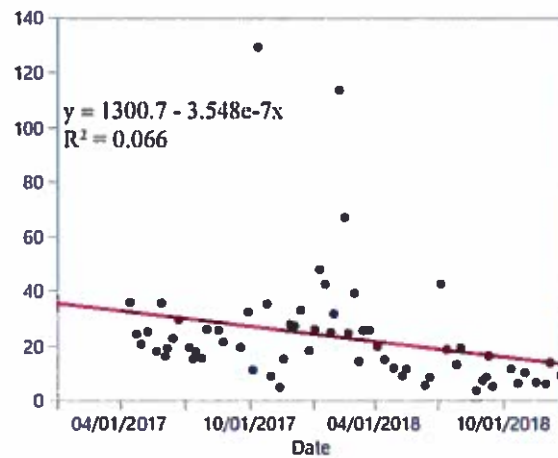


Figure 10. Bivariate fit of SSC over time in the As Liyog River.

The mean Hach turbidity measured during this project was 11.8 ± 1.16 NTU (Table 6). Turbidity varied by site (Chi-Square = 82.7, $p < 0.0001$). The highest average turbidity by site, as with SSC, was in the As Liyog River with a mean turbidity of 17 NTU, followed by the Ajayan (10.6 NTU) and the Sumay (7.53 NTU) rivers (Table 6). Not surprisingly, turbidity was highly correlated with SSC (Spearman's Rho = 0.7336, $p < 0.0001$). Turbidity was also correlated with water level in the

ivers. An analysis of the data indicated significant correlations between water level and turbidity for both the Ajayan (Spearman's Rho = 0.2204, $p < 0.0010$) and the As Liyog (Spearman's Rho = 0.1369, $p < 0.0445$) sites.

Graphs of the results of the Hach turbidity measurements over time can be seen in Figures 12a and 12b. The Guam turbidity standards for M1/S1 waters have been designated

as 0.5 NTU above the ambient concentration (Table 7). For the other two marine (M2 and M3) and surface water (S2 and S3) classifications, the standard is 1.0 NTU above the ambient turbidity. As an estimate of ambient turbidity for the three sites in the project, the mean turbidity value (11.5 NTU) was used.

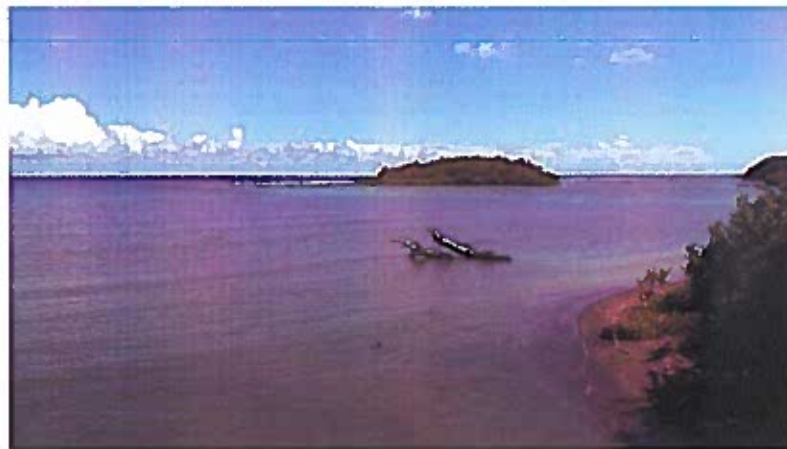


Figure 11. Outflow from the Ajayan River into the Achang Preserve. Note the turbidity plume going out into the Preserve.

As with SSC, there were a number of occasions when turbidity exceeded the estimated Guam turbidity standards. The highest turbidity recorded as with SSC, was during the collection of the 14 October 2017 samples (Figure 12a), and as noted, was also during a period of increased (approximately 25 cm) rain. The highest turbidity measured during this time was at the

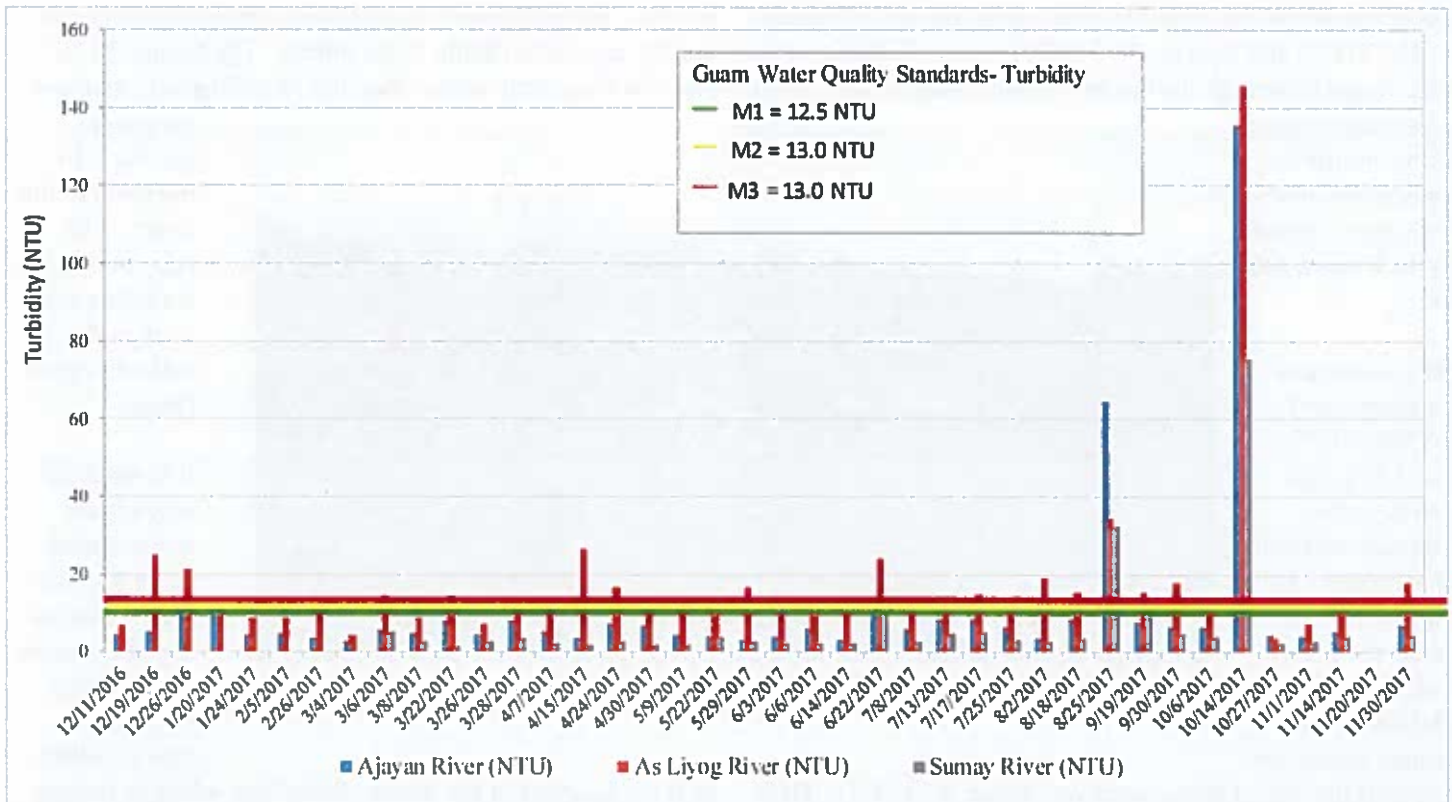


Figure 12a. Turbidity (NTU) in the three rivers for the period 12/11/2016 - 11/30/17 compared to Guam water quality standards.

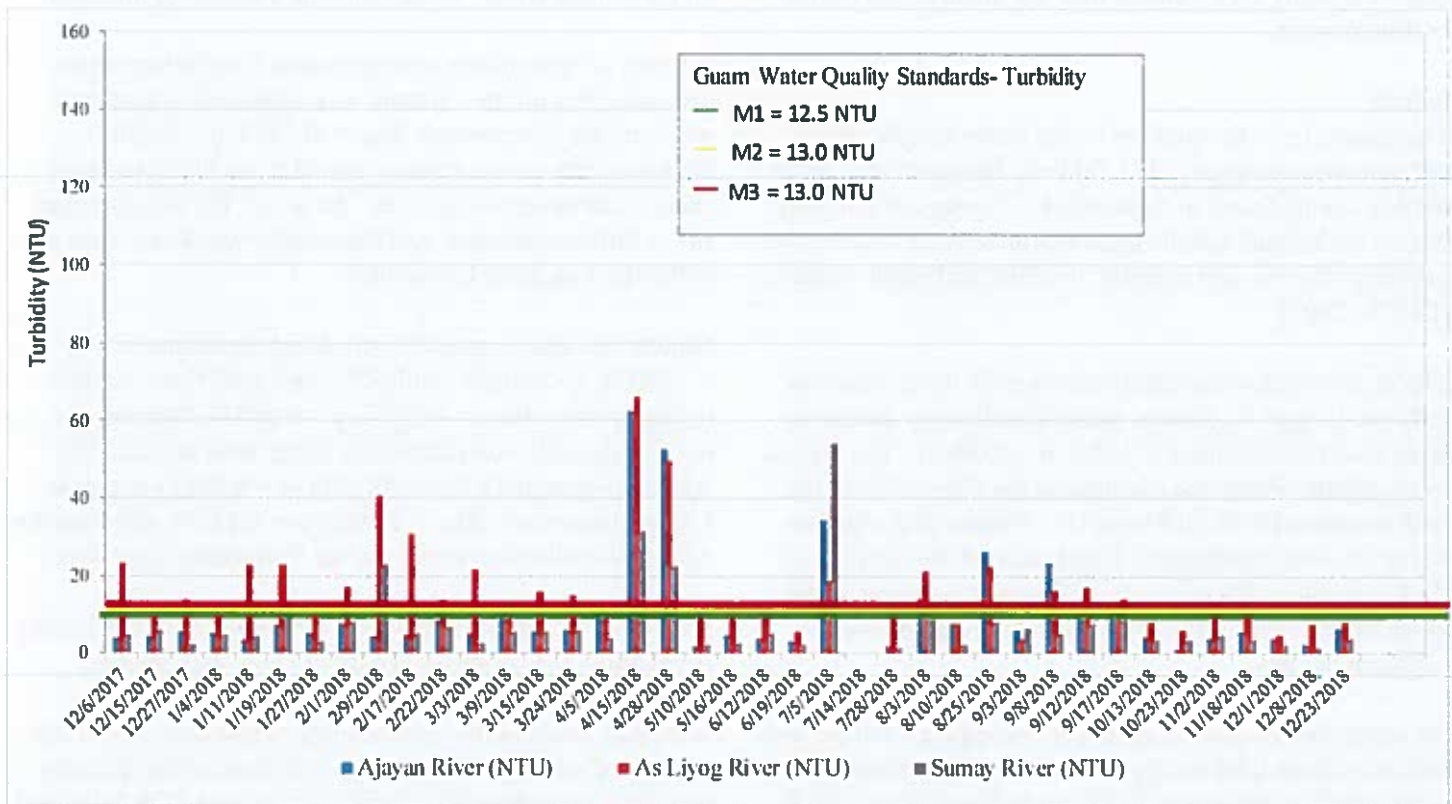


Figure 12b. Turbidity (NTU) in the three rivers for the period 12/6/2017 - 12/23/2018 compared to Guam water quality standards.

As Liyog River site with 146 NTU, followed by the Ajayan (135.5 NTU) and Sumay (75.6 NTU) rivers. Turbidity was also found to be high during the 15 April and 28 April 2018 sampling (Figure 12b). Rainfall during this time was approximately 18.6 cm or 6.62 in.

The town (and municipality) of Merizo (Figure 13) is split between the Manell and Geus watersheds. In the Geus watershed, the mean turbidity from the nine samplings conducted towards the end of this project was lower, 3.34 NTU. Data from EPA's STORET (WQP, 2019) for Guam was assessed for turbidity measurements. The mean turbidity for Guam was 33.6 ± 4.01 NTU, higher than the mean found for the current project.

Nitrate

The results from the analysis of the water samples for nitrate are also summarized in Table 6. More detailed information can be found in Appendix C. Sources of nitrogen can be varied and include agricultural activities such as the growing of crops and animals, wildlife, and septic systems (CUCE, 2005).

The mean nitrate concentration across all three sites was 0.07 ± 0.01 mg/l N. Nitrate varied significantly across the three rivers (Chi-Square = 165.3, $p < 0.0001$). The highest mean nitrate of any site occurred at the Sumay River site with a mean of 0.19 ± 0.01 mg/l N. Nitrate at the Sumay River site was significantly higher than at the Ajayan and As Liyog sites. There was no difference, however, in the mean nitrate concentration between the Ajayan and As Liyog river sites.

As noted earlier, the Sumay River site appears to have less influence from tidal waters (Table 3), being located further upstream than either the Ajayan or As Liyog sites, which may limit mixing with waters from the Achang Preserve. Results of monitoring nitrogen in the Manell watershed can be seen in Figures 14a and 14b. The Sumay River site had some of the higher nitrate values recorded during the

project. Superimposed on the graphs are the Guam water quality standards (Table 7) for nitrate. The Sumay River site was frequently higher than the M2/S2 (good) standard



Figure 13. Houses in the town of Merizo bordering Cocos Lagoon.

for nitrate, putting it in the good to fair range. The Ajayan and the As Liyog sites were in the excellent to good range.

It is not clear why nitrate at the Sumay River site was higher than either the Ajayan or the Liyog river sites.

One possibility is the location of the Sumay River site, which is further upstream, and likely experiences less mixing with tidal waters. Another possibility are the few dwellings near the site on the Sumay River, which could be a source of nitrogen.

A series of Spearman's nonparametric correlations were calculated for nitrate. Nitrate was negatively correlated with salinity (Spearman's Rho = -0.7853, $p < 0.0001$). Higher nitrate concentrations would more likely be associated with terrestrial sources. As noted, the Sumay River site is further upstream, and the salinity was lower than at either the Ajayan or Liyog sites.

Nitrate was also negatively correlated (Spearman's Rho = -0.3038, $p < 0.0001$) with SSC, and with Hach turbidity (Spearman's Rho = -0.3926, $p < 0.0001$). Nitrate was not significantly correlated with water level at either the Ajayan (Spearman's Rho = 0.1243, $p = 0.0664$) or the As Liyog (Spearman's Rho = 0.0965, $p = 0.1594$), although the Ajayan correlation was not too far from being significant.

Nadeau and Denton (2016) assessed nutrients in the Togcha River along the southeast coast of Guam, and detected nitrate at concentrations up to 0.36 mg/l N, similar to what was found in the current study. Denton *et al.* (2005) conducted a study in Agana Bay and Tumon Bay, popular tourist areas on the west coast of Guam, and found elevated levels of nitrate, ranging from 1.34 to 4.01 mg/l N in Agana Bay, and in Tumon Bay nitrate concentrations ranged from 0.08 to 0.79 mg/l N, higher than was found in the current

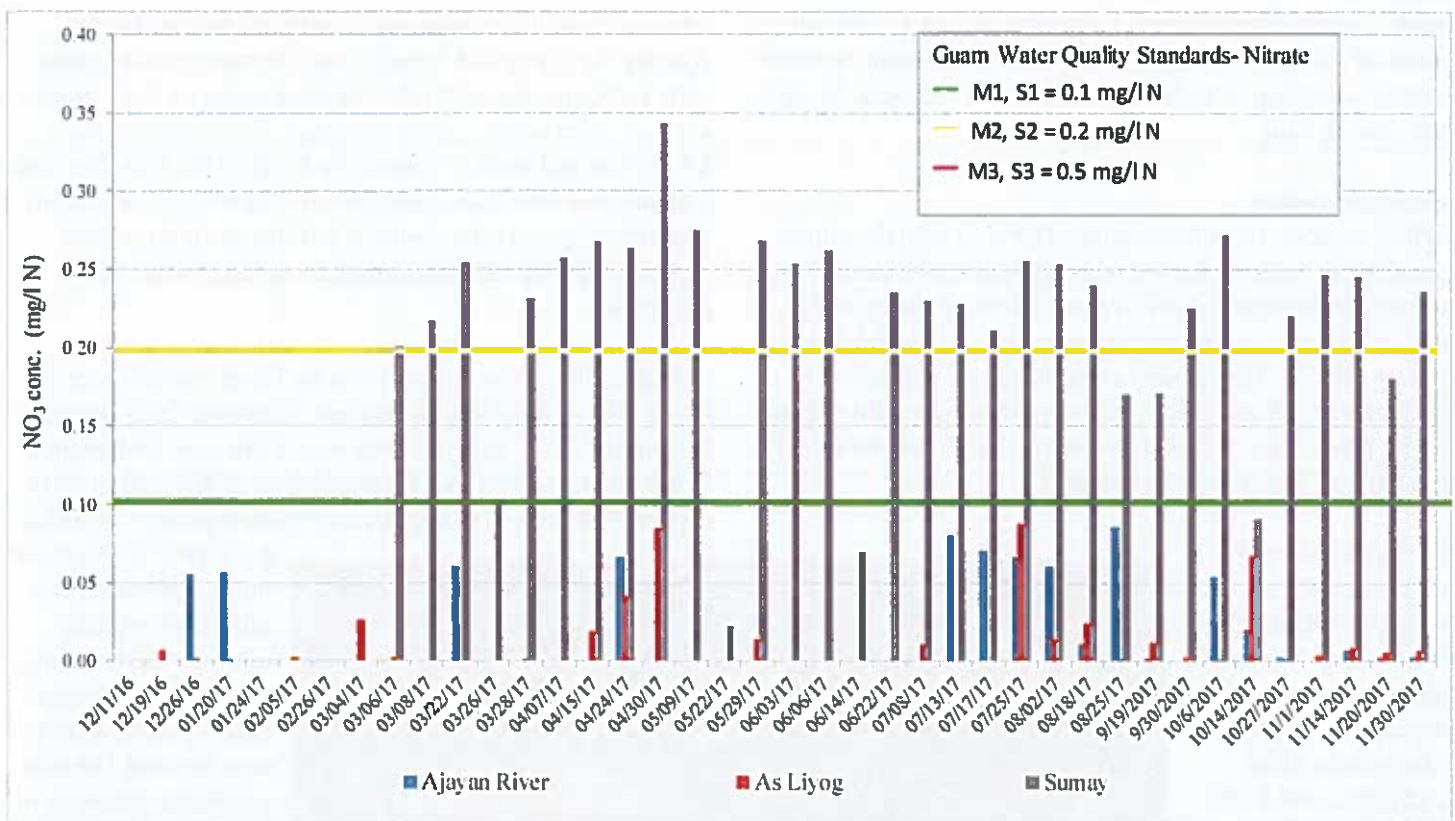


Figure 14a. Nitrate concentrations (mg/l) in the three rivers for the period 12/11/2016-11/30/17 compared to Guam water quality standards.

