Southeast Florida and Florida Keys Antibiotic Resistance Study

Final project report

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Objective

Antibiotic resistance has recently been recognized as an emerging contaminant and has molecular methods that identify these genes in microbial populations that have proven to be useful tools in assessing anthropogenic impacts on terrestrial and aquatic environments (1). Analyzing microbial communities for the presence of antibiotics enable researchers to demonstrate at the genetic level, the influences of antibiotic laden sources such as septic systems, shallow injection wells, storm sewer overflows and outfalls on regional ecosystems. As antibiotic resistance can affect pathogen virulence, these sources of antibiotic laden pollutants can sustain the presence of these pathogens that can in turn present human recreational and ecosystem health risks.

The objective of this project is to determine the prevalence of antibiotic resistance genes in bacterial populations in impacted (close to and alongside outfall pipes and within the wastewater stream) and reference (along transect extending away from the outfalls) sediment samples around the Hollywood, FL wastewater treatment plant's ocean outfall. Two sample sets of coral mucus, the water column and reef sediment were also collected from a Florida Keys coral reef, pre, and post treatment with amoxicillin, and were also analyzed for the presence of antibiotic resistance genes. Sediment and wastewater samples were analyzed for 15 different antibiotic resistant gene targets via polymerase chain reaction presence/absence assays in Southeast Florida coral reef environments. Approximately 38 samples per season (June = wet season, March = dry season) were collected by the Florida Department of Environmental Protection, Broward County and NOAA research teams and shipped to and analyzed at the USGS St. Petersburg Environmental and Public Health Microbiology Laboratory.

"*This work is a follow-on to the pilot study results from 14ESFUSAFDEP3519 completed between 10/2014 & 9/2016. This project is a continuation of the SE Florida Coral Reef Initiative (SEFCRI) Technical Advisory Committee's recently concluded Outfall Biomarker Pilot Project (LBSP 28/29 Phase I) and builds upon the results and outcomes of that initial work.*" (current FLDEP/USGS FUSA agreement for this project)

Equipment

1. Applied Biosystems StepOne and StepOnePlus Real-Time PCR Systems. Life Technologies catalog #'s 4376373 and 4376598, respectively.
    a. TaqMan Fast Advanced Master Mix, catalog # 4444556 (ABR PCR).
    b. TaqMan Fast Virus 1-Step Master Mix, catalog # 4444432 (Viral PCR).
    c. MicroAmp Fast 96-Well Reaction Plate (0.1ml), catalog # 4346907.
    d. MicroAmp Fast Optical 48-Well Reaction Plate (0.1ml), catalog # 4375816.
    e. MicroAmp Optical 8-Cap Strip, catalog # 4323032.
2. Eppendorf Reference Pipettes, 0.1-2.5µl, 0.5-10µl, 2-20µl, 20-100µl, 50-200µl, 100-1000µl and 500-2500µl.
3. Microfuge tube and 96-well microplate cold plates.
4. Master mix boats/Reagent Reservoir. Corning Incorporated COSTAR # 4870 or equivalent.
5. Sterile pipette tips and 1.8ml or 2.0ml microfuge tubes.
6. Fifty and 15ml conical tubes. Sterile Falcon or Fisher brand equivalent.
7. Tube racks for 15, 50 and 2.0ml tubes.
8. Ten, 25 and 50ml pipettes with autopipette and/or bulb.
9. IEC Centra MP4R centrifuge with swinging 250ml bucket rotor and 50ml tube adapters.
10. Beckman Coulter, Avanti J-E centrifuge with rotor/adaptors for 50ml conical tubes.
11. Eppendorf 5415 D microcentrifuge (24 place).
12. Incubator capable of 56^{o}C.
13. Mettler Toledo AL54 scale and weigh boats.
14. MoBio PowerSoil DNA Isolation Kit, MoBio's catalog # 12888-100 (now owned by Qiagen).
15. MoBio Vortex Genie 2, catalog # 13111-V and 24 place tube holder, catalog # 13000-V1-24.
16. pH Meter with reference solutions at 4.0, 7.0 and 10.0 pH.
17. Millipore MF membrane filters, 0.45um HA. Fisher Scientific catalog # HAWP04700.
18. Sulfuric Acid, Fisher Scientific catalog # A300-500.
19. Sodium Hydroxide, Fisher Scientific catalog # S392-212.
20. Potassium Citrate, Fisher Scientific catalog # P218-500.
21. Potassium phosphate, monobasic, ACROS # 42420-5000.
22. Sodium Phosphate Dibasic Heptahydrate, Fisher Scientific catalog # S373-500.
23. Tris, Fisher Scientific catalog # T393-212.
24. Sodium (Tetra) Ethylenediamine Tetraacetate, Fisher Scientific catalog # S657-500.