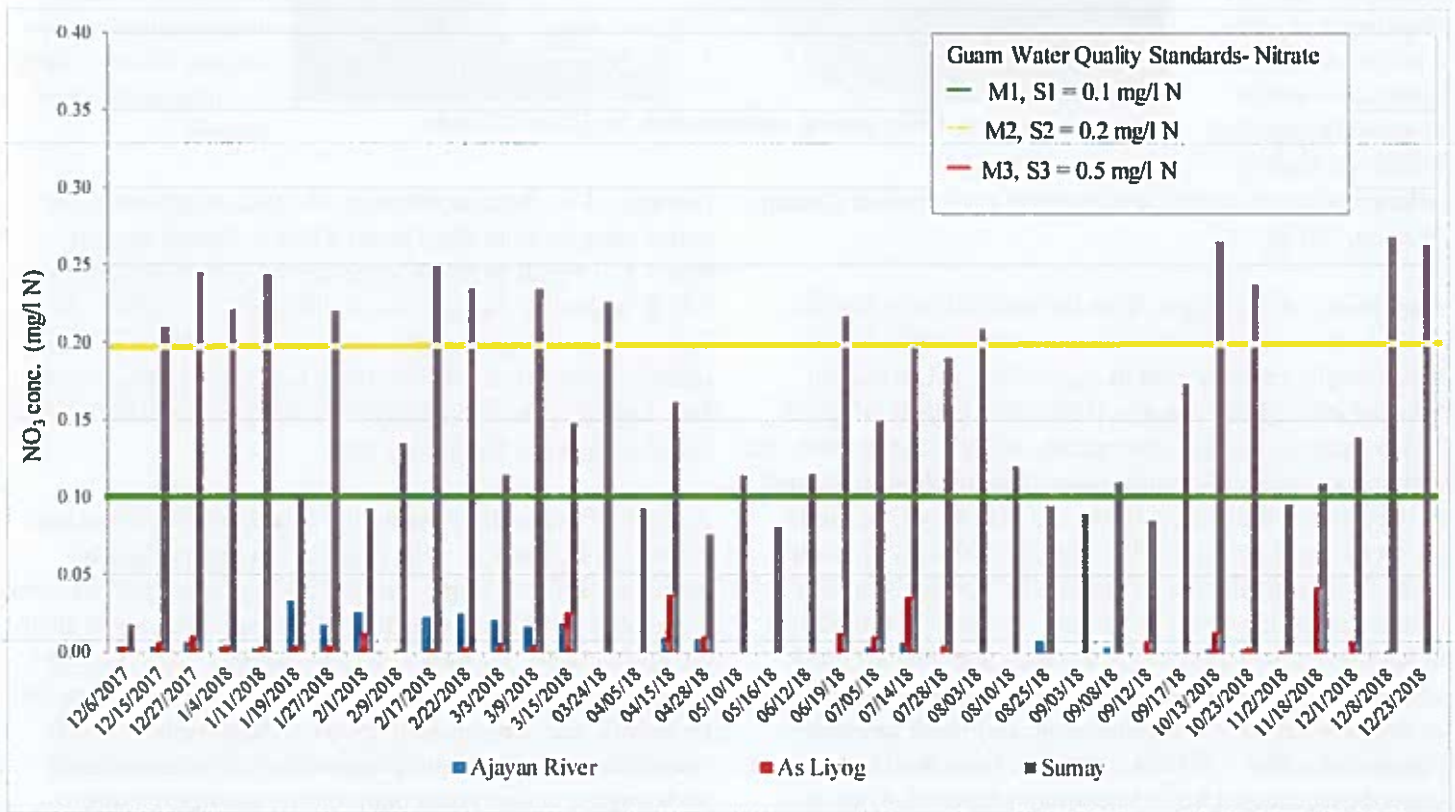


Figure 14b. Nitrate concentrations (mg/l) in the three rivers for the period 12/6/2017-12/23/18 compared to Guam water quality standards.

study. A review of STORET data (WQP, 2019), indicated a mean of 0.010 ± 0.00 mg/g N for nitrate for Guam environmental sampling, which is slightly above the mean found in the current study.

Orthophosphate

While sources of orthophosphate (HPO_4^{3-}) include natural weathering from rocks, the major human-influenced sources include sewage, runoff from agricultural sites (fertilizers and farm animals), and application of lawn fertilizers (Oram, 2014). Phosphorus is an important element for both plants and animals. Orthophosphate is readily taken up by plants and in unpolluted waters, the concentration is typically at very low concentrations.

Elevated levels of nutrients such as orthophosphate in freshwater and marine environments can lead to an overabundance of phytoplankton and algae, and in the marine environment can shade out corals and seagrasses. In seagrasses, an overabundance of nutrients has also been shown to result in increased epiphytes, which can lead to reduced seagrass growth and survival as a result of shading (Nelson, 2017).

A summary of the results from the analysis of orthophosphate in the water samples are included in Table 6. Detailed results can be found in Appendix C. The overall mean of orthophosphate was 0.08 ± 0.00 mg/l P, which is higher than the Guam water quality M2/S2 (good) limit. As with nitrate, the highest orthophosphate reading (0.46 mg/l P) occurred at the Sumay River site. However, the highest mean orthophosphate (0.1 mg/l P) (Table 6) occurred at the As Liyog River site (Figure 15). Orthophosphate varied across the sites (Chi-Square = 54.076, $p < 0.0001$). In addition, a Kruskal-Wallis test indicated that all three sites were different from one another. There was a positive correlation between orthophosphate and Hach turbidity (Spearman's Rho = 0.1714, $p = 0.0114$), as well as between orthophosphate and SSC (Spearman's Rho = 0.4148, $p < 0.0001$).



Figure 15. As Liyog River looking upstream from the Route 4 bridge.

The results of the monitoring of orthophosphate for the Ajayan, As Liyog and Sumay river sites are shown graphically in Figure 16a and 16b. Superimposed on both graphs are the Guam water quality standards for orthophosphate for marine and surface waters. In Figure 16a, it can be seen that all three sites exceeded not only the M1/S1 (excellent) and M2/S2 (good) standards, but at times, all three sites exceeded the M3/S3 (fair) standard of 0.025 mg/l orthophosphate.

In Figure 16b, there appear to be far fewer exceedences of the M3/S3 standard for the period December 2017 through December 2018. It is not clear why there was a difference in the two time periods. A nonparametric Wilcoxon test indicated that there was a significant (Chi-Square = 74.0285,

$p < 0.0001$) difference in the concentrations of orthophosphate between the two time periods. The separation of results in Figures 16a and 16b was somewhat arbitrary in order to better present the data, however, it appears there was a difference in the orthophosphate concentrations between these two designated time periods.

In Figure 17a, the concentration of orthophosphate in the water samples from the Ajayan River is plotted against time. An overall negative slope can be seen in this graph. The R^2 value for this line was 0.408, and $p < 0.0001$. In Figure 17b, orthophosphate in the As Liyog River is also plotted against time. Again, there is a negative slope to the line, however the R^2 value ($R^2 = 0.149019$, $p = 0.0006$) was less than that for the Ajayan site.

A graph of the rainfall during the course of the project can be seen in Figure 17c. The slope of the line is slightly positive. If, for example, rainfall during the period between November 2017 and December 2018 was much lower than the period from December 2016 to October 2017, it might be expected that less rainfall could result in less surface water runoff, and subsequently lower orthophosphate levels. As noted, orthophosphate is more likely to be associated with surface water runoff than nitrate, as nitrate tends to percolate through the soil into groundwater.

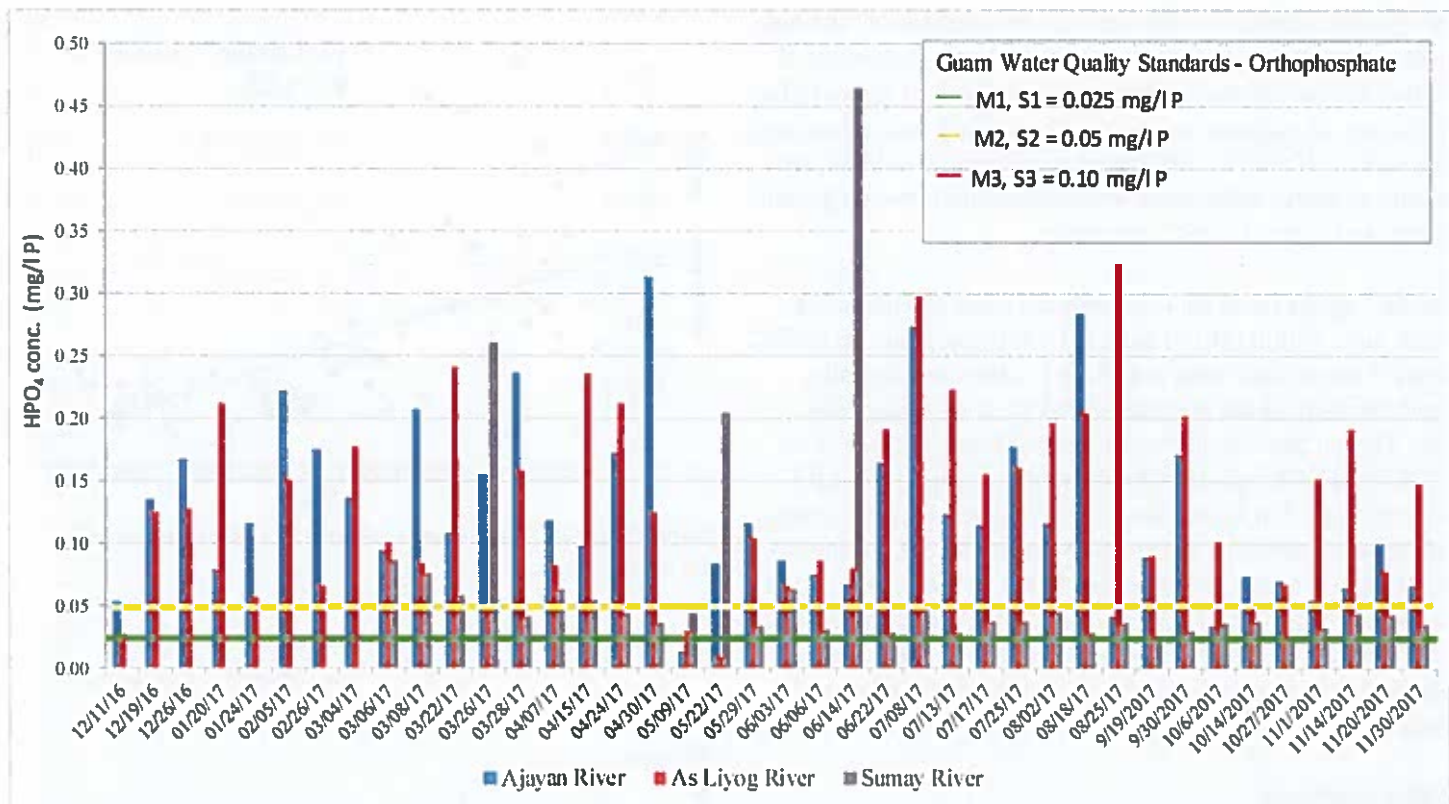


Figure 16a. Orthophosphate (HPO₄⁻) concentrations (mg/l) in the three rivers for the period 12/11/2016-11/30/17 compared to Guam water quality standards.

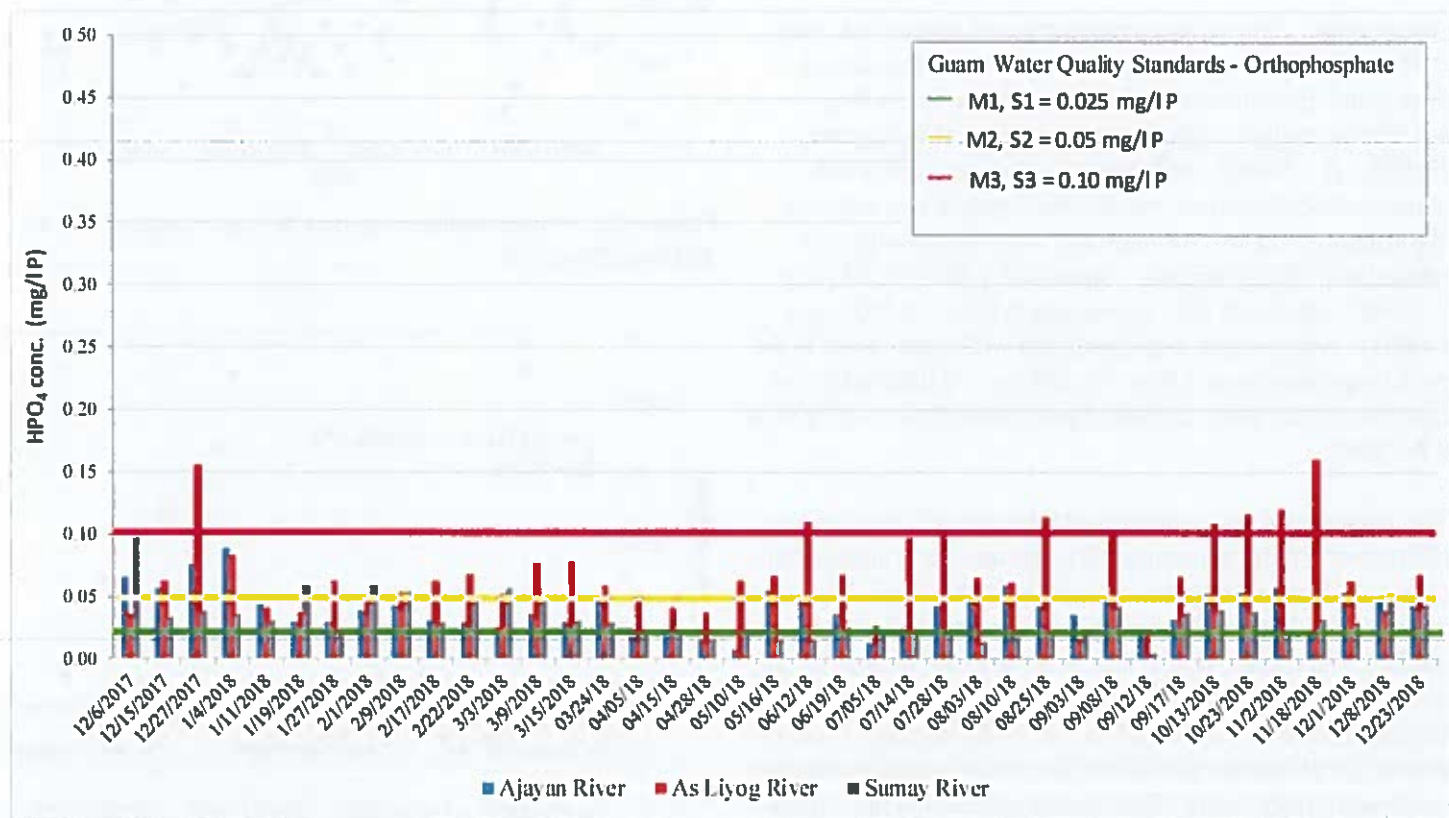


Figure 16b. Orthophosphate (HPO₄⁻) concentrations (mg/l) in the three rivers for the period 12/6/2017 - 12/23/2018 compared to Guam water quality standards

It's possible there is a link between revegetation of the hill-sides either through restoration efforts (e.g., replanting of native species of plants) or natural regrowth of grasses after wildfires, to reduced inputs of SSC and orthophosphate into the rivers. However, additional monitoring would be necessary to better understand the relationship between ground cover and inputs of SSC over time.

In the Togcha River on the southeast coast of Guam, Nadeau and Denton (2016) detected orthophosphate up to 0.02 mg/l P, lower than what was found in the current study. In their study in the popular tourist areas of Agana Bay and Tumon Bay on the west coast of Guam, Denton *et al.* (2005) found levels of orthophosphate, ranging from 0.13 to 0.31 mg/l P in Agana Bay, and in Tumon Bay orthophosphate concentrations ranged from below detection limit to 0.39 mg/l P, similar to the range found in the current study. A review of STORET data (WQP, 2019), indicated a mean orthophosphate concentration of 0.22 ± 0.01 mg/g P for Guam environmental sampling, slightly above the mean found in the current study.

Other Nutrients

The results from the analysis of other nutrients can be seen in Table 6 and in Appendix C. Results for a couple of the additional nutrients are summarized below.