25. Sodium Thiosulfate, Fisher Scientific catalog # S474-500.
26. Ethanol 200 proof, Decon Labs Inc. catalog # 2716.
27. Water, D.I.U.F., Fisher Scientific catalog # W2-20.
28. Qiagen AE buffer (10mM Tris-Cl, 0.5mM EDTA, pH 9.0).
29. Primer/probe sets and corresponding gene target clone (target sequence with ~15bp of random sequences on each end – Integrated DNA Technologies (IDT) gBlock) synthesized by Eurofins, IDT or Applied Biosystems.

Sample sites and collection

Sample sites are illustrated in the wet and dry season heatmap figures (Figures 1 and 3). Samples included sediment and water column samples collected along transects centered on a regional ocean outfall and from regional wastewater treatment plants.

Sediment samples were collected by SCUBA divers who navigated to the sample site by descending an anchored center-point buoy line (centroid) and attaching a measuring tape to the anchor. Divers collected one sediment sample at the centroid of the array near the anchor (beneath the outfall pipe) and then navigated to the cardinal and ordinal directions (N, NW, W, SW, S, SE, E, NE) by dive scooter or by self-propulsion to collect samples at 25m and 50m from the centroid. One diver ran out the tape to 50m. The other diver collected sediment at 25m then the 50m site. Samples at 100, 200, 400 and 800m north and south of the centroid were collected on single dives at those intervals. Samples collected at 100, 200, and 400m, west of the centroid were collected by one diver team using scooters to move from site to site along the pipeline with anchored floats deployed from the boat marking the collection location. Samples at 800, and 1,600m from the centroid were collected on individual dives at those sites. Sediment samples were collected by divers using a 50ml centrifuge tube to scoop sediment from the seabed. Divers wore single-use nitrile gloves to prevent cross-contamination of sediment samples. Nitrile gloves were changed between samples (underwater) if more than one sample was collected on a dive. Outfall water samples were collected by opening an empty sterile 50ml centrifuge tube in the plume at the mouth of the pipe. Wastewater treatment samples were collected using sterile technique.

Sample storage and shipping

Wet Season:
Field sediment and water samples were brought to the surface where they were kept on ice on the boat and during transport to the FDEP CRCP office in Miami, FL, where they were placed in a freezer. Effluent water samples from the three wastewater treatment plants were collected while wearing Nitrile gloves. They were collected from spigots directly into sterile 50ml centrifuge tubes. Samples were placed in plastic Ziploc bags, and kept on ice during transport to the FDEP CRCP office where they were placed in a freezer with the field samples. Once all samples were collected, they were shipped frozen, on dry ice, overnight to the USGS lab in St. Petersburg, FL.

Dry Season:
Field sediment and water samples were brought to the surface where they were kept on ice on the boat and during transport to the FDEP CRCP office in Miami, FL, where they were placed in a refrigerator. Effluent and influent water samples from the three wastewater treatment plants were collected while wearing Nitrile gloves. They were taken from spigots directly into sterile 50ml centrifuge tubes. Samples were placed in Ziploc bags, and kept on ice during transport in the car to the FDEP CRCP office where they were placed in a refrigerator with the field samples. Once all samples were collected, they were shipped on ice, overnight to the USGS lab in St. Petersburg, FL.