Ammonium. The mean concentration of ammonium was 0.07 ± 0.00 mg/l N. The highest concentration detected during this project was 0.25 mg/l N (Table 6). A Kruskal-Wallis analysis indicated a significant (Chi-Square = 54.0382, $p < 0.0001$) difference in the ammonium concentration among sites, and that the Liyog River site was significantly higher. Ammonium was significantly correlated with Hach turbidity (Spearman's Rho = 0.2776, $p < 0.0001$), and with SSC (Spearman's Rho = 0.5052, $p < 0.0001$). Ammonium was correlated with water level in the As Liyog (Spearman's Rho = 0.3295, $p < 0.0001$), but not correlated with daily rainfall (Spearman's Rho = -0.0324, $p = 0.7809$).

The presence of ammonium is pH dependent. At a lower pH (below 7), the ammonia (NH_3) is ionized to ammonium (NH_4^+). The reverse is true at a higher pH. Guam EPA does not have standards for ammonium, but does have standards for ammonia, however it is for toxicity to aquatic animals. The first ammonia standard is the Criteria Chronic Concentration (CCC), which is the 30 day average concentration for all waters that should not be exceeded more than once every three years. The second standard is the Criteria Maximum Concentration or CMC, which is the one hour concentration that should not be exceeded more than once

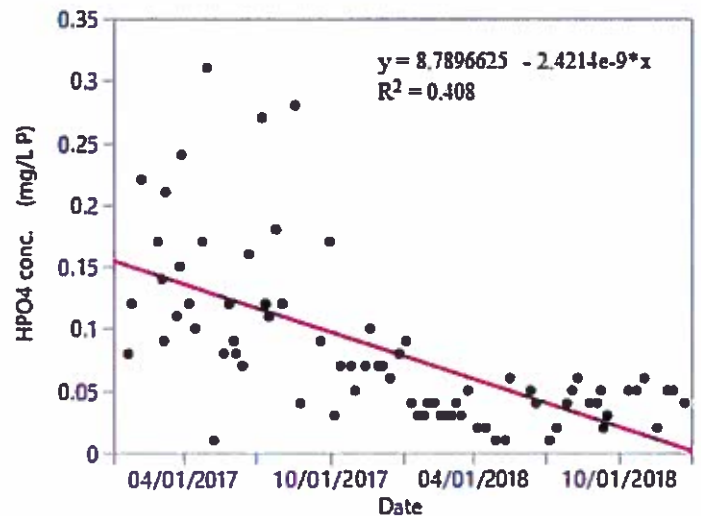


Figure 17a. Orthophosphate over time in water samples from the Ajayan River site.

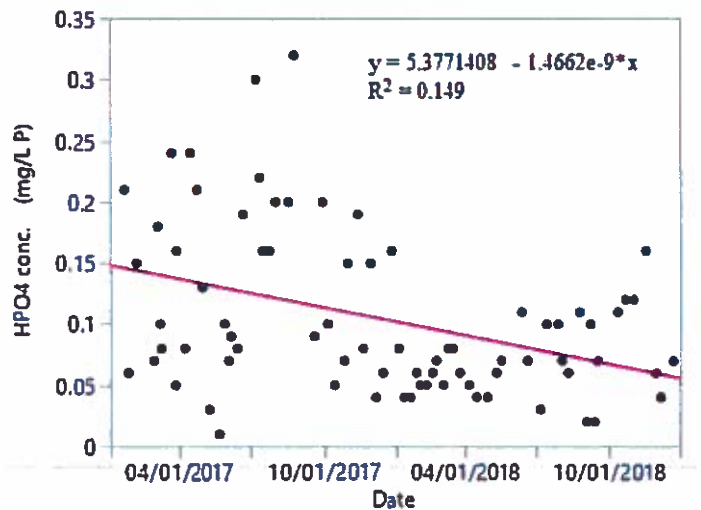


Figure 17b. Orthophosphate over time in water samples from the As Liyog River site.

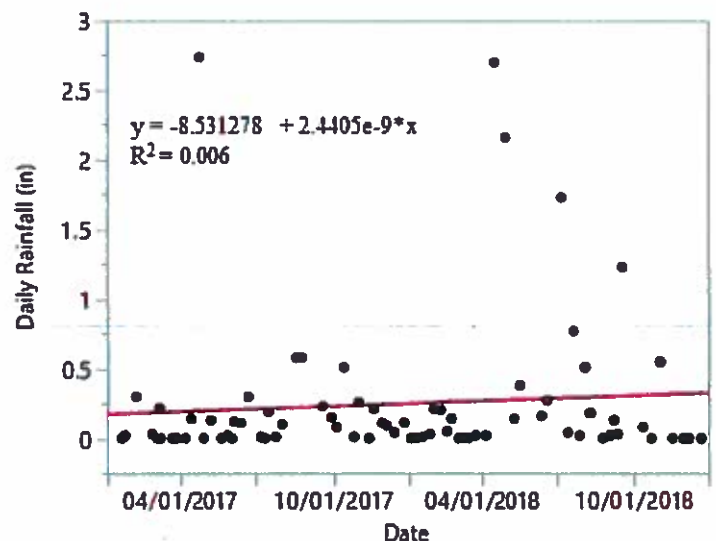


Figure 17c. Rainfall in the Manell watershed study area over time.

every three years. At a pH of 7.5, the CCC is 2.28 mg/l N; at a pH of 8, the CCC is 1.27 mg/l N. At a pH of 7.5, the CMC is 19.9 mg/l N; at a pH of 8, the CMC is 8.40. The highest concentration of ammonium found in this study was 0.25 mg/l N. At a pH between 7 and 8, approximately 10 to 20 percent of the ammonium would be in the ammonia form. Given that, the estimated amount of ammonia present would be below both the CMC and CCC.

As with orthophosphate, ammonium in the water samples varied over time. Plots of the ammonium concentration during the project by river can be seen in Appendix D. The slopes of the lines for ammonium were negative, although the R² values for the Ajayan (R² = 0.2834), As Liyog (R² = 0.1638), and the Sumay (R² = 0.1939) river sites were not high.

In the Togcha River in Guam, Nadeau and Denton (2016) detected ammonium ranging from below the detection limit to 2.54 mg/l N, higher than what was found in the current study. In their study in Agana Bay and Tumon Bay, Denton *et al.* (2005) found ammonium concentrations ranging from below the detection limit to 0.23 mg/l N in Agana Bay, and in Tumon Bay ammonium concentrations ranged from below detection limit to 0.39 mg/l N, higher than that found in the current study.

Silica. The mean concentration of silica (HSiO₃⁻) was 13.5 ±0.68 mg/l. The highest concentration detected during this study was 40 mg/l at the Sumay River site (Table 6). A Kruskal-Wallis analysis indicated that silica varied by site (Chi-Square = 76.2688, p < 0.0001), with all sites being significantly different from one another.

Somewhat surprisingly, silica was negatively correlated with both SSC (Spearman's Rho = -0.2979, p < 0.0001) and Hach turbidity (Spearman's Rho = -0.3190, p < 0.0001). As silica is sometimes used as an indicator of surface water

runoff and erosion from terrestrial areas, it was thought that these two parameters might be linked to silica.

As with ammonium, silica in the water samples varied over time. Plots of the silica concentration by river can be seen in Appendix E. The slopes of the lines for silica over time were negative, but the R² values for the Ajayan (R² = 0.17528), As Liyog (R² = 0.02224), and the Sumay (R² = 0.23116) river sites were relatively small.

Urea. Urea (CH₄N₂O) is a product of the metabolism of nitrogen-containing compounds like proteins, formed in the liver from ammonia, and excreted in the urine by mammals. Urea is also used in fertilizers.

The mean concentration of urea in the water samples from the Manell watershed was 0.02 ±0.00 mg/l N. The highest concentration (0.08 mg/l N) was from the Sumay River site. A Kruskal-Wallis non-parametric analysis indicated a significant difference between sites (Chi-Square = 37.1476, p < 0.0001), with all sites being significantly different from one another. Urea, however, was not correlated with daily rainfall (Spearman's Rho = 0.0241, p = 0.8360).

Table 8. Spearman correlations between nutrient species.

Nutrient	by Nutrient	Spearman Rho	Prob> p
HPO4 ⁻	NO3 ⁻	-0.3913	< 0.0001
HSiO3 ⁻	HPO4 ⁻	-0.0395	0.5578
HSiO3 ⁻	NO3 ⁻	0.7228	<0.0001
NH4	HPO4 ⁻	0.7089	<0.0001
NH4	HSiO3 ⁻	-0.1249	0.0626
NH4 ⁺	NO3 ⁻	-0.3905	<0.0001
NO2 ⁻	NO3 ⁻	-0.5531	<0.0001
NO2 ⁻	NH4 ⁺	0.7416	<0.0001
NO2 ⁻	HPO4 ⁻	0.5922	<0.0001
NO2 ⁻	HSiO3 ⁻	-0.3556	<0.0001
Total N	NO3 ⁻	0.3999	<0.0001
Total N	NO2 ⁻	-0.1871	0.0051
Total N	NH4 ⁺	0.0982	0.1436
Total N	HPO4 ⁻	0.0682	0.3103
Total N	HSiO3 ⁻	0.2705	<0.0001
Total P	NO3 ⁻	-0.1894	0.0045
Total P	NO2 ⁻	0.2792	<0.0001
Total P	NH4 ⁺	0.4439	<0.0001
Total P	HPO4 ⁻	0.6976	<0.0001
Total P	HSiO3 ⁻	-0.0596	0.3758
Urea	NO3 ⁻	-0.369	<0.0001
Urea	NO2 ⁻	0.6729	<0.0001
Urea	NH4 ⁺	0.6582	<0.0001
Urea	HPO4 ⁻	0.5837	<0.0001
Urea	HSiO3 ⁻	-0.1825	0.0063

Relationships Between Nutrients

Spearman's rank correlation coefficient analysis between nutrients for the three sites in this project can be seen in Table 8. Whittall *et al.* (2013) examined correlations between nutrient species, leaving out those that are autocorrelated (e.g., orthophosphorus and total phosphorus), and found that ammonium, urea and orthophosphorus are associated with runoff, while the oxidized forms of nitrogen (e.g., nitrate) tend to percolate into soils during rainfall events, and eventually into groundwater. The results of the analysis indicated significant correlations between ammonium and urea (Spearman's Rho = 0.6582, p < 0.001), ammonium and orthophosphate (Spearman's Rho = 0.7089, p < 0.0001), and urea and orthophosphate (Spearman's Rho = 0.5837,

$p < 0.0001$), nutrients more associated with runoff. As noted by Whitall *et al.* (2013), urea and ammonia should be tightly linked as the urea can be converted to ammonium through enzymatic processes.

The more oxidized nitrate were not positively correlated with any of these nutrients. Somewhat surprisingly, however, nitrite (NO_2^-) was negatively correlated with nitrate, and positively correlated with urea, ammonia, and orthophosphate (Table 8). However, this could be a function of the very low detections for nitrite throughout the study (Appendix C).

Silica (HSiO_3^-) can be a useful indicator of erosion, as silica accounts for nearly 60 percent of the composition of the Earth's crust. A series of correlations carried out for silica indicated that it was correlated with stream height in the Ajayan (Spearman's Rho = 0.4431, $p < 0.0001$), and the As Liyog (Spearman's Rho = 0.3295, $p < 0.0044$), however, it was not significantly correlated with either Hach turbidity (Spearman's Rho = 0.2242, $p = 0.0548$), or SSC (Spearman's Rho = -0.0563, $p = 0.6559$), which could be an indication of the confounding factors of tidal flow into and out of the Ajayan and As Liyog river sites. Another factor could be a possible decreasing trend in silica concentration over time. Appendix E contains graphs showing the concentration of silica over time at the three sites for this project.

While it appears that a number of nutrient species were predictively positively correlated, there were some that were not correlated as expected. More local sources of nutrient input could also be a factor for the differences. Additional monitoring in these rivers would help in better understanding the sources of the nutrients as well as the possibility of more local inputs.

SUMMARY AND CONCLUSIONS

This project, funded by NOAA's Coral Reef Conservation Program, and requested by local partners, resulted in the monitoring of water quality in three rivers that drain to the Achang Reef Flat Marine Preserve. Local resource managers had expressed concern that inputs from the Manell watershed could be impacting the Achang Preserve. This study described the spatial and temporal variation for a series of parameters including turbidity, suspended sediment concentration (SSC), and nutrients at sites on the Ajayan, As Liyog, and Sumay rivers. The project was carried out to provide a baseline of conditions for these parameters, so that local resource managers could better understand the condition of these rivers, and also provide the means for

assessing the efficacy of restoration activities being carried out or planned for the Manell watershed.

The sites monitored on the Ajayan and As Liyog rivers were near where these rivers empty into the Achang Preserve. Because of this, there was some mixing of seawater with the freshwater flowing down the rivers. The Sumay River site was a little further upstream of where it empties into the Achang Preserve. The salinity/conductivity measurements taken reflected the influence of the saline waters from the Achang Preserve at the sites, with much higher readings in the Ajayan and As Liyog site, compared to the Sumay River site. Sites further upstream for the Ajayan and the As Liyog rivers were not possible due to dense jungle that could not be penetrated even with a kayak, along with uncertainties regarding land ownership. A total of 76 sampling events occurred in the Ajayan and As Liyog rivers sites. The Sumay River (71 sampling events) site was added somewhat later in the project. There were times during the project when the collection of water samples for SSC and nutrient analyses could not be carried out due to flood waters covering the roads to the sites, making it too difficult and too dangerous to attempt sample collection.

Automated stream level monitoring was carried out during the course of the project in the Ajayan and As Liyog rivers; the water level in the Sumay River was too low for the automated stream level monitoring. At the Ajayan River site, an automated turbidity logger was also put in place. An automated rain gauge was established further up in the Manell watershed as well. Results indicated that stream level, which was used as a proxy for stream flow, was correlated positively with rainfall in the Ajayan and As Liyog rivers. At the Ajayan River site, rainfall was correlated with turbidity from the automated logger. Stream level also varied during the year. Results for stream level were classified as occurring either in the wet season (approximately May through November), or the dry season (approximately December through April). It was found that stream level for both the Ajayan and As Liyog river sites were positively correlated with this grouping, that is, higher stream levels were associated with the wet season.

The results from the monitoring of SSC, turbidity, nitrate, and orthophosphate were compared to the Guam Environmental Protection Agency water quality standards. The marine and surface waters in Guam have been classified according to their expected water quality. Waters are classified as either excellent, good or fair, and have assigned water quality standards for various parameters, and the concentrations of those parameters needed to achieve the expected or desired water quality. The area where this

project was carried out was classified in the excellent to good range.

SSC was found to vary across the sites in the three rivers. The mean concentration of SSC at the As Liyog River site was in the good to fair range for SSC. The mean SSC values for the Ajayan and Sumay river sites were in the excellent to good water quality range.

In addition to the automated turbidity readings at the Ajayan River site, turbidity readings were also taken with a Hach turbidimeter during each round of water sample collections. Results indicated that turbidity was correlated with SSC and water level. The Guam water quality standard for turbidity is set between 0.5 and 1.0 NTUs (nephelometric turbidity units) above the ambient concentration. For this study, the mean turbidity measurement across sites was used as the ambient concentration. Given that, there were a number of times when the water quality was only in the good to fair range for turbidity in both the Ajayan and the As Liyog river sites. In some cases, the exceedence of the standard appeared to be linked to significant rainfall events.

Measurements of nitrate and orthophosphate from the three sites were also compared to the Guam water quality standards. The overall mean nitrate concentration for the three river sites was in the excellent range. However, for the Sumay River, nitrate was frequently only in the good to fair range. It is not clear why the nitrate concentration in the Sumay River site was higher than at the Ajayan and As Liyog sites. Because the Sumay River site was a little further upstream than the sites on either the Ajayan or As Liyog rivers, there may have been less mixing with seawater.

For orthophosphate, the mean concentration across sites was in the good to fair range. The As Liyog River site had the highest mean orthophosphate concentration, and was in the fair range.

During the project, a possible temporal trend was seen for a number of parameters. The concentrations of SSC, orthophosphate, and perhaps ammonium and silica showed some evidence of a negative or downward slope in concentrations over time. Although the R^2 or coefficient of determination for these parameters was not high, there was at least the suggestion of a downward trend in the concentration of these parameters across sites over time. Rainfall amounts during the two years of this project appeared to be normal, with typical wet and dry season rainfall amounts.

Erosion has been a serious issue in the Manell watershed. Grazing by feral animals, the presence of off-roading vehicles, and in particular wildfires set by poachers, all likely promote the erosion of soils through the removal of vegetative cover from the steep hillsides, and along stream banks. There have been various watershed restoration efforts including public education and outreach, planting of native species, and the installation of riparian buffer strips. In addition to these restoration efforts, the effects of wildfires leaving the soil bare, followed by revegetation either naturally or enhanced as a part of ongoing restoration efforts, could all result in a decrease of bare soils present, and perhaps a detectable reduction in parameters related to erosion.

If the apparent decreases in SSC, orthophosphate, ammonium and silica seen during the course of this project are real and are linked to decreases in runoff from the watershed, it would suggest the need for continued restoration efforts, as well as public education and outreach. Even if a reduction in erosion was due to natural revegetation of the hillsides following wildfires, it would still point to the need for continued public education and outreach, to further reduce the incidence of the wildfires, and also highlight the need for a continuation of the replanting efforts in the watershed. As noted earlier, since the establishment of the Achang Preserve, fish stocks there have increased relative to the rest of Cocos Lagoon. Given that, reductions in the input of sediments and nutrients into the Achang Preserve would likely benefit the Preserve, and the rest of Cocos Lagoon.

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Appendix A. Results of water quality monitoring in the Manell Watershed, Guam.

Location	ID	Date	Time	Temperature (°C)	Dissolved Oxygen (mg/l)	Dissolved Oxygen (%)	Conductivity (µs/cm)	Salinity (ppt)	Turbidity (NTU)	SSC (mg/l)
Liyog Bridge	L001-01	12/11/2016	1625	32.3	7.39	100	48,124	30.90	6.99	
Ajayan Bridge	A001-01	12/11/2016	1700	31.0	5.15	73.5	40,274	26.88	4.51	
Liyog Bridge	L002-01	12/19/2016	1640	29.1	5.99	90.1	44,106	28.36	24.80	
Ajayan Bridge	A002-01	12/19/2016	1700	29.3	5.80	88.4	41,448	26.73	5.31	
Liyog Bridge	L003-01	12/26/2016	1615	31.6	6.02	95.1	46,435	30.13	21.35	
Ajayan Bridge	A003-01	12/26/2016	1650	30.1	5.46	75.5	14,515	11.54	11.35	
Liyog Bridge	L004-01	1/20/2017	1525	31.0	6.48	100	48,122	31.25	13.95	
Ajayan Bridge	A004-01	1/20/2017	1610	28.8	5.83	80.5	14,681	8.83	9.50	
Liyog Bridge	L005-01	1/24/2017	1610	30.4	6.11	97.7	49,081	31.89	8.73	
Ajayan Bridge	A005-01	1/24/2017	1635	29.2	5.79	86.2	40,965	25.50	5.29	
Liyog Bridge	L006-01	2/5/2017	1610	30.7	6.20	95.1	47,160	30.55	8.88	
Ajayan Bridge	A006-01	2/5/2017	1625	29.0	5.36	78.2	33,349	20.83	4.95	
Liyog Bridge	L007-01	2/26/2017	1650	31.8	5.65	89.9	49,420	32.07	9.14	
Ajayan Bridge	A007-01	2/26/2017	1710	30.5	5.48	82.7	36,655	22.54	3.78	
Liyog Bridge	L008-01	3/4/2017	1540	28.9	3.54	54.0	41,469	26.51	4.27	
Ajayan Bridge	A008-01	3/4/2017	1605	30.4	4.53	70.7	42,315	27.12	2.70	
Liyog Bridge	L009-01	3/6/2017	925	27.0	78.70	78.7	49,559	32.44	14.65	
Ajayan Bridge	A009-01	3/6/2017	1000	27.8	4.10	60.9	40,800	26.55	5.45	
Sumay Bridge	S009-01	3/6/2017	1010	28.2	4.30	59.5	21,723	12.74	5.41	
Liyog Bridge	L010-01	3/8/2017	1700	30.3	5.02	79.8	47,887	31.05	8.90	
Ajayan Bridge	A010-01	3/8/2017	1720	31.2	3.12	48.6	40,277	25.52	4.98	
Sumay Bridge	S010-01	3/8/2017	1735	28.8	3.94	52.5	9,360	4.09	2.87	
Liyog Bridge	L011-01	3/22/2017	1700	34.1	5.75	97.6	48,966	31.64	14.85	
Ajayan Bridge	A011-01	3/22/2017	1735	30.9	4.39	65.4	26,624	16.37	8.03	
Sumay Bridge	S011-01	3/22/2017	1755	28.4	3.71	48.1	1,412	0.70	1.57	
Liyog Bridge	L012-01	3/26/2017	1735	32.4	5.87	96.5	49,443	32.64	7.32	
Sumay Bridge	S012-01	3/26/2017	1750	32.1	5.23	80.4	36,239	24.31	2.67	
Ajayan Bridge	A012-01	3/26/2017	1800	33.1	4.29	70.4	44,141	28.61	4.40	
Liyog Bridge	L013-01	3/28/2017	1545	34.4	5.66	95.5	49,323	31.82	13.60	
Sumay Bridge	S013-01	3/28/2017	1605	28.3	3.81	49.0	1,674	0.84	3.68	
Ajayan Bridge	A013-01	3/28/2017	1620	31.9	4.41	66.6	34,327	21.23	8.12	
Liyog Bridge	L014-01	4/7/2017	1550	33.7	5.39	90.1	49,585	32.15	9.54	
Sumay Bridge	S014-01	4/7/2017	1605	30.1	4.57	64.6	15,004	8.52	2.44	
Ajayan Bridge	A014-01	4/7/2017	1625	34.5	3.89	64.6	39,793	25.31	5.31	
Liyog Bridge	L015-01	4/15/2017	1650	30.8	1.39	21.1	36,039	22.69	26.80	35.60
Sumay Bridge	S015-01	4/15/2017	1720	28.3	3.51	45.5	1,635	0.82	2.04	4.80
Ajayan Bridge	A015-01	4/15/2017	1735	33.3	4.26	68.0	45,418	29.23	3.39	11.20
Liyog Bridge	L016-01	4/24/2017	1330	30.2	4.50	65.5	36,144	22.61	16.70	24.00
Sumay Bridge	S016-01	4/24/2017	1405	28.6	3.68	47.7	1,683	0.84	2.82	3.40
Ajayan Bridge	A016-01	4/24/2017	1430	31.3	2.24	32.6	23,766	14.62	7.11	9.60
Liyog Bridge	L017-01	4/30/2017	1805	31.6	4.27	62.8	21,809	12.77	14.10	20.40
Sumay Bridge	S017-01	4/30/2017	1820	28.4	3.60	46.5	1,802	0.90	1.84	2.30
Ajayan Bridge	A017-01	4/30/2017	1840	31.9	4.77	73.7	33,584	21.07	6.75	11.40
Liyog Bridge	L018-01	5/9/2017	1540	35.0	5.80	95.0	46,410	29.94	13.60	24.80
Sumay Bridge	S018-01	5/9/2017	1550	28.7	3.48	45.3	2,265	1.15	1.86	2.60
Ajayan Bridge	A018-01	5/9/2017	1615	35.0	4.07	69.9	49,298	32.04	4.40	12.20
Liyog Bridge	L019-01	5/22/2017	1600	32.7	5.62	93.5	51,121	33.32	9.62	17.60
Sumay Bridge	S019-01	5/22/2017	1615	31.6	5.43	83.1	34,197	21.33	4.16	8.93
Ajayan Bridge	A019-01	5/22/2017	1630	33.1	5.07	82.7	45,331	29.22	4.08	8.40
Liyog Bridge	L020-01	5/29/2017	1250	30.6	3.68	57.9	45,400	29.28	16.90	35.40
Sumay Bridge	S020-01	5/29/2017	1305	29.3	2.79	37.1	6,448	3.52	2.90	8.20
Ajayan Bridge	A020-01	5/29/2017	1320	31.7	3.72	58.4	40,303	25.46	3.34	8.40
Liyog Bridge	L021-01	6/3/2017	1705	33.7	4.95	82.9	48,714	31.48	9.34	16.00
Sumay Bridge	S021-01	6/3/2017	1730	30.0	3.91	53.5	10,583	6.00	2.45	3.40
Ajayan Bridge	A021-01	6/3/2017	1755	34.6	5.32	88.1	44,737	28.62	4.08	8.60
Ajayan Bridge	A022-01	6/6/2017	1025	31.2	2.50	39.5	45,083	29.01	6.10	12.80
Liyog Bridge	L022-01	6/6/2017	1045	31.2	2.36	36.9	42,650	27.23	11.25	18.80
Sumay Bridge	S022-01	6/6/2017	1105	29.0	3.29	43.2	2,732	1.42	2.15	3.00
Sumay Bridge	S023-01	6/14/2017	1112	30.0	3.35	51.6	33,345	21.09	2.53	3.33
Liyog Bridge	L023-01	6/14/2017	1132	30.6	3.34	53.5	49,788	32.35	12.10	22.40
Ajayan Bridge	A023-01	6/14/2017	1144	31.4	3.98	65.7	50,862	33.35	3.29	5.60
Ajayan Bridge	A024-01	6/22/2017	1344	33.3	5.02	77.2	29,617	18.79	9.27	10.27
Liyog Bridge	L024-01	6/22/2017	1412	31.2	0.96	16.1	50,240	32.72	24.10	29.40
Sumay Bridge	S024-01	6/22/2017	1435	28.6	3.56	45.4	1,976	1.00	14.25	36.80
Liyog Bridge	L025-01	7/8/2017	1235	32.7	2.53	38.8	29,777	18.29	10.55	19.00

Notes: mg/l, milligrams/liter; µs/cm, microsiemens/centimeter; ppt, parts per thousand; NTU, nephelometric turbidity unit; SSC, suspended sediment concentration

Appendix A. Results of water quality monitoring in the Manell Watershed, Guam (cont.).

Location	ID	Date	Time	Temperature (°C)	Dissolved Oxygen (mg/l)	Dissolved Oxygen (%)	Conductivity (µs/cm)	Salinity (ppt)	Turbidity (NTU)	SSC (mg/l)
Sumay Bridge	S025-01	7/8/2017	1250	29.0	3.16	41.4	2,315	1.09	2.85	12.40
Ajayan Bridge	A025-01	7/8/2017	1310	33.1	2.75	42.1	33,010	20.48	6.20	8.60
Liyog Bridge	L026-01	7/13/2017	1210	30.4	4.83	72.4	45,075	29.96	14.00	14.93
Sumay Bridge	S026-01	7/13/2017	1240	28.8	2.63	34.4	3,245	1.67	4.65	3.33
Ajayan Bridge	A026-01	7/13/2017	1305	32.2	2.83	41.2	17,359	9.94	8.52	4.67
Liyog Bridge	L027-01	7/17/2017	1610	30.1	6.21	95.3	46,992	30.64	15.20	17.60
Sumay Bridge	S027-01	7/17/2017	1625	28.3	3.41	44.0	1,457	0.72	5.37	4.27
Ajayan Bridge	A027-01	7/17/2017	1640	30.3	3.57	52.0	26,571	16.60	8.39	5.60
Liyog Bridge	L028-01	7/25/2017	1415	33.5	3.42	53.4	26,441	16.01	13.10	15.20
Sumay Bridge	S028-01	7/25/2017	1430	29.1	1.82	23.8	2,006	1.02	2.99	3.20
Ajayan Bridge	A028-01	7/25/2017	1445	33.4	2.01	31.1	29,204	17.86	6.28	6.80
Liyog Bridge	L029-01	8/2/2017	955	30.2	2.25	34.3	37,425	23.51	19.10	25.80
Sumay Bridge	S029-01	8/2/2017	1010	28.6	2.81	36.2	2,421	1.25	2.92	2.67
Ajayan Bridge	A029-01	8/2/2017	1030	31.3	2.19	32.4	26,353	15.92	3.43	3.87
Liyog Bridge	L030-01	8/18/2017	920	30.4	1.60	25.4	49,865	32.48	15.55	25.49
Sumay Bridge	S030-01	8/18/2017	930	28.5	3.03	39.7	3,322	1.73	3.39	2.50
Ajayan Bridge	A030-01	8/18/2017	940	31.1	2.20	33.0	31,338	19.25	8.53	6.73
Liyog Bridge	L031-01	8/25/2017	1310	31.9	4.45	71.0	42,447	26.73	34.35	21.06
Sumay Bridge	S031-01	8/25/2017	1325	28.3	3.53	45.9	3,321	1.61	32.35	5.00
Ajayan Bridge	A031-01	8/25/2017	1340	28.5	3.65	48.7	8,508	4.77	64.55	8.87
Sumay Bridge	S032-01	9/19/2017	830	28.4	2.83	37.1	12,060	6.75	9.81	5.33
Liyog Bridge	L032-01	9/19/2017	850	29.4	2.44	37.4	49,149	32.35	15.45	19.21
Ajayan Bridge	A032-01	9/19/2017	910	30.1	2.73	41.4	35,882	21.56	7.62	13.27
Liyog Bridge	L033-01	9/30/2017	855	29.8	1.35	20.4	42,137	27.38	17.80	32.14
Sumay Bridge	S033-01	9/30/2017	910	28.2	3.74	48.0	1,838	0.91	4.74	1.52
Ajayan Bridge	A033-01	9/30/2017	925	30.7	2.91	45.0	44,898	28.52	6.45	6.83
Liyog Bridge	L034-01	10/6/2017	1120	29.9	2.94	24.6	39,551	24.97	10.30	10.99
Sumay Bridge	S034-01	10/6/2017	1135	28.9	2.86	38.5	10,998	5.40	4.06	3.01
Ajayan Bridge	A034-01	10/6/2017	1150	31.1	3.66	54.4	24,593	14.90	6.63	3.39
Liyog Bridge	L035-01	10/14/2017	1055	27.2	6.70	83.3	683	0.33	146.00	129.06
Sumay Bridge	S035-01	10/14/2017	1110	27.3	4.79	60.2	586	0.28	75.55	50.60
Ajayan Bridge	A035-01	10/14/2017	1145	26.6	6.26	78.1	498	0.24	135.50	82.17
Liyog Bridge	L036-01	10/27/2017	1655	33.5	4.41	72.8	43,386	27.26	3.38	35.00
Sumay Bridge	S036-01	10/27/2017	1710	28.6	3.30	43.2	1,469	0.73	2.24	3.43
Ajayan Bridge	A036-01	10/27/2017	1735	30.8	4.86	73.5	35,236	22.04	4.33	5.98
Liyog Bridge	L037-01	11/1/2017	835	28.9	2.33	36.3	43,922	27.67	7.46	8.60
Sumay Bridge	S037-01	11/1/2017	850	28.3	3.11	40.4	1,619	0.81	2.96	11.89
Ajayan Bridge	A037-01	11/1/2017	900	29.6	2.86	43.0	39,094	24.77	3.91	4.77
Liyog Bridge	L038-01	11/14/2017	1100	29.4	2.87	41.3	28,834	18.81	9.61	4.53
Sumay Bridge	S038-01	11/14/2017	1125	28.5	3.39	43.9	1,549	0.77	3.99	2.68
Ajayan Bridge	A038-01	11/14/2017	1155	30.4	3.76	58.4	44,017	28.24	5.30	7.48
Liyog Bridge	L039-01	11/20/2017	1405	30.2	3.57	55.6	49,862	29.30	---	14.93
Sumay Bridge	S039-01	11/20/2017	1420	28.9	2.84	37.1	1,693	0.85	---	4.33
Ajayan Bridge	A039-01	11/20/2017	1430	30.7	3.73	59.1	47,551	30.78	---	5.36
Liyog Bridge	L040-01	11/30/2017	1040	28.2	2.55	36.9	34,725	21.75	18.00	27.61
Sumay Bridge	S040-01	11/30/2017	1050	28.3	3.04	39.2	1,479	0.74	4.60	4.15
Ajayan Bridge	A040-01	11/30/2017	1105	28.9	3.91	59.5	44,894	28.92	7.05	11.28
Liyog Bridge	L041-01	12/6/2017	1145	28.8	5.30	82.3	50,645	33.10	23.20	27.06
Sumay Bridge	S041-01	12/6/2017	1210	29.2	5.07	73.0	29,252	17.89	4.61	6.50
Ajayan Bridge	A041-01	12/6/2017	1220	29.5	4.90	74.5	42,091	26.89	4.16	5.43
Liyog Bridge	L042-01	12/15/2017	1105	28.6	4.62	70.2	45,593	29.43	13.15	32.80
Sumay Bridge	S042-01	12/15/2017	1115	26.6	3.59	46.6	1,474	0.73	5.96	9.39
Ajayan Bridge	A042-01	12/15/2017	1130	29.3	3.67	54.1	34,664	21.68	4.54	4.92
Liyog Bridge	L043-01	12/27/2017	905	28.4	3.53	50.8	32,888	20.48	13.95	17.97
Sumay Bridge	S043-01	12/27/2017	935	28.0	3.77	48.3	1,455	0.72	2.18	3.53
Ajayan Bridge	A043-01	12/27/2017	955	29.1	2.61	39.1	39,566	25.12	5.58	5.88
Liyog Bridge	L044-01	1/4/2018	1540	29.2	4.13	63.0	44,230	28.43	13.40	25.81
Sumay Bridge	S044-01	1/4/2018	1600	28.5	3.49	45.3	2,008	1.02	4.68	7.89
Ajayan Bridge	A044-01	1/4/2018	1610	30.3	3.15	48.4	41,131	26.19	5.09	5.89
Liyog Bridge	L045-01	1/11/2018	1045	27.8	6.35	95.5	46,100	29.82	22.85	47.65
Sumay Bridge	S045-01	1/11/2018	1100	28.1	3.64	46.8	1,527	0.76	4.51	3.82
Ajayan Bridge	A045-01	1/11/2018	1115	30.3	3.51	54.3	43,138	27.62	3.41	6.25
Liyog Bridge	L046-01	1/19/2018	1605	29.0	5.04	77.6	47,090	30.50	22.90	42.24
Sumay Bridge	S046-01	1/19/2018	1620	28.7	4.00	52.5	4,553	2.41	9.22	8.24
Ajayan Bridge	A046-01	1/19/2018	1635	30.4	3.64	50.7	14,121	8.11	7.26	5.53

Notes: mg/l, milligrams/liter; µs/cm, microsiemens/centimeter; ppt, parts per thousand; NTU, nephelometric turbidity unit; SSC, suspended sediment concentration

Appendix A. Results of water quality monitoring in the Manell Watershed, Guam (cont.).