Table 1. Primer and Probe Sequences

	Upstream Primer	Downstream Primer	Label-Probe	Reference
Antibiotic Resistance Genes				
tetB	ACACTCAGTATTCCAAGCCTTTG	GATAGACATCACTCCCTGTAATGC	FAM- AAAGCGATCCCACCACCAGCCAAT	(2)
tetL	GGTTTTGAACGTCTCATTACCTGAT	CCAATGGAAAAGGTTAACATAAAGG	FAM- CCACCTGCGAGTACAAACTGGGTGAAC	(2)
tetM	GGTTTCTCTTGGATACTTAAATCAATCR	CCAACCATAYAATCCTTGTTCRC	VIC- ATGCAGTTATGGARGGGATACGCTATGGY	(2)
tetO	AAGAAAACAGGAGATTCCAAAACG	CGAGTCCCCAGATTGTTTTTAGC	FAM- ACGTTATTTCCCGTTTATCACGG	(2)
tetQ	AGGTGCTGAACCTTGTTTGATTC	GGCCGGACGGAGGATTT	VIC- TCGCATCAGCATCCCGCTC	(2)
tetW	GCAGAGCGTGGTTCAGTCT	GACACCGTCTGCTTGATGATAAT	VIC-TTCGGGATAAGCTCTCCGCCGA	(2)
ampC	GGGAATGCTGGATGCACAA	CATGACCCAGTTCGCCATATC	VIC- CCTATGGCGTGAAAACCAACGTGCA	(2)
vanA	CTGTGAGGTCGGTTGTGCG	TTTGGTCCACCTCGCCA	VIC- CAACTAACGCGGCACTGTTTCCCAAT	(2)
ermB	GGATTCTACAAGCGTACCTTGGA	GCTGGCAGCTTAAGCAATTGCT	FAM- CACTAGGGTTGCTCTTGCACACTCAAGTC	(3)
mecA	CATTGATCGCAACGTTCAATTTAAT	TGGTCTTTCTGCATTCCTGGA	VIC- CTATGATCCCAATCTAACTTCCACATACC	(3)
blaSHV	AACAGCTGGAGCGAAAGATCCA	TGTTTTTCGCTGACCGGCGAG	FAM- TCCACCAGATCCTGCTGGCGATAG	(3)
blaPSE	GATTTGGTGCTCGGAGTATT	CATTGAAGCCTGTGTTTGAG	VIC- CTTGATGCTCACTCCA	(4)
floR	GGCAGGCGATATTCATTACT	CGAGAAGAAGACGAAGAAGG	FAM- CTAAAGCCGACAGTGTA	(4)
aadA2	CAGCCAYGATCGACATTGATCT	CCAAGGCAACGCTATGTTCTC	VIC- CTGCTTACAAAAGC	(4)
tetG	CGGTACTTCTGGCTTCTCTT	GAATCGGCAATGGTTGAG	FAM- CAGGAGCCGCAGTCGATTACACG	(4)

Positive controls were gene target sequences synthesized with ~15-25 base pair extensions beyond the 5’ and 3’ ends of the primer binding sequences. The controls were synthesized by Integrated DNA Technologies (gBlock double-stranded DNA fragments).

Master mix recipes

Single probe reactions – 20µl reactions - row 1 Internal Positive Control (IPC) control

			13rxns
a.	2x TaqMan Fast Universal Master Mix –	10µl	130µl
b.	Template –	2µl	
c.	Primer/probe (10µM/5µM working stock)	2µl	26µl
d.	10x Exo Internal Positive Control	2µl	26µl
e.	50x Exo IPC DNA	0.4µl	5.2µl
f.	PCR grade H2O	3.6µl	46.8µl

IPC control reactions row 8 (4 reactions)

			5rxns
a.	2x TaqMan Fast Universal Master Mix –	10µl	50µl
b.	Template –	2µl	
c.	10x Exo Internal Positive Control	2µl	10µl
d.	50x Exo IPC DNA	0.4µl	2µl
e.	PCR grade H_2O	5.6µl	28µl

Single probe reactions (when utilized for startup testing and repeats if needed) – 20µl reactions - rows 2 - 7

a.	2x TaqMan Fast Universal Master Mix –	10µl
b.	Template –	2µl
c.	Primer/probe (10µM/5µM working stock)	2µl
d.	PCR grade H_2O	6.0µl