Location	ID	Date	Time	Temperature (°C)	Dissolved Oxygen (mg/l)	Dissolved Oxygen (%)	Conductivity (µs/cm)	Salinity (ppt)	Turbidity (NTU)	SSC (mg/l)
Liyog Bridge	L047-01	1/27/2018	900	25.9	4.86	70.8	46,212	29.96	12.45	24.73
Sumay Bridge	S047-01	1/27/2018	910	27.9	3.78	48.2	1,450	0.72	3.08	5.09
Ajayan Bridge	A047-01	1/27/2018	925	28.3	4.29	56.3	7,870	4.33	5.30	4.76
Liyog Bridge	L048-01	2/1/2018	1445	28.8	4.98	76.3	47,018	30.46	17.25	31.49
Sumay Bridge	S048-01	2/1/2018	1455	28.5	4.21	54.6	1,816	0.91	7.97	8.18
Ajayan Bridge	A048-01	2/1/2018	1510	30.1	3.54	50.5	22,179	13.26	7.66	5.45
Liyog Bridge	L049-01	2/9/2018	1600	30.1	5.33	84.3	49,747	32.40	40.55	113.25
Sumay Bridge	S049-01	2/9/2018	1615	28.2	4.31	55.8	3,667	1.92	23.10	34.27
Ajayan Bridge	A049-01	2/9/2018	1650	30.9	4.61	71.4	41,318	26.30	4.39	6.17
Liyog Bridge	L050-01	2/17/2018	1440	29.7	5.63	88.2	48,944	31.83	30.80	66.67
Sumay Bridge	S050-01	2/17/2018	1455	28.7	3.38	43.9	1,600	0.80	5.14	4.20
Ajayan Bridge	A050-01	2/17/2018	1505	31.3	3.28	46.3	14,188	8.14	4.73	4.71
Liyog Bridge	L051-01	2/22/2018	1620	28.5	4.31	65.4	45,598	29.44	13.85	24.40
Sumay Bridge	S051-01	2/22/2018	1635	28.6	1.16	15.2	7,089	3.87	6.67	8.21
Ajayan Bridge	A051-01	2/22/2018	1650	31.1	5.17	72.2	11,358	6.40	8.41	6.18
Liyog Bridge	L052-01	3/3/2018	1405	29.3	4.91	76.4	48,731	31.69	21.75	39.00
Sumay Bridge	S052-01	3/3/2018	1420	28.5	0.97	12.7	7,429	4.07	2.81	4.17
Ajayan Bridge	A052-01	3/3/2018	1430	32.0	2.99	43.2	17,448	10.18	5.14	6.37
Liyog Bridge	L053-01	3/9/2018	1645	27.9	3.61	54.1	44,906	28.96	12.15	14.03
Sumay Bridge	S053-01	3/9/2018	1700	28.4	0.72	9.5	9,061	5.04	5.60	11.49
Ajayan Bridge	A053-01	3/9/2018	1720	32.4	3.63	52.3	14,705	8.45	10.25	5.90
Ajayan Bridge	A054-01	3/15/2018	1035	29.7	3.71	50.7	12,293	6.99	5.58	2.73
Liyog Bridge	L054-01	3/15/2018	1050	28.0	4.02	63.6	49,187	12.06	16.00	25.27
Sumay Bridge	S054-01	3/15/2018	1115	28.4	2.97	38.4	2,337	1.19	5.76	4.96
Liyog Bridge	L055-01	3/24/2018	1640	28.7	3.65	55.4	44,821	28.87	15.05	25.49
Sumay Bridge	S055-01	3/24/2018	1650	28.6	2.01	26.1	2,210	1.12	5.96	11.88
Ajayan Bridge	A055-01	3/24/2018	1700	31.5	4.39	64.7	25,190	15.22	5.88	4.98
Liyog Bridge	L056-01	4/5/2018	1605	32.0	3.30	52.3	42,298	26.97	11.10	19.68
Sumay Bridge	S056-01	4/5/2018	1620	29.1	0.53	7.0	5,540	2.97	4.18	6.34
Ajayan Bridge	A056-01	4/5/2018	1640	31.8	3.73	52.8	12,391	7.03	9.69	10.41
Liyog Bridge	L057-01	4/15/2018	1405	26.8	6.24	78.4	1,469	0.73	66.05	14.67
Sumay Bridge	S057-01	4/15/2018	1415	27.2	4.31	54.5	917	0.45	31.55	12.93
Ajayan Bridge	A057-01	4/15/2018	1625	26.4	6.11	76.1	765	0.37	62.45	9.79
Sumay Bridge	S058-01	4/28/2018	1340	28.3	0.29	3.8	1,438	0.72	22.60	8.26
Liyog Bridge	L058-01	4/28/2018	1350	29.2	1.70	23.1	12,280	6.98	49.60	11.52
Ajayan Bridge	A058-01	4/28/2018	1410	28.7	5.60	72.7	1,340	0.66	52.70	4.14
Liyog Bridge	L059-01	5/10/2018	1725	33.8	5.22	84.8	41,533	26.36	8.77	8.74
Sumay Bridge	S059-01	5/10/2018	1740	28.6	3.29	42.6	1,620	0.81	2.30	3.70
Ajayan Bridge	A059-01	5/10/2018	1755	31.7	6.15	91.8	27,768	16.93	1.99	3.64
Liyog Bridge	L060-01	5/16/2018	1345	32.2	3.32	52.2	39,553	25.02	9.73	11.21
Sumay Bridge	S060-01	5/16/2018	1405	29.9	6.47	88.2	10,434	5.85	2.57	2.01
Ajayan Bridge	A060-01	5/16/2018	1420	33.3	3.44	55.5	41,556	26.40	4.70	6.78
Liyog Bridge	L061-01	6/12/2018	1105	29.2	6.51	96.9	37,911	24.12	12.95	5.23
Sumay Bridge	S061-01	6/12/2018	1120	28.4	3.67	47.3	1,354	0.67	5.22	2.90
Ajayan Bridge	A061-01	6/12/2018	1140	30.6	4.37	66.9	39,194	24.81	4.13	5.23
Liyog Bridge	L062-01	6/19/2018	1540	31.0	4.18	63.5	35,417	22.16	5.82	8.12
Sumay Bridge	S062-01	6/19/2018	1600	28.6	2.86	37.1	1,808	0.91	2.49	2.06
Ajayan Bridge	A062-01	6/19/2018	1615	32.3	4.61	71.8	36,569	22.93	3.21	3.93
Liyog Bridge	L063-01	7/5/2018	1625	26.7	6.23	78.1	1,391	0.69	18.75	42.29
Sumay Bridge	S063-01	7/5/2018	1645	27.6	4.60	58.5	978	0.48	54.35	71.57
Ajayan Bridge	A063-01	7/5/2018	1700	26.8	5.95	74.6	1,061	0.52	34.60	4.34
Liyog Bridge	L064-01	7/14/2018	1255	32.4	2.52	36.0	12,576	7.13	---	18.45
Sumay Bridge	S064-01	7/14/2018	1310	28.9	3.02	39.3	1,617	0.81	---	4.95
Ajayan Bridge	A064-01	7/14/2018	1320	30.9	4.21	57.9	8,162	4.49	---	2.40
Geus End of E. R.	G03-65	7/20/2018	1000	26.3	8.05	99.8	265	0.12	---	2.32
Geus Dam	G01-65	7/20/2018	1030	26.2	8.73*	100	246	0.12	---	2.54
Geus Bridge (E. R.)	G00-65	7/20/2018	1100	26.7	7.43	92.8	296	0.14	---	0.72
Geus Bridge (Rt.4)	G02-65	7/20/2018	1115	27.1	6.92	87.1	351	0.17	---	2.73
Liyog Bridge	L065-01	7/28/2018	1255	32.5	3.41	55.1	45,789	29.45	10.75	12.85
Sumay Bridge	S065-01	7/28/2018	1310	29.1	3.81	49.9	1,554	0.78	1.57	1.99
Ajayan Bridge	A065-01	7/28/2018	1330	32.4	3.56	55.2	34,833	21.95	1.74	3.61
Liyog Bridge	L066-01	8/3/2018	1545	30.6	4.24	65.6	42,286	27.00	21.25	19.00
Sumay Bridge	S066-01	8/3/2018	1550	27.9	4.02	51.4	1,110	0.55	9.63	5.71
Ajayan Bridge	A066-01	8/3/2018	1610	29.1	4.66	70.3	41,632	26.58	14.30	8.39
Geus Bridge (Rt.4)	G02-66	8/3/2018	1630	27.2	7.04	89.6	367	0.17	3.55	2.46

Notes: mg/l, milligrams/liter; µs/cm, microsiemens/centimeter; ppt, parts per thousand; NTU, nephelometric turbidity unit; SSC, suspended sediment concentration

Appendix A. Results of water quality monitoring in the Manell Watershed, Guam (cont.).

Location	ID	Date	Time	Temperature (°C)	Dissolved Oxygen (mg/l)	Dissolved Oxygen (%)	Conductivity (µs/cm)	Salinity (ppt)	Turbidity (NTU)	SSC (mg/l)
Geus Bridge (E.R.)	G00-66	8/3/2018	1650	27.0	7.41	93.0	304	0.14	12.65	14.58
Geus End of E.R.	G03-66	8/3/2018	1700	26.8	7.93	99.2	258	0.12	40.75	24.49
Liyog Bridge	L067-01	8/10/2018	1440	32.8	6.06	98.9	46,636	30.05	7.77	
Sumay Bridge	S067-01	8/10/2018	1455	28.8	4.2	54.5	937	0.46	2.51	4.13
Ajayan Bridge	A067-01	8/10/2018	1510	33.3	8.47*	100	46,896	30.22	7.66	9.23
Geus Bridge (Rt.4)	G02-67	8/10/2018	1525	29.4	7.44	97.6	493	0.23	0.68	1.30
Geus Bridge (E.R.)	G00-67	8/10/2018	1545	28.0	6.57	84.0	429	0.2	0.83	0.97
Geus End of E.R.	G03-67	8/10/2018	1555	27.6	7.28	92.3	414	0.2	0.86	0.98
Geus Dam	G01-67	8/10/2018	1625	26.8	7.7	96.4	384	0.18	1.60	1.37
Liyog Bridge	L068-01	8/25/2018	1435	31.7	2.07	33.4	48,238	31.25	22.55	3.32
Sumay Bridge	S068-01	8/25/2018	1500	28.8	3.88	50.4	1,144	0.56	8.26	4.33
Ajayan Bridge	A068-01	8/25/2018	1525	30.4	7.68*	100	6,448	3.49	26.35	1.40
Liyog Bridge	L069-01	9/3/2018	1550	32.9	6.46*	100	51,180	33.36	4.03	6.92
Sumay Bridge	S069-01	9/3/2018	1605	29.7	4.4	60.0	11,528	6.52	6.33	4.63
Ajayan Bridge	A069-01	9/3/2018	1620	32.9	6.83*	100	48,916	31.7	6.10	4.32
Geus Bridge (Rt.4)	G02-69	9/3/2018	1635	28.9	7.39	96.1	496	0.24	0.95	0.42
Geus Bridge (E.R.)	G00-69	9/3/2018	1650	27.0	6.72	84.4	428	0.2	1.01	0.38
Geus End of E.R.	G03-69	9/3/2018	1700	26.4	6.82	84.7	417	0.2	1.62	0.75
Geus Dam	G01-69	9/3/2018	1735	26.4	7.67	95.3	415	0.2	1.90	0.56
Liyog Bridge	L070-01	9/8/2018	1225	30.0	3.2	50.2	47,730	30.94	16.35	8.17
Sumay Bridge	S070-01	9/8/2018	1240	29.2	6.7	88.0	2,129	1.08	5.17	1.81
Ajayan Bridge	A070-01	9/8/2018	1250	30.4	3.51	50.4	23,061	13.83	23.40	5.81
Sumay Bridge	S071-01	9/12/2018	1350	28.6	4.28	55.4	1,066	0.52	7.76	2.77
Ajayan Bridge	A071-01	9/12/2018	1410	28.7	5.2	70.5	14,895	8.61	13.40	5.29
Liyog Bridge	L071-01	9/12/2018	1425	29.3	4.67	62.2	6,082	3.28	17.20	16.14
Geus Bridge (Rt.4)	G02-71	9/12/2018	1440	28.8	6.55	84.9	391	0.19	2.69	1.02
Geus Bridge (E.R.)	G00-71	9/12/2018	1450	27.7	6.81	86.7	3,397	0.16	3.30	0.39
Geus End of E.R.	G03-71	9/12/2018	1500	27.6	7.68	97.6	309	0.15	5.15	2.98
Geus Dam	G01-71	9/12/2018	1535	27.6	7.78	98.7	279	0.13	3.66	1.77
Liyog Bridge	L072-01	9/17/2018	1625	32.6	7.57*	100	50,334	32.75	12.60	4.86
Sumay Bridge	S072-01	9/17/2018	1640	28.6	4.41	57.1	1,051	0.52	14.40	1.81
Ajayan Bridge	A072-01	9/17/2018	1650	31.3	7.19*	100	39,445	24.97	9.33	3.29
Liyog Bridge	L073-01	10/13/2018	1455	32.3	5.33	87.2	49,349	32.04	8.13	11.21
Sumay Bridge	S073-01	10/13/2018	1510	28.8	4.41	57.3	1,324	0.66	3.38	1.62
Ajayan Bridge	A073-01	10/13/2018	1555	31.3	4.47	71.2	46,189	29.78	4.34	5.63
Liyog Bridge	L074-01	10/23/2018	1000	28.9	3.69	56.9	48,018	31.18	6.17	5.94
Sumay Bridge	S074-01	10/23/2018	1010	28.3	3.57	46.1	1,220	0.6	3.43	1.45
Ajayan Bridge	A074-01	10/23/2018	1025	29.7	2.77	43.6	50,159	32.72	1.13	4.72
Liyog Bridge	L075-01	11/2/2018	830	29.7	2.00	31.0	46,150	29.8	9.99	9.84
Sumay Bridge	S075-01	11/2/2018	850	28.2	3.73	47.9	1,255	0.62	4.97	3.83
Ajayan Bridge	A075-01	11/2/2018	900	29.8	3.24	51.1	49,453	32.2	4.12	4.82
Geus Bridge (Rt.4)	G02-75	11/2/2018	930	27.7	7.70	98.0	470	0.22	1.32	0.51
Geus Bridge (E.R.)	G00-75	11/2/2018	945	27.2	6.77	85.2	400	0.19	2.24	0.38
Geus End of E.R.	G03-75	11/2/2018	955	26.9	7.03	88.1	379	0.18	2.99	0.19
Geus Dam	G01-75	11/2/2018	1030	26.7	7.65	95.6	354	0.17	3.14	0.59
Liyog Bridge	L076-01	11/18/2018	1035	31.0	0.77	12.3	47,912	31.03	12.30	6.27
Sumay Bridge	S076-01	11/18/2018	1050	28.6	3.77	48.8	1,170	0.58	3.42	2.85
Ajayan Bridge	A076-01	11/18/2018	1105	30.0	3.34	52.9	50,821	33.2	5.54	2.89
Geus Bridge (Rt.4)	G02-77	12/1/2018	1525	29.4	8.93*	100	412	0.2	1.12	1.77
Geus Bridge (E.R.)	G00-77	12/1/2018	1545	27.5	7.06	89.5	351	0.17	3.04	1.44
Ajayan Bridge	A077-01	12/1/2018	1620	31.6	5.02	80.6	47,351	30.61	3.97	4.10
Liyog Bridge	L077-01	12/1/2018	1635	34.3	7.69*	100	45,178	28.95	4.81	5.76
Sumay Bridge	S077-01	12/1/2018	1650	28.5	3.53	45.7	1,202	0.59	2.48	2.91
Liyog Bridge	L078-01	12/8/2018	1135	28.3	4.91	74.4	45,828	29.61	7.53	13.59
Sumay Bridge	S078-01	12/8/2018	1150	28.6	4.33	56.0	736	0.36	1.43	3.16
Ajayan Bridge	A078-01	12/8/2018	1205	29.0	4.36	67.1	46,892	30.36	2.37	4.46
Geus Bridge (Rt.4)	G02-78	12/8/2018	1215	28.5	11.57*	100	437	0.21	1.76	3.32
Geus Bridge (E.R.)	G00-78	12/8/2018	1235	27.6	6.83	86.7	437	0.21	2.48	3.33
Liyog Bridge	L079-01	12/23/2018	1310	29.1	6.31	96.1	43,913	28.21	8.13	8.79
Sumay Bridge	S079-01	12/23/2018	1315	28.2	3.91	50.3	1,266	0.63	4.15	1.61
Ajayan Bridge	A079-01	12/23/2018	1335	29.0	6.08	93.6	47,191	30.57	6.27	4.73
Geus Bridge (Rt.4)	G02-79	12/23/2018	1350	27.6	7.02	89.2	428	0.2	1.17	0.83
Geus Bridge (E.R.)	G00-79	12/23/2018	1405	26.9	6.90	86.6	379	0.18	1.19	0.78

Notes: mg/l, milligrams/liter; µs/cm, microsiemens/centimeter; ppt, parts per thousand; NTU, nephelometric turbidity unit; SSC, suspended sediment concentration

Appendix B. Spearman correlation coefficients between parameters.

Variable	Variable	Spearman's Rho	p value
Ajayan Water Level	SSC (mg/L)	0.3670	<0.0001
Ajayan Water Level	Turbidity (NTU)	0.2204	0.001
As Liyog Water Level	SSC (mg/L)	0.2944	<0.0001
As Liyog Water Level	Turbidity (NTU)	0.1369	0.0445
HPO ₄ ⁼ (mg/l)	Turbidity (NTU)	0.1714	0.0114
HPO ₄ ⁼ (mg/l)	SSC (mg/L)	0.4148	<0.0001
As Liyog Water Level	Ajayan Water Level	0.5607	<0.0001
NH ₄ ⁺ (mg/l-N)	Turbidity (NTU)	0.2776	<0.0001
NH ₄ ⁺ (mg/l-N)	SSC (mg/L)	0.5052	<0.0001
NH ₄ ⁺ (mg/l-N)	Ajayan Water Level	0.3295	<0.0001
NH ₄ ⁺ (mg/l-N)	As Liyog Water Level	0.0335	<0.0001
NH ₄ ⁺ (mg/l-N)	Rainfall (in)	-0.0324	0.7809
NH ₄ ⁺ (mg/l-N)	DO (mg/L)	-0.2348	0.0004
NO ₃ ⁻ (mg/l-N)	Salinity (ppt)	-0.7853	<0.0001
NO ₃ ⁻ (mg/l-N)	SSC (mg/L)	-0.3038	<0.0001
NO ₃ ⁻ (mg/l-N)	Turbidity (NTU)	-0.3926	<0.0001
NO ₃ ⁻ (mg/l-N)	Ajayan Water Level	0.1243	0.0664
NO ₃ ⁻ (mg/l-N)	As Liyog Water Level	0.0965	0.1594
Rainfall (in)	Ajayan Water Level	0.0147	0.0001
Rainfall (in)	As Liyog Water Level	0.0335	<.0001
Rainfall (in)	Turbidity (NTU)	0.0880	<.0001
Rainfall (in)	SSC (mg/L)	0.1434	0.0412
Rainfall (in)	Salinity (ppt)	-0.1503	0.022
SSC (mg/L)	Turbidity (NTU)	0.7336	<0.0001

Appendix C. Results of nutrient monitoring in the Manell Watershed, Guam.

Sample ID	Site	Collection Date	NO ₃ ⁻ conc. (umol/l)	NO ₂ ⁻ conc. (mg/l N)	HPO ₄ ³⁻ conc. (umol/l)	HPO ₄ ²⁻ conc. (mg/l P)	HSiO ₃ ⁻ conc. (umol/l)	HSiO ₂ ⁻ conc. (mg/l SiO ₂)	NH ₄ ⁺ conc. (umol/l)	NH ₄ ⁺ conc. (mg/l N)	NO ₂ ⁻ conc. (umol/l)	NO ₂ ⁻ conc. (mg/l N)	Urea conc. (umol/l)	Urea conc. (mg/l N)	NO ₃ ⁻ +NO ₂ ⁻ conc. (umol)	Total N conc. (umol/l)	Total N conc. (mg/l N)	Total P conc. (umol/l)	Total P conc. (mg/l P)
A001-01	Ajayan	12/11/16	0.00	0.00	1.73	0.05	41.84	3.16	2.58	0.04	0.18	0.00	0.42	0.01	0.18	22.66	0.32	2.21	0.07
A002-01	Ajayan	12/19/16	0.00	0.00	4.37	0.14	168.51	12.82	5.81	0.08	0.39	0.01	1.12	0.02	0.39	19.14	0.27	4.58	0.14
A003-01	Ajayan	12/26/16	3.97	0.08	5.39	0.17	145.36	11.06	3.34	0.05	0.58	0.01	2.54	0.04	4.54	24.26	0.34	7.78	0.24
A004-01	Ajayan	01/20/17	4.01	0.08	2.54	0.08	277.08	21.08	2.99	0.04	0.32	0.00	1.02	0.01	4.32	22.91	0.32	5.37	0.17
A005-01	Ajayan	01/24/17	0.00	0.00	3.76	0.12	116.51	8.88	3.81	0.05	0.29	0.00	0.81	0.01	0.29	34.51	0.48	4.16	0.13
A006-01	Ajayan	02/05/17	0.00	0.00	7.20	0.22	207.37	15.78	6.85	0.10	0.46	0.01	1.32	0.02	0.46	21.06	0.29	7.03	0.22
A007-01	Ajayan	02/26/17	0.00	0.00	5.64	0.17	211.78	16.11	4.62	0.07	0.41	0.01	1.42	0.02	0.41	26.94	0.38	5.70	0.18
A008-01	Ajayan	03/04/17	0.00	0.00	4.39	0.14	158.96	12.09	4.35	0.06	0.34	0.00	1.15	0.02	0.34	22.39	0.31	5.02	0.16
A009-01	Ajayan	03/06/17	0.00	0.00	3.06	0.09	110.56	8.41	3.86	0.05	0.28	0.00	0.85	0.01	0.28	24.71	0.35	3.79	0.12
A010-01	Ajayan	03/08/17	0.00	0.00	6.69	0.21	272.47	20.73	6.59	0.09	0.51	0.01	1.71	0.02	0.51	19.70	0.28	7.83	0.24
A011-01	Ajayan	03/22/17	4.33	0.08	3.48	0.11	283.05	21.54	8.25	0.09	0.38	0.01	1.26	0.02	4.89	54.83	0.77	5.45	0.17
A012-01	Ajayan	03/26/17	0.00	0.00	5.00	0.15	197.91	15.06	5.88	0.08	0.38	0.01	1.28	0.02	0.38	43.31	0.61	5.78	0.18
A013-01	Ajayan	03/28/17	0.00	0.00	7.82	0.24	277.42	21.11	8.86	0.12	0.53	0.01	2.08	0.03	0.53	28.84	0.40	9.23	0.29
A014-01	Ajayan	04/07/17	0.00	0.00	3.84	0.12	134.50	10.23	3.95	0.06	0.31	0.00	1.08	0.02	0.31	29.03	0.41	4.13	0.13
A015-01	Ajayan	04/15/17	0.00	0.00	3.16	0.10	116.53	8.87	3.23	0.05	0.30	0.00	0.70	0.01	0.30	30.98	0.43	3.98	0.12
L001-01	As Liyog	12/11/16	0.00	0.00	0.88	0.03	48.97	3.57	2.02	0.03	0.13	0.00	0.31	0.00	0.13	29.61	0.41	2.19	0.07
L002-01	As Liyog	12/19/16	0.48	0.01	4.04	0.13	81.07	6.17	4.78	0.07	0.41	0.01	0.68	0.01	0.88	37.92	0.53	5.48	0.17
L003-01	As Liyog	12/26/16	0.10	0.00	4.12	0.13	98.51	7.34	4.60	0.08	0.28	0.00	0.78	0.01	0.36	26.48	0.37	3.98	0.12
L004-01	As Liyog	01/20/17	0.10	0.00	6.83	0.21	161.48	12.29	6.87	0.10	0.43	0.01	1.31	0.02	0.52	44.28	0.62	6.71	0.21
L005-01	As Liyog	01/24/17	0.00	0.00	1.82	0.06	58.88	4.48	2.36	0.03	0.13	0.00	0.22	0.00	0.13	43.39	0.61	1.76	0.05
L006-01	As Liyog	02/05/17	0.19	0.00	4.85	0.15	105.70	8.04	4.84	0.07	0.31	0.00	0.98	0.01	0.50	48.32	0.68	4.78	0.15
L007-01	As Liyog	02/28/17	0.00	0.00	2.14	0.07	58.60	4.48	2.42	0.03	0.18	0.00	0.40	0.01	0.18	48.67	0.68	2.36	0.07
L008-01	As Liyog	03/04/17	1.90	0.03	5.75	0.18	148.75	11.32	7.33	0.10	0.53	0.01	1.28	0.02	2.43	38.30	0.54	5.79	0.18
L009-01	As Liyog	03/06/17	0.23	0.00	3.27	0.10	81.66	6.21	2.65	0.04	0.20	0.00	0.42	0.01	0.43	39.88	0.56	4.35	0.13
L010-01	As Liyog	03/08/17	0.00	0.00	2.71	0.08	65.63	4.99	2.91	0.04	0.19	0.00	0.53	0.01	0.19	41.82	0.59	3.15	0.10
L011-01	As Liyog	03/22/17	0.08	0.00	7.81	0.24	177.33	13.49	7.45	0.10	0.51	0.01	1.57	0.02	0.58	29.44	0.41	7.84	0.24
L012-01	As Liyog	03/28/17	0.00	0.00	1.69	0.05	58.96	4.33	2.42	0.03	0.12	0.00	0.28	0.00	0.12	22.94	0.32	2.13	0.07
L013-01	As Liyog	03/28/17	0.12	0.00	5.11	0.16	135.76	10.33	6.29	0.09	0.39	0.01	0.96	0.01	0.51	52.62	0.74	5.03	0.16
L014-01	As Liyog	04/07/17	0.00	0.00	2.69	0.08	72.27	5.50	2.85	0.04	0.17	0.00	0.38	0.01	0.17	51.84	0.73	2.89	0.09
L015-01	As Liyog	04/15/17	1.37	0.02	7.65	0.24	252.13	19.18	10.49	0.15	0.60	0.01	1.47	0.02	1.97	58.10	0.81	8.12	0.25
S009-01	Sumay	03/08/17	14.36	0.20	2.78	0.09	355.88	27.08	2.36	0.03	0.36	0.00	1.50	0.02	14.72	48.74	0.68	5.51	0.17
S010-01	Sumay	03/08/17	15.53	0.22	2.47	0.08	395.31	30.06	2.55	0.04	0.23	0.00	0.64	0.01	15.76	49.09	0.69	4.78	0.15
S011-01	Sumay	03/22/17	18.19	0.25	1.88	0.06	398.48	30.32	2.35	0.03	0.17	0.00	0.32	0.00	18.37	49.08	0.69	3.58	0.11
S012-01	Sumay	03/26/17	7.28	0.10	8.40	0.28	392.27	29.85	7.62	0.11	0.53	0.01	1.69	0.02	7.81	28.78	0.40	9.03	0.28
S013-01	Sumay	03/28/17	16.56	0.23	1.35	0.04	400.42	30.47	2.93	0.04	0.17	0.00	0.32	0.00	16.73	49.73	0.70	3.57	0.11
S014-01	Sumay	04/07/17	18.41	0.26	2.06	0.06	372.03	28.31	3.57	0.05	0.28	0.00	1.01	0.01	18.70	57.70	0.81	4.31	0.13
S015-01	Sumay	04/15/17	19.15	0.27	1.75	0.05	389.30	29.62	2.72	0.04	0.18	0.00	0.17	0.00	19.33	49.43	0.69	3.98	0.12
L016-01	As Liyog	04/24/17	3.02	0.04	8.86	0.21	218.82	16.50	13.63	0.19	0.81	0.01	1.78	0.02	3.83	52.15	0.73	8.53	0.26
A016-01	Ajayan	04/24/17	4.74	0.07	5.55	0.17	195.49	14.87	3.94	0.06	0.60	0.01	2.68	0.04	5.33	66.97	0.94	8.30	0.26
S016-01	Sumay	04/24/17	18.81	0.28	1.44	0.04	400.84	30.50	3.03	0.04	0.19	0.00	0.29	0.00	19.00	91.02	1.27	3.81	0.12
L017-01	As Liyog	04/30/17	6.07	0.09	4.04	0.13	217.19	16.52	6.84	0.09	0.32	0.00	1.41	0.02	6.39	51.92	0.73	6.16	0.19
A017-01	Ajayan	04/30/17	0.00	0.00	10.10	0.31	64.91	4.94	12.87	0.18	0.83	0.01	3.30	0.05	0.83	48.04	0.67	11.36	0.35
S017-01	Sumay	04/30/17	24.48	0.34	1.18	0.04	358.30	27.26	4.81	0.06	0.17	0.00	0.90	0.01	24.65	48.74	0.68	3.75	0.12
L018-01	As Liyog	05/09/17	0.00	0.00	0.96	0.03	57.87	4.39	10.60	0.15	0.73	0.01	2.75	0.04	0.73	41.89	0.59	3.62	0.11
A018-01	Ajayan	05/09/17	0.00	0.00	0.44	0.01	30.57	2.33	6.54	0.12	0.57	0.01	2.04	0.03	0.57	36.21	0.51	2.09	0.06
S018-01	Sumay	05/09/17	19.67	0.28	1.42	0.04	398.84	30.35	3.14	0.04	0.23	0.00	0.43	0.01	19.90	49.18	0.69	3.88	0.12
L019-01	As Liyog	05/22/17	0.00	0.00	0.31	0.01	10.64	0.81	9.92	0.14	0.64	0.01	2.43	0.03	0.64	34.54	0.48	2.27	0.07
A019-01	Ajayan	05/22/17	0.00	0.00	2.70	0.08	37.83	2.88	7.41	0.10	0.51	0.01	1.68	0.02	0.51	43.55	0.61	2.35	0.07
S019-01	Sumay	05/22/17	1.56	0.02	6.60	0.20	68.03	5.18	10.26	0.14	0.70	0.01	2.78	0.04	2.26	30.97	0.43	7.68	0.24
L020-01	As Liyog	05/28/17	1.03	0.01	3.38	0.10	106.55	8.11	8.09	0.09	0.43	0.01	0.80	0.01	1.46	50.24	0.70	4.00	0.12
A020-01	Ajayan	05/29/17	0.00	0.00	3.75	0.12	51.33	3.91	10.10	0.14	0.73	0.01	2.55	0.04	0.73	27.86	0.39	4.06	0.13
S020-01	Sumay	05/29/17	19.14	0.27	1.11	0.03	364.62	27.74	3.82	0.05	0.24	0.00	0.51	0.01	19.38	50.48	0.71	4.22	0.13
L021-01	As Liyog	06/03/17	0.00	0.00	2.14	0.07	44.88	3.41	9.18	0.13	0.81	0.01	2.28	0.03	0.81	50.95	0.71	2.54	0.08
A021-01	Ajayan	06/03/17	0.00	0.00	2.80	0.09	116.18	8.84	2.88	0.04	0.23	0.00	0.64	0.01	0.23	28.13	0.39	3.96	0.12
S021-01	Sumay	06/03/17	18.12	0.25	2.04	0.06	390.88	29.74	3.07	0.04	0.30	0.00	0.79	0.01	18.42	49.95	0.70	4.70	0.15
L022-01	As Liyog	06/06/17	0.00	0.00	2.78	0.09	86.55	5.06	12.38	0.17	0.80	0.01	2.54	0.04	0.80	67.12	0.94	5.30	0.16
A022-01	Ajayan	06/06/17	0.00	0.00	2.43	0.08	60.10	4.57	9.79	0.14	0.87	0.01	2.53	0.04	0.87	45.05	0.63	3.71	0.11
S022-01	Sumay	06/06/17	18.72	0.26	1.02	0.03	406.06	30.89	2.90	0.04	0.19	0.00	0.30	0.00	18.82	53.80	0.75	3.71	0.11
L023-01	As Liyog	06/14/17	0.00	0.00	2.58	0.08	56.11	4.27	8.56	0.12	0.60	0.01	1.82	0.03	0.60	36.07	0.51	3.02	0.09
A023-01	Ajayan	06/14/17	0.00	0.00	2.17	0.07	67.89	5.17	9.69	0.14	0.88	0.01	2.24	0.03	0.88	25.24	0.35	4.17	0.13
S023-01	Sumay	06/14/17	4.93	0.07	15.01	0.46	144.84	11.02	11.77	0.16	0.90	0.01	3.31	0.05	5.83	37.77	0.53	26.89	0.83
L024-01	As Liyog	06/22																	

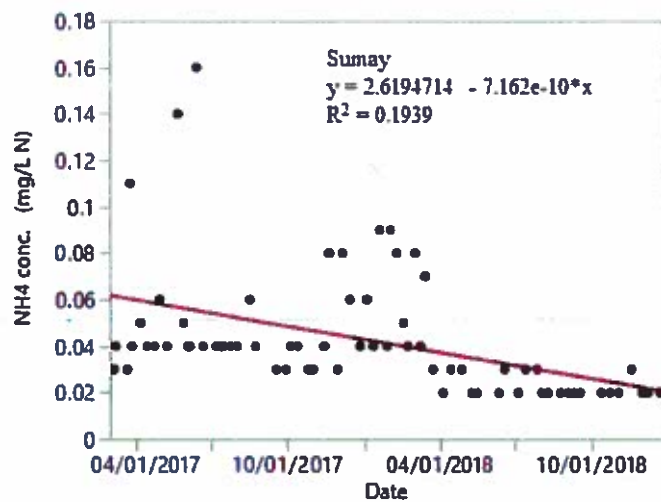
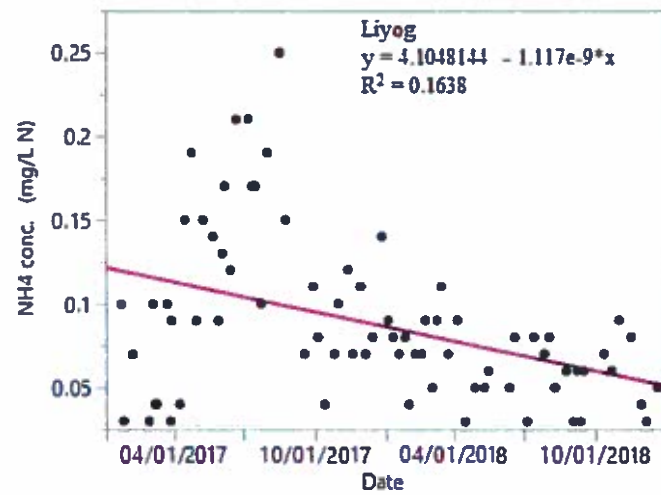
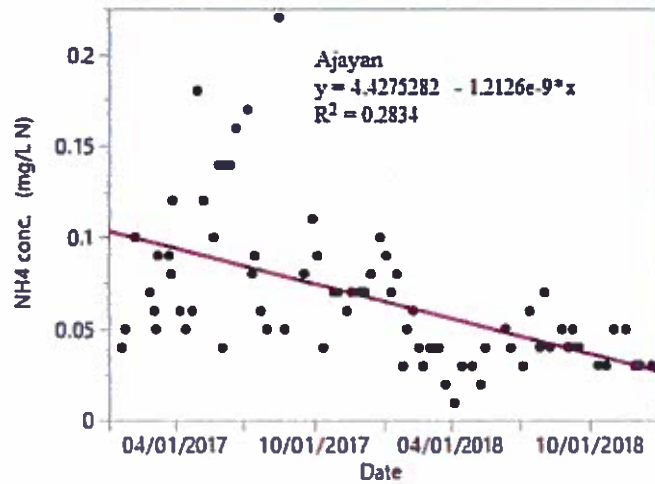
Appendix C. Results of nutrient monitoring in the Manell Watershed, Guam (cont.).