Multiplex (two primer and probe sets) – 20µl reactions

			13rxns
a.	2x TaqMan Fast Universal Master Mix –	10µl	130µl
b.	Template –	2µl	
c.	Primer/probe (10µM/5µM working stock)	2µl each	26µl
d.	PCR grade H_2O	4.0µl	52µl

Amplification profile

Sixty^{o}C for 0.5min, 50^{o}C for 2min and 95^{o}C for 5min, then 45 cycles of 95^{o}C for 0.25min and 60^{o}C for 1min, then a final step at 60^{o}C for 0.5min

PCR plate layouts (wet season)

Plate 1 multiplex duplicate sample layout (tetB/IPC, tetL/M, tetO/W, ampC/vanA, ermB/mecA, blaSHV/blaPSE, floR/aadA2) and Plate 2 (tetG/tetQ) are samples in columns x2 and rows of gene target using a 96-well plate. Use bottom right four wells for negative control (PCR grade water for template – columns 9 and 10, row 8) and IPC negative control (2µl of IPC block – columns 11 and 12, row 8). Two dyes used, FAM and VIC. FAM labeled probes are tetB, tetL, tetO, ampC, ermB, blaSHV, floR, and tetG. VIC labeled probes are the IPC, TetM, TetW, vanA, mecA, blaPSE, aadA2, and tetQ.

PCR plate layouts (dry season)

Each combination of target/IPC or target/target were run for all the samples in duplicate on an individual 96-well plates with negative (PCR grade H_2O) and positive (gBlock) controls.

Work Flow

27 June 2018

Florida Keys pre-treatment sample set

Received samples (Florida Keys coral antibiotic treatment study, #'s 1-12. These are pretreatment samples, meaning the sample set collected prior to the diseased corals within the reef being treated with amoxicillin) and placed in the refrigerator.

6 July 2018

Centrifuged 25mls of water and mucus samples at 5900g x 30 min at 15^{o}C and pipetted off all but ~500µl of supernatant. Vortexed the samples and transferred 250µl to Qiagen's PowerSoil bead beating tubes. Also transferred ~0.25g of sediment from each sediment sample to bead beating tubes. Extracted DNA from the samples using the PowerSoil protocol and eluted DNA in 100µl of Qiagen's AE buffer. Placed the extracts in the -20^{o}C freezer until analysis.

Florida Keys post-treatment sample set

Received samples 13 – 24 and placed in the refrigerator.

9 July 2018

Set up new primer and probe mixes for the 15 targets. Then set up a polymerase chain reaction (PCR) assay using the above single and multiplex reactions to verify the gBlock synthetic positive control templates and the new primer/probe mixes. First reaction all checked OK except tetL and floR. Remake 10^{-3} dilutions of positive controls from the 10^{-2} frozen stock and rerun both dilutions for these targets. All amplified as expected. Set up and run plate 1 for samples 1 through 6. Extract DNA from samples 13 – 24 using the same protocol as utilized for the first sample set and placed in the refrigerator.

10 July 2018

Set up and run plate 1 for samples 7-12, 13-18 and 19-24. Set up and run plate 2 for samples 1 through 24.

24 July 2018

Received 38 frozen samples from DEP and placed in -20^{o}C freezer. Samples included 6 water and 32 sediment samples collected near three ocean outfalls and their corresponding wastewater treatment plants (WWTP) during Southeast Florida's wet season. Three effluent water samples were taken at the North Broward, Hollywood, and North Miami-Dade WWTP. At the mouth of the ocean outfall pipes associated with each of the three WWTP a sediment sample was taken from directly underneath the pipe, and a water sample was taken from inside the mouth of the pipe. At the Hollywood outfall only, sediment samples were taken in a rosette pattern 25 and 50 m N, S, E, W, NE, NW, SE and SW from the mouth of the pipe. Sediment samples were also taken along N and S transects at 100, 200, 400 and 800 m from the mouth of the pipe, and along W transect at 100, 200, 400, 800 and 1,600 m from the mouth of the pipe. The W transect was meant to follow along the outfall pipe.

Table 2. Field and laboratory sample data spreadsheet (wet season)

Collection Date	Site	Sample/Site ID	Site Lat	Site Long	Depth	Water Temp.	Weather	Note (where sample collected)	Divers
6/11/2018	Broward North WWTP Outfall (pipe)	BRN_Sed	26.251347	-80.062208	111'	79F	Seas 1-2'/Wind<5 mph	Sandy bottom beneath outfall pipe	AR/PQ
6/11/2018	Broward North WWTP Outfall pipe)	BRN_H2O	26.251347	-80.062208	111'	79F	Seas 1-2'/Wind<5 mph	From inide mouth of outfall pipe	AR/PQ
6/11/2018	Hollywood outfall (south transect)	HOL_S_100	26.018319	-80.086023	84'	82F	Seas<1'/Wind<5mph	Sandy bottom next to patch reef	DC/KG
6/11/2018	Hollywood outfall (south transect)	HOL_S_200	26.017518	-80.086005	84'	82F	Seas<1'/Wind<5mph	Sandy bottom next to patch reef	JW/KB
6/11/2018	Hollywood outfall (south transect)	HOL_S_400	26.015895	-80.086023	85'	80F	Seas<1'/Wind<5mph	Sandy bottom next to patch reef	DC/KG
6/11/2018	Hollywood outfall (south transect)	HOL_S_800	26.011918	-80.085936	90'	80F	Seas<1'/Wind<5mph	Sandy bottom next to patch reef	JW/KB
6/12/2018	Hollywood outfall (pipe)	HOL_Sed	26.019126	-80.08602	86'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom beneath outfall pipe	KB/MW
6/12/2018	Hollywood outfall (pipe)	HOL_H2O	26.019126	-80.08602	86'	81F	Seas 2-3'/Wind SE 5-10mph	From inide mouth of outfall pipe	KB/MW
6/12/2018	Hollywood outfall (rosette)	HOL_E_25	n/a	n/a	92'	77F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, adjacent to/next to patch reef	KG/PQ
6/12/2018	Hollywood outfall (rosette)	HOL_E_50	n/a	n/a	95'	78F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, adjacent to/next to patch reef	KG/PQ
6/12/2018	Hollywood outfall (rosette)	HOL_SE_25	n/a	n/a	89'	77F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, adjacent to/next to patch reef	KG/PQ
6/12/2018	Hollywood outfall (rosette)	HOL_SE_50	n/a	n/a	94'	77F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, adjacent to/next to patch reef	KG/PQ
6/12/2018	Hollywood outfall (rosette)	HOL_N_25	n/a	n/a	85'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom near minimal patch rreef	KB/MW
6/12/2018	Hollywood outfall (rosette)	HOL_N_50	n/a	n/a	86'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, open	KB/MW
6/12/2018	Hollywood outfall (rosette)	HOL_NE_25	n/a	n/a	90'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom next to patch reef	KB/MW
6/12/2018	Hollywood outfall (rosette)	HOL_NE_50	n/a	n/a	94'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom next to patch reef	KB/MW
6/12/2018	Hollywood outfall (rosette)	HOL_NW_25	n/a	n/a	82'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, adjacent to/next to patch reef	ND/MS