Sample ID	Site	Collection Date	NO ₃ ⁻ conc. (µmol/l)	NO ₂ ⁻ conc. (mg/l N)	HPO ₄ ⁻ conc. (µmol/l)	HPO ₄ ⁻ conc. (mg/l P)	HSiO ₃ ⁻ conc. (µmol/l)	HSiO ₃ ⁻ conc. (mg/l SiO ₂)	NH ₄ ⁺ conc. (µmol/l)	NH ₄ ⁺ conc. (mg/l N)	NO ₂ ⁻ conc. (µmol/l)	NO ₂ ⁻ conc. (mg/l N)	Urea conc. (µmol/l)	Urea conc. (mg/l N)	NO ₃ ⁻ +NO ₂ ⁻ conc. (µM)	Total N conc. (µmol/l)	Total N conc. (mg/l N)	Total P conc. (µmol/l)	Total P conc. (mg/l P)
A030-01	Ajayan	08/18/17	0.78	0.01	9.16	0.26	232.80	17.71	15.48	0.22	0.62	0.01	2.22	0.03	1.38	42.99	0.60	10.05	0.31
S030-01	Sumay	08/18/17	17.17	0.24	0.94	0.03	367.45	27.96	4.53	0.06	0.30	0.00	0.38	0.01	17.47	49.08	0.69	3.34	0.10
L031-01	As Liyog	08/25/17	0.16	0.00	10.43	0.32	251.70	19.15	10.75	0.15	0.65	0.01	2.22	0.03	0.81	38.69	0.54	10.57	0.33
A031-01	Ajayan	08/25/17	6.16	0.09	1.34	0.04	140.40	10.68	3.88	0.05	0.21	0.00	0.72	0.01	6.37	61.09	0.86	3.91	0.12
S031-01	Sumay	08/25/17	12.18	0.17	1.16	0.04	351.35	26.73	2.79	0.04	0.17	0.00	0.83	0.01	12.35	43.78	0.61	3.28	0.10
L032-01	As Liyog	9/19/2017	0.88	0.01	2.90	0.09	171.20	13.03	4.69	0.07	0.43	0.01	1.21	0.02	1.31	25.53	0.36	3.59	0.11
A032-01	Ajayan	9/19/2017	0.16	0.00	2.87	0.09	213.59	16.25	5.56	0.08	0.93	0.01	2.05	0.03	1.08	23.77	0.33	3.72	0.12
S032-01	Sumay	9/19/2017	12.22	0.17	0.86	0.03	390.60	29.72	2.45	0.03	0.15	0.00	0.27	0.00	12.37	31.52	0.44	1.30	0.04
L033-01	As Liyog	9/30/2017	0.20	0.00	6.51	0.20	68.14	5.16	7.56	0.11	0.93	0.01	2.10	0.03	1.12	30.55	0.43	8.45	0.26
A033-01	Ajayan	9/30/2017	0.00	0.00	5.52	0.17	53.91	4.10	7.85	0.11	0.93	0.01	2.33	0.03	0.93	47.34	0.66	8.25	0.26
S033-01	Sumay	9/30/2017	16.12	0.23	0.99	0.03	498.12	37.90	2.15	0.03	0.15	0.00	0.19	0.00	16.27	33.45	0.47	1.95	0.06
L034-01	As Liyog	10/6/2017	0.12	0.00	3.29	0.10	157.55	11.99	5.88	0.08	0.80	0.01	1.63	0.03	0.92	35.65	0.50	3.76	0.12
A034-01	Ajayan	10/6/2017	3.84	0.05	1.11	0.03	361.34	29.01	6.50	0.09	0.47	0.01	1.77	0.02	4.31	22.25	0.31	1.12	0.03
S034-01	Sumay	10/6/2017	19.40	0.27	1.19	0.04	525.09	39.95	3.13	0.04	0.21	0.00	0.32	0.00	19.61	28.58	0.40	1.23	0.04
L035-01	As Liyog	10/14/2017	4.83	0.07	1.55	0.05	315.28	23.99	3.05	0.04	0.16	0.00	0.29	0.00	4.99	22.44	0.31	1.76	0.05
A035-01	Ajayan	10/14/2017	1.44	0.02	2.36	0.07	179.02	13.82	2.98	0.04	0.14	0.00	0.22	0.00	1.58	43.78	0.61	2.44	0.08
S035-01	Sumay	10/14/2017	6.53	0.09	1.21	0.04	281.10	21.39	2.53	0.04	0.17	0.00	0.12	0.00	6.89	49.57	0.69	3.01	0.09
L036-01	As Liyog	10/27/2017	0.14	0.00	2.18	0.07	98.64	7.50	4.79	0.07	0.71	0.01	2.37	0.03	0.85	35.49	0.50	2.52	0.08
A036-01	Ajayan	10/27/2017	0.17	0.00	2.28	0.07	180.41	13.73	5.26	0.07	0.81	0.01	2.23	0.03	0.97	29.07	0.41	3.27	0.10
S036-01	Sumay	10/27/2017	15.77	0.22	0.82	0.03	342.99	26.10	1.99	0.03	0.17	0.00	0.18	0.00	15.94	35.76	0.50	1.46	0.05
L037-01	As Liyog	11/1/2017	0.28	0.00	4.90	0.15	262.54	21.50	7.49	0.10	0.82	0.01	1.88	0.03	1.09	30.67	0.43	5.39	0.17
A037-01	Ajayan	11/1/2017	0.06	0.00	1.78	0.05	84.62	6.44	5.32	0.07	0.77	0.01	1.67	0.02	0.83	19.55	0.27	2.44	0.08
S037-01	Sumay	11/1/2017	17.57	0.25	1.06	0.03	440.11	33.49	2.42	0.03	0.17	0.00	0.13	0.00	17.74	37.60	0.53	1.94	0.06
L038-01	As Liyog	11/14/2018	0.61	0.01	6.17	0.19	89.39	5.28	8.56	0.12	0.98	0.01	2.14	0.03	1.59	43.17	0.60	12.09	0.37
A038-01	Ajayan	11/14/2018	0.51	0.01	2.10	0.07	181.89	13.84	4.31	0.06	0.59	0.01	1.08	0.02	1.10	26.27	0.37	2.79	0.09
S038-01	Sumay	11/14/2018	17.51	0.25	1.44	0.04	490.46	37.32	2.70	0.04	0.17	0.00	0.13	0.00	17.68	35.97	0.50	2.27	0.07
L039-01	As Liyog	11/20/2018	0.40	0.01	2.52	0.08	99.83	7.60	5.29	0.07	0.74	0.01	1.89	0.03	1.14	30.82	0.43	3.14	0.10
A039-01	Ajayan	11/20/2018	0.19	0.00	3.20	0.10	92.93	7.07	5.22	0.07	0.78	0.01	1.70	0.02	0.97	22.31	0.31	4.21	0.13
S039-01	Sumay	11/20/2018	12.92	0.18	1.40	0.04	328.31	24.98	5.37	0.08	0.43	0.01	1.31	0.02	13.35	33.57	0.47	1.43	0.04
L040-01	As Liyog	11/30/2017	0.46	0.01	4.77	0.15	251.51	19.14	7.85	0.11	0.82	0.01	1.75	0.02	1.27	39.30	0.55	5.68	0.18
A040-01	Ajayan	11/30/2017	0.22	0.00	2.14	0.07	116.55	8.67	5.13	0.07	0.79	0.01	1.58	0.02	1.02	26.75	0.37	2.85	0.09
S040-01	Sumay	11/30/2017	16.83	0.24	1.15	0.04	488.92	37.20	2.44	0.03	0.18	0.00	0.35	0.00	17.01	36.84	0.52	1.95	0.06
L041-01	As Liyog	12/6/2017	0.22	0.00	1.16	0.04	12.16	0.93	5.03	0.07	0.81	0.01	1.74	0.02	1.03	28.27	0.40	2.01	0.06
A041-01	Ajayan	12/6/2017	0.21	0.00	2.11	0.07	128.20	9.75	5.19	0.07	0.80	0.01	1.74	0.02	1.00	26.74	0.37	2.77	0.09
S041-01	Sumay	12/6/2017	1.23	0.02	3.16	0.10	178.28	13.41	5.82	0.08	0.90	0.01	1.94	0.03	2.14	28.69	0.40	4.03	0.12
L042-01	As Liyog	12/15/2017	0.45	0.01	2.04	0.06	49.33	3.75	6.05	0.08	0.79	0.01	2.12	0.03	1.09	46.34	0.65	3.00	0.09
A042-01	Ajayan	12/15/2017	0.25	0.00	1.82	0.06	78.65	5.98	5.91	0.08	0.81	0.01	1.68	0.02	1.08	35.51	0.50	2.29	0.07
S042-01	Sumay	12/15/2017	14.97	0.21	1.07	0.03	249.81	18.99	4.22	0.06	0.27	0.00	0.90	0.01	15.24	41.21	0.58	1.17	0.04
L043-01	As Liyog	12/27/2017	0.74	0.01	5.02	0.16	153.57	11.84	10.21	0.14	0.90	0.01	2.07	0.03	1.64	44.03	0.62	7.70	0.24
A043-01	Ajayan	12/27/2017	0.45	0.01	2.46	0.08	170.81	12.98	7.07	0.10	0.80	0.01	1.84	0.02	1.25	35.05	0.49	3.11	0.10
S043-01	Sumay	12/27/2017	17.46	0.24	1.26	0.04	258.88	19.70	2.87	0.04	0.13	0.00	0.24	0.00	17.59	36.48	0.51	2.06	0.06
L044-01	As Liyog	1/4/2018	0.32	0.00	2.89	0.08	65.86	5.01	6.67	0.09	0.83	0.01	1.77	0.02	1.15	40.13	0.56	3.42	0.11
A044-01	Ajayan	1/4/2018	0.23	0.00	2.87	0.09	149.84	11.40	6.19	0.09	0.87	0.01	1.74	0.02	1.10	29.02	0.41	3.63	0.11
S044-01	Sumay	1/4/2018	15.77	0.22	1.14	0.04	270.11	20.55	4.01	0.06	0.30	0.00	0.66	0.01	16.07	35.74	0.50	1.35	0.04
L045-01	As Liyog	1/11/2018	0.21	0.00	1.34	0.04	29.89	2.27	5.45	0.08	0.88	0.01	1.47	0.02	0.89	32.30	0.45	2.42	0.08
A045-01	Ajayan	1/11/2018	0.16	0.00	1.42	0.04	68.96	5.25	5.27	0.07	0.71	0.01	1.42	0.02	0.87	29.52	0.41	2.38	0.07
S045-01	Sumay	1/11/2018	17.40	0.24	1.01	0.03	301.59	22.95	2.86	0.04	0.18	0.00	0.43	0.01	17.58	38.42	0.54	1.03	0.03
L046-01	As Liyog	1/19/2018	0.29	0.00	1.20	0.04	19.58	1.48	4.95	0.07	0.77	0.01	1.71	0.02	1.05	29.97	0.42	2.29	0.07
A046-01	Ajayan	1/19/2018	2.38	0.03	0.98	0.03	177.42	13.50	5.52	0.08	0.24	0.00	0.82	0.01	2.82	39.55	0.55	1.24	0.04
S046-01	Sumay	1/19/2018	7.19	0.10	1.91	0.06	337.09	25.65	6.16	0.09	0.58	0.01	1.84	0.03	7.77	28.36	0.40	2.15	0.07
L047-01	As Liyog	1/27/2018	0.27	0.00	2.04	0.08	48.29	3.67	5.79	0.08	0.79	0.01	1.77	0.02	1.06	28.93	0.41	2.81	0.09
A047-01	Ajayan	1/27/2018	1.25	0.02	0.93	0.03	248.39	18.90	1.92	0.03	0.09	0.00	0.43	0.01	1.34	33.71	0.47	0.93	0.03
S047-01	Sumay	1/27/2018	15.69	0.22	0.73	0.02	304.04	23.13	2.98	0.04	0.18	0.00	0.57	0.01	15.87	35.69	0.50	0.87	0.03
L048-01	As Liyog	2/1/2018	0.88	0.01	1.51	0.05	75.29	5.73	2.51	0.04	0.26	0.00	2.38	0.03	1.14	39.96	0.56	2.81	0.09
A048-01	Ajayan	2/1/2018	1.86	0.03	1.28	0.04	267.37	20.34	3.81	0.05	0.32	0.00	1.13	0.02	2.18	38.05	0.53	1.44	0.04
S048-01	Sumay	2/1/2018	6.57	0.09	1.89	0.06	358.73	27.14	6.20	0.09	0.58	0.01	2.09	0.03	7.15	30.59	0.43	2.14	0.07
L049-01	As Liyog	2/9/2018	0.08	0.00	1.74	0.05	14.45	1.10	5.07	0.07	0.82	0.01	1.83	0.03	0.89	39.55	0.55	3.06	0.09
A049-01	Ajayan	2/9/2018	0.00	0.00	1.35	0.04	39.71	3.02	4.62	0.06	0.78	0.01	1.64	0.02	0.76	35.79	0.50	1.87	0.06
S049-01	Sumay	2/9/2018	9.63	0.13	1.73	0.05	308.13	23.44	5.68	0.06	0.48	0.01	1.64	0.02	10.11	41.41	0.58	1.73	0.05
L050-01	As Liyog	2/17/2018	0.12	0.00	2.02	0.06	37.77	2.87	4.92	0.07	0.72	0.01	1.41	0.02	0.84	34.36	0.48	3.34	0.10
A050-01	Ajayan	2/17/2018	1.60	0.02	0.98	0.03	298.08	22.53	2.56	0.04	0.16	0.00	0.73	0.01	1.				

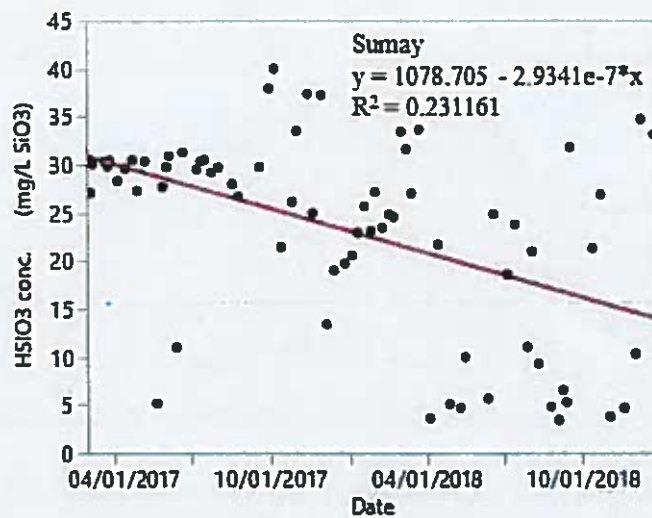
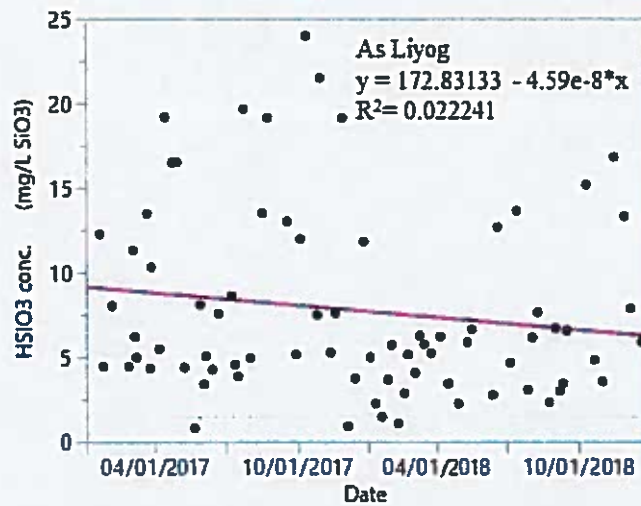
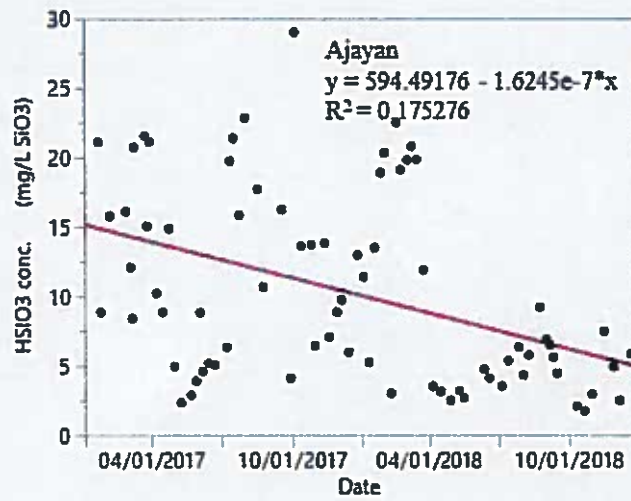
Appendix C. Results of nutrient monitoring in the Manell Watershed, Guam (cont.).