6/12/2018	Hollywood outfall (rosette)	HOL_NW_50	n/a	n/a	79'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom, adjacent to/next to patch reef	ND/MS
6/12/2018	Hollywood outfall (rosette)	HOL_W_25	n/a	n/a	77'	81F	Seas 2-3'/Wind SE 5-10mph	Pipe trench, sandy spot	ND/MS
6/12/2018	Hollywood outfall (rosette)	HOL_W_50	n/a	n/a	73'	81F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom next to and on north side of pipe	ND/MS
6/12/2018	Hollywood outfall (rosette)	HOL_SW_25	n/a	n/a	78'	82F	Seas 2-3'/Wind SE 5-10mph	Sandy spot in open depression on reef ridge	DC/SW
6/12/2018	Hollywood outfall (rosette)	HOL_SW_50	n/a	n/a	70'	82F	Seas 2-3'/Wind SE 5-10mph	Sandy spot in open depression on reef ridge	DC/SW
6/12/2018	Hollywood outfall (rosette)	HOL_S_25	n/a	n/a	85'	82F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom next to reef	DC/SW
6/12/2018	Hollywood outfall (rosette)	HOL_S_50	n/a	n/a	82'	82F	Seas 2-3'/Wind SE 5-10mph	Sandy bottom in channel between reef ridges	DC/SW
6/12/2018	Hollywood outfall (west transect)	HOL_W_100	26.019111	-80.086922	64'	81F	Seas 1-2'/Wind ESE<5mph	Pipe trench, sandy spot	AR/MW
6/12/2018	Hollywood outfall (west transect)	HOL_W_200	26.019094	-80.087816	60'	81F	Seas 1-2'/Wind ESE<5mph	Pipe trench, sandy spot	AR/MW
6/12/2018	Hollywood outfall (west transect)	HOL_W_400	26.019055	-80.089598	60'	81F	Seas 1-2'/Wind ESE<5mph	Pipe trench, sandy spot	AR/MW
6/12/2018	Hollywood outfall (west transect)	HOL_W_800	26.018996	-80.093201	67'	82F	Seas 1-2'/Wind ESE<5mph	Sandy bottom next to concrete mat covering pipe	KG/PQ
6/12/2018	Hollywood outfall (west transect)	HOL_W_1600	26.018778	-80.100384	25'	82F	Seas 1-2'/Wind ESE<5mph	Next to trench, sandy bottom	KG/PQ
6/12/2018	Hollywood outfall (north transect)	HOL_N_100	26.019935	-80.086031	83'	81F	Seas 1-2'/Wind ESE<5mph	Sandy bottom, next to patch reef	ND/MS
6/12/2018	Hollywood outfall (north transect)	HOL_N_200	26.020742	-80.086049	83'	81F	Seas 1-2'/Wind ESE<5mph	Sandy bottom, next to patch reef	DC/SW
6/12/2018	Hollywood outfall (north transect)	HOL_N_400	26.022358	-80.086091	82'	81F	Seas 1-2'/Wind ESE<5mph	Sandy bottom, next to patch reef	ND/MS
6/12/2018	Hollywood outfall (north transect)	HOL_N_800	26.026376	-80.086081	85'	81F	Seas 1-2'/Wind ESE<5mph	Sandy bottom, next to patch reef	DC/SW
6/19/2018	Haulover outfall (Miami-Dade North)	MDN_Sed	25.92005	-80.086467				Sandy bottom beneath outfall diffuser pipe	
6/19/2018	Haulover outfall (Miami-Dade North)	MDN_H2O	25.92005	-80.086467				From inide mouth of 1 of 12 outfall diffuser pipes	
6/20/2018	Broward North WWTP	BRN_WWTP_H2O	n/a	n/a	n/a	n/a	n/a	From WWTP	DC
6/20/2018	Hollywood WWTP	HOL_WWTP_H2O	n/a	n/a	n/a	n/a	n/a	From WWTP	DC
6/20/2018	Miami-Dade North WWTP	MDN_WWTP_H2(	n/a	n/a	n/a	n/a	n/a	From WWTP	DC

7 August 2018

Thaw the water samples (Broward North, Hollywood and Miami-Dade North WWTP and matching water column samples collected from the outfall pipe labeled H2O). Start lab sample number system below. Centrifuge 12ml of each sample using Amicon Ultra 15s at 5000 x g for 30 minutes. Transfer retentate ~100µl to 1.5ml microfuge tube and freeze the sample at -20^{o}C. Additionally, centrifuge 2ml at 16000 x g for 10 minutes and pipette off 1.8ml. Resuspend the pellet in the remaining 200µl and freeze the samples at -20^{o}C.

1. Broward North WWTP
2. Broward North outfall H_2O
3. Hollywood WWTP
4. Hollywood outfall H_2O
5. Miami-Dade North WWTP
6. Miami-Dade North outfall H_2O

Week of 6 and 13 August 2018

Extract DNA (same DNA extraction protocol as used for the Florida Keys sample sets) from ~0.26g of each sediment sample and the 7 August concentrated wastewater samples. Elute each in 100µl of Qiagen's AE buffer and store at -20^{o}C.

27 August 2018

Set up and run gBlock positive controls for each antibiotic gene primer set. All target controls amplified.

28 August 2018

Set up and run ABR plate 1 for samples 1 through 6.

29 August 2018

Set up and run ABR plate 1 for samples 7 through 12, 13 through 18 and 19 through 24.

30 August 2018

Set up and run ABR plate 1 for samples 25 through 30, 31 through 36 and 37 and 38.

31 August 2018

Set up and run ABR plate 2 for samples 1 through 38.

6 September 2018

Repeat samples 1 through 6 to see if same detects occur without using the Amicon Ultra centrifugation units with these wastewater stream samples. Extract DNA using centrifugation of 15ml at 5900 x g for 30 minutes. Discard all but ~200µl of the supernatant. Vortex the sample to suspend the pellet and extract DNA from the entire volume. Store the 100µl AE extracts in the -20^{o}C.

13 September 2018

Set up and run ABR PCR for the 6 September 2018 samples.

12 March 2019

Received 42 samples on ice (not frozen) from DEP and placed in the refrigerator. Samples included 10 water and 32 sediment samples collected during Southeast Florida's dry season. The samples were collected in the same manner as the Southeast Florida wet season samples, except 4 influent water samples were also taken from the Hollywood and North Miami-Dade WWTP. An image of the sample data spreadsheet with our laboratory identification numbers is pasted below.

Table 3. Field and laboratory sample data spreadsheet (dry season)