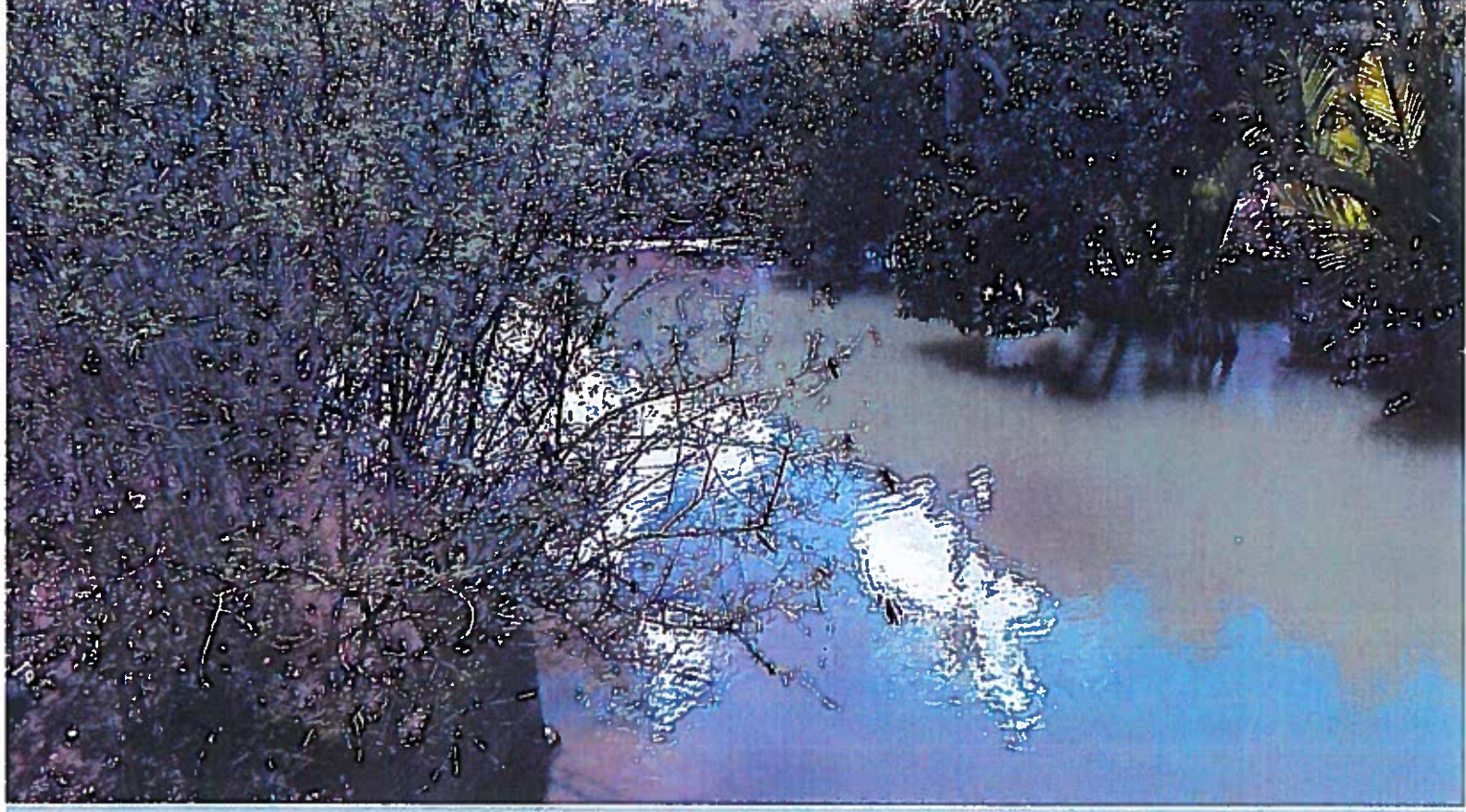
Sample ID	Site	Collection Date	NO ₃ ⁻ conc. (umol/l)	NO ₂ ⁻ conc. (mg/l N)	HPO ₄ ⁻ conc. (umol/l)	HPO ₄ ⁻ conc. (mg/l P)	HSiO ₃ ⁻ conc. (umol/l)	HSiO ₃ ⁻ conc. (mg/l SiO ₂)	NH ₄ ⁺ conc. (umol/l)	NH ₄ ⁺ conc. (mg/l N)	NO ₂ ⁻ conc. (umol/l)	NO ₂ ⁻ conc. (mg/l N)	Urea conc. (umol/l)	Urea conc. (mg/l N)	NO ₃ ⁻ +NO ₂ ⁻ conc. (umol/l)	Total N conc. (umol/l)	Total N conc. (mg/l N)	Total P conc. (umol/l)	Total P conc. (mg/l P)
S058-01	Sumay	04/05/18	6.59	0.09	0.70	0.02	47.54	3.62	1.39	0.02	0.08	0.00	0.15	0.00	6.65	34.67	0.49	1.64	0.05
L057-01	As Liyog	04/15/18	2.66	0.04	1.31	0.04	45.22	3.44	2.22	0.03	0.08	0.00	0.04	0.00	2.74	49.65	0.70	5.43	0.17
A057-01	Ajayan	04/15/18	0.66	0.01	0.66	0.02	41.38	3.15	2.40	0.03	0.08	0.00	0.00	0.00	0.75	40.81	0.57	2.99	0.09
S057-01	Sumay	04/15/18	11.56	0.16	0.69	0.02	285.18	21.70	2.46	0.03	0.26	0.00	0.19	0.00	11.81	37.33	0.52	1.61	0.05
L058-01	As Liyog	04/28/18	0.77	0.01	1.21	0.04	29.75	2.26	3.26	0.05	0.08	0.00	0.00	0.00	0.83	36.28	0.51	3.98	0.12
A058-01	Ajayan	04/28/18	0.63	0.01	0.48	0.01	32.95	2.51	2.27	0.03	0.08	0.00	0.00	0.00	0.88	42.88	0.60	2.83	0.09
S058-01	Sumay	04/28/18	5.43	0.08	0.49	0.02	66.92	5.09	2.21	0.03	0.11	0.00	0.00	0.00	5.54	40.44	0.57	2.99	0.09
L059-01	As Liyog	05/10/18	0.00	0.00	2.04	0.08	77.47	5.89	3.38	0.05	0.81	0.01	1.48	0.02	0.81	23.61	0.33	1.98	0.06
A059-01	Ajayan	05/10/18	0.00	0.00	0.24	0.01	41.95	3.19	1.10	0.02	0.10	0.00	0.82	0.01	0.10	20.76	0.29	3.89	0.12
S059-01	Sumay	05/10/18	8.19	0.11	0.89	0.02	61.99	4.72	1.59	0.02	0.10	0.00	0.32	0.00	8.29	30.43	0.43	2.95	0.09
L060-01	As Liyog	05/16/18	0.00	0.00	2.18	0.07	87.58	6.66	4.55	0.06	0.68	0.01	1.46	0.02	0.66	34.00	0.48	2.83	0.09
A060-01	Ajayan	05/16/18	0.00	0.00	1.80	0.06	35.24	2.68	2.72	0.04	0.57	0.01	1.29	0.02	0.57	27.42	0.38	1.90	0.06
S060-01	Sumay	05/16/18	5.70	0.08	0.50	0.02	131.26	9.99	1.70	0.02	0.08	0.00	0.42	0.01	5.78	31.48	0.44	2.73	0.08
L061-01	As Liyog	06/12/18	0.00	0.00	3.58	0.11	36.39	2.77	3.85	0.05	0.80	0.01	1.94	0.03	0.80	26.42	0.37	6.96	0.22
A061-01	Ajayan	06/12/18	0.00	0.00	1.64	0.05	62.42	4.75	3.29	0.05	0.52	0.01	1.01	0.01	0.52	31.92	0.45	2.00	0.06
S061-01	Sumay	06/12/18	5.20	0.11	0.51	0.02	74.62	5.68	1.36	0.02	0.08	0.00	0.00	0.00	8.29	29.00	0.41	2.58	0.08
L062-01	As Liyog	06/19/18	0.91	0.01	2.19	0.07	166.94	12.70	5.36	0.08	0.22	0.00	2.10	0.03	1.13	32.25	0.45	3.08	0.10
A062-01	Ajayan	06/19/18	0.00	0.00	1.16	0.04	54.10	4.12	2.86	0.04	0.58	0.01	1.19	0.02	0.58	22.07	0.31	1.47	0.05
S062-01	Sumay	06/19/18	15.52	0.22	0.83	0.03	326.70	24.86	2.03	0.03	0.18	0.00	0.29	0.00	15.70	31.37	0.44	2.88	0.09
L063-01	As Liyog	07/05/18	0.75	0.01	0.89	0.03	61.54	4.68	1.90	0.03	0.08	0.00	1.47	0.02	0.83	49.47	0.69	5.27	0.16
A063-01	Ajayan	07/05/18	0.25	0.00	0.43	0.01	46.38	3.53	1.85	0.03	0.05	0.00	0.00	0.00	0.30	38.68	0.54	2.78	0.09
S063-01	Sumay	07/05/18	10.65	0.15	0.68	0.02	244.84	18.63	1.70	0.02	0.18	0.00	1.33	0.02	10.81	39.57	0.55	2.80	0.09
L064-01	As Liyog	07/14/18	2.54	0.04	3.16	0.10	179.40	13.65	5.70	0.08	0.16	0.00	0.41	0.01	2.70	31.97	0.45	4.53	0.14
A064-01	Ajayan	07/14/18	0.42	0.01	0.80	0.02	70.62	5.37	4.37	0.06	0.07	0.00	0.03	0.00	0.49	26.97	0.38	3.25	0.10
S064-01	Sumay	07/14/18	14.02	0.20	0.67	0.02	312.29	23.76	1.97	0.03	0.15	0.00	2.21	0.03	14.17	31.86	0.45	2.74	0.08
L065-01	As Liyog	07/28/18	0.32	0.00	3.18	0.10	40.52	3.06	5.12	0.07	0.80	0.01	1.83	0.02	1.11	27.60	0.39	6.70	0.21
A065-01	Ajayan	07/28/18	0.00	0.00	1.35	0.04	83.47	6.35	2.97	0.04	0.55	0.01	1.22	0.02	0.55	23.33	0.33	1.38	0.04
S065-01	Sumay	07/28/18	13.61	0.19	0.71	0.02	145.32	11.06	1.89	0.03	0.14	0.00	0.11	0.00	13.75	34.97	0.49	2.73	0.08
G00-85	Geus	07/20/18	7.81	0.11	0.34	0.01	102.72	7.82	0.96	0.01	0.06	0.00	0.18	0.00	7.87	26.48	0.37	1.89	0.06
L066-01	As Liyog	08/03/18	0.00	0.00	2.11	0.07	60.92	6.16	5.60	0.08	0.65	0.01	1.46	0.02	0.65	36.51	0.51	2.35	0.07
A066-01	Ajayan	08/03/18	0.00	0.00	1.64	0.05	56.79	4.32	4.77	0.07	0.69	0.01	1.50	0.02	0.69	29.88	0.42	1.69	0.05
S066-01	Sumay	08/03/18	14.88	0.21	0.44	0.01	275.69	20.88	1.39	0.02	0.18	0.00	1.43	0.02	15.08	50.31	0.70	2.46	0.08
G00-66	Geus	08/03/18	5.00	0.07	0.21	0.01	188.82	14.21	0.78	0.01	0.09	0.00	0.39	0.01	5.09	24.58	0.34	2.26	0.07
L067-01	As Liyog	08/10/18	0.00	0.00	1.98	0.06	100.55	7.65	3.58	0.05	0.61	0.01	1.48	0.02	0.81	28.53	0.40	2.26	0.07
A067-01	Ajayan	08/10/18	0.00	0.00	1.90	0.06	75.88	5.77	2.55	0.04	0.53	0.01	1.15	0.02	0.53	26.29	0.37	1.75	0.05
S067-01	Sumay	08/10/18	6.56	0.12	0.57	0.02	122.26	9.30	1.49	0.02	0.09	0.00	0.00	0.00	6.65	31.41	0.44	2.71	0.08
G00-67	Geus	08/10/18	3.83	0.05	0.32	0.01	60.44	6.12	0.99	0.01	0.05	0.00	1.60	0.02	3.87	17.42	0.24	2.12	0.07
L068-01	As Liyog	08/25/18	0.02	0.00	3.70	0.11	30.60	2.34	4.51	0.08	0.88	0.01	1.96	0.03	0.90	33.60	0.47	7.02	0.22
A068-01	Ajayan	08/25/18	0.50	0.01	1.37	0.04	121.03	9.21	3.90	0.05	0.08	0.00	1.23	0.02	0.58	30.87	0.43	2.94	0.09
S068-01	Sumay	08/25/18	6.75	0.09	0.83	0.02	83.37	4.82	1.34	0.02	0.09	0.00	0.85	0.01	6.84	32.11	0.45	2.33	0.07
L069-01	As Liyog	09/03/18	0.00	0.00	0.53	0.02	88.18	6.71	2.48	0.03	0.00	0.00	1.65	0.02	0.00	23.24	0.33	1.60	0.05
A069-01	Ajayan	09/03/18	0.00	0.00	1.17	0.04	90.44	6.88	2.99	0.04	0.65	0.01	1.30	0.02	0.65	37.83	0.53	1.47	0.05
S069-01	Sumay	09/03/18	6.38	0.09	0.72	0.02	45.07	3.43	1.70	0.02	0.08	0.00	0.70	0.01	6.46	33.54	0.47	2.54	0.08
G00-69	Geus	09/03/18	5.23	0.07	0.56	0.02	208.09	15.83	0.87	0.01	0.05	0.00	1.98	0.03	5.28	23.33	0.33	1.99	0.06
L070-01	As Liyog	09/08/18	0.00	0.00	3.30	0.10	39.50	3.01	4.36	0.06	0.81	0.01	1.63	0.02	0.81	28.32	0.40	6.91	0.21
A070-01	Ajayan	09/08/18	0.23	0.00	1.49	0.05	85.38	6.50	3.90	0.05	0.34	0.00	1.50	0.02	0.57	38.59	0.54	3.27	0.10
S070-01	Sumay	09/08/18	7.82	0.11	1.35	0.04	66.38	6.57	1.47	0.02	0.10	0.00	0.44	0.01	7.92	35.41	0.50	2.73	0.08
L071-01	As Liyog	09/12/18	0.52	0.01	0.69	0.02	45.28	3.44	1.85	0.03	0.07	0.00	0.21	0.00	0.59	35.99	0.50	3.72	0.12
A071-01	Ajayan	09/12/18	0.00	0.00	0.76	0.02	73.86	5.82	2.75	0.04	0.30	0.00	0.70	0.01	0.30	22.91	0.32	3.74	0.12
S071-01	Sumay	09/12/18	6.10	0.09	0.17	0.01	69.51	5.29	1.23	0.02	0.07	0.00	0.38	0.01	6.17	32.93	0.46	2.19	0.07
G00-71	Geus	09/12/18	5.22	0.07	0.42	0.01	279.07	21.23	0.68	0.01	0.05	0.00	1.35	0.02	5.27	23.36	0.33	1.67	0.05
L072-01	As Liyog	09/17/18	0.00	0.00	2.16	0.07	66.40	6.57	4.04	0.06	0.64	0.01	1.51	0.02	0.64	42.26	0.59	2.14	0.07
A072-01	Ajayan	09/17/18	0.00	0.00	1.02	0.03	58.56	4.46	3.14	0.04	0.57	0.01	1.29	0.02	0.57	26.44	0.37	1.22	0.04
S072-01	Sumay	09/17/18	12.38	0.17	1.20	0.04	417.80	31.79	1.49	0.02	0.14	0.00	2.08	0.03	12.51	39.35	0.55	2.77	0.09
L073-01	As Liyog	10/13/2018	0.94	0.01	3.53	0.11	199.81	15.20	5.10	0.07	0.31	0.00	4.41	0.08	1.25	33.50	0.47	4.22	0.13
A073-01	Ajayan	10/13/2018	0.13	0.00	1.88	0.05	27.58	2.10	1.84	0.03	0.45	0.01	0.90	0.01	0.58	19.63	0.27	3.69	0.11
S073-01	Sumay	10/13/2018	18.97	0.27	1.28	0.04	279.86	21.29	1.48	0.02	0.13	0.00	5.57	0.08	19.10	43.46	0.61	2.96	0.09
L074-01	As Liyog	10/23/2018	0.21	0.00	3.78	0.12	63.68	4.85	4.00	0.06	0.75	0.01	2.51	0.04	0.96	31.89	0.45	3.84	0.12
A074-01	Ajayan	10/23/2018	0.15	0.00	1.75	0.05	22.84	1.74	1.95	0.03	0.44	0.01	0.59	0.01	0.59	22.38	0.31	3.75	0.12
S074-01	Sumay	10/23/2018	16.94	0.24	1.24	0.04	353.44	26.69	1.77	0.02	0.14	0.00	1.35	0.02	17.08	33.98	0.48	2.74	0.08
L075-01	As Liyog	11/2/2018	0.06	0.00	3.91	0.12	48.86	3.57	6.37	0.09	0.76	0.01	2.14	0.03	0.82	46.82	0.66	4.81	0.15
A075-01	Ajayan	11/2/2018	0.00	0.00	1.86	0.06	36.67												

Appendix D. Ammonium concentrations over time at the three sites in the Manell watershed.



Appendix E. Silica (HSiO_3^-) concentrations over time at the three sites in the Manell watershed.





U.S. Department of Commerce
Wilbur Ross, Secretary

National Oceanic and Atmospheric Administration
Neil Jacobs, Acting Administrator

National Ocean Service
Nicole LeBoeuf, Acting Assistant Administrator for Ocean Service and Coastal Zone Management



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