Collection Date	Site	Sample/Site ID	Depth	Water Temp.	Weather	Note (where sample collected)	Divers/Sampler		USGS Laboratory sample number	wt/volume assayed
3/4/19	Haulover outfall (Miami-Dade North)	MDN_Sed	98	75	Seas 1-2;sunny/clear skies;wind SW 10-15k	In between rubble below mouth of pipe	KK/AR		1	0.25g
3/4/19	Haulover outfall (Miami-Dade North)	MDN_H2O	98	75	Seas 1-2;sunny/clear skies;wind SW 10-15k	From inside mouth of pipe	KK/AR		2	250ul
3/4/19	Hollywood outfall (south transect)	HOL_S_800	89	79	Seas 1-2;sunny/clear skies;wind SW 10-15k		MS/ND		3	0.25g
3/4/19	Hollywood outfall (south transect)	HOL_S_400	84	78	Seas 1-2;sunny/clear skies;wind SW 10-15k	Sandy spot next to reef ledge	AS/KK		4	0.25g
3/4/19	Hollywood outfall (south transect)	HOL_S_200	86	77	Seas 1-2;sunny/clear skies;wind SW 10-15k		KK/PQ		5	0.25g
3/4/19	Hollywood outfall (south transect)	HOL_S_100	82	77	Seas 1-2;sunny/clear skies;wind SW 10-15k		MS/ND		6	0.25g
3/4/19	Hollywood outfall (north transect)	HOL_N_200	82	75	Seas 1-2;sunny/clear skies;wind SW 10-15k		AS/KK		7	0.25g
3/4/19	Hollywood outfall (north transect)	HOL_N_100	87	75	Seas 1-2;sunny/clear skies;wind SW 10-15k		PQ/AR		8	0.25g
3/4/19	Hollywood outfall (north transect)	HOL_N_400	77	78	Seas 1-2;sunny/clear skies;wind SW 10-15k		MS/ND		9	0.25g
3/4/19	Hollywood outfall (north transect)	HOL_N_800	78	77	Seas 1-2;sunny/clear skies;wind SW 10-15k		AS/KK		10	0.25g
3/4/19	Hollywood outfall (west transect)	HOL_W_800	69		Seas 1-2;sunny/clear skies;wind SW 10-15k	Couldn't see pipe, sampled in sand next to coordinate	KK/PQ		11	0.25g
3/4/19	Hollywood outfall (west transect)	HOL_W_1600	20		Seas 1-2;sunny/clear skies;wind SW 10-15k	Could see pipe, sampled in sand next to it	KK/PQ		12	0.25g
3/5/19	Broward North WWTP Outfall (pipe)	BRN_Sed	108	73	Seas 1-2;sunny/part cloud;wind W/NW 5-10k	Below mouth of pipe	KK/KG		13	0.25g
3/5/19	Broward North WWTP Outfall pipe)	BRN_H2O	106	73	Seas 1-2;sunny/part cloud;wind W/NW 5-10k	From inside mouth of pipe	KK/KG		14	250ul
3/5/19	Hollywood outfall (pipe)	HOL_Sed	99	75	Seas 1-2;sunny/part cloud;wind W/NW 5-10k	Below mouth of pipe	AR/PQ		15	0.25g
3/5/19	Hollywood outfall (pipe)	HOL_H2O	99	75	Seas 1-2;sunny/part cloud;wind W/NW 5-10k	From inside mouth of pipe	AR/PQ		16	250ul
3/5/19	Hollywood outfall (rosette)	HOL_E_25	~99		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		17	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_E_50	~99		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		18	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_SE_25	~99		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		19	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_SE_50	~99		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		20	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_S_25	~99		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		21	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_S_50	~99		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		22	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_N_25	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		ND/KK		23	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_N_50	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		ND/KK		24	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_NW_25	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		ND/KK		25	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_NW_50	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		ND/KK		26	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_SW_25	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AS/EH		27	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_SW_50	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AS/EH		28	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_W_25	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AS/EH		29	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_W_50	88		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AS/EH		30	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_NE_25	95		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		KK/KG		31	0.25g
3/5/19	Hollywood outfall (rosette)	HOL_NE_50	95		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		KK/KG		32	0.25g
3/5/19	Hollywood outfall (west transect)	HOL_W_100	85		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		33	0.25g
3/5/19	Hollywood outfall (west transect)	HOL_W_200	85		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		34	0.25g
3/5/19	Hollywood outfall (west transect)	HOL_W_400	85		Seas 1-2;sunny/part cloud;wind W/NW 5-10k		AR/PQ		35	0.25g
3/7/19	Broward North WWTP	BRN WWTP effluent	n/a	n/a	n/a	effluent, right before goes to outfall pipe, outside tank	AS		36	250ul
3/7/19	Hollywood WWTP	HOL WWTP effluent	n/a	n/a	n/a	effluent, from spigot inside building	AS		37	250ul
3/7/19	Hollywood WWTP	HOL WWTP influent	n/a	n/a	n/a	influent, from spigot inside building	AS		38	250ul
3/11/19	Miami-Dade North WWTP	MDN WWTP effluent	n/a	n/a	n/a	effluent, from spigot inside building	AS		39	250ul
3/11/19	Miami-Dade North WWTP	MDN WWTP influent high	n/a	n/a	n/a	influent, from spigot outside*	AS	*these th	40	250ul
3/11/19	Miami-Dade North WWTP	MDN WWTP influent med	n/a	n/a	n/a	influent, from spigot outside*	AS		41	250ul
3/11/19	Miami-Dade North WWTP	MDN WWTP influent low	n/a	n/a	n/a	influent, from spigot outside*	AS		42	250ul
										water samples = 250ul out of a 500ul concen
										sediment samples = ~0.25g

20 March 2019

Weighed out ~0.25 grams of each sediment sample and placed in Qiagen bead beating tubes. Placed in the -20°C freezer. Processed the 10 water/sewage samples as described on 6 July 2018 and placed in the -20°C freezer.

22 March 2019

Extracted DNA from all the samples using Qiagen's Powersoil Kit. Eluted DNA with 100µl of Qiagen's AE buffer and placed in -20°C freezer.

4 April 2019

Set up and run positive control reactions for each primer/probe target set and its corresponding IDT gBlock target clone. aadA2 gBlock did not amplify. Reordered primers/probe and gBlock clone. All other target clones resulted in amplification.

15 April 2019

Set up and run positive control reactions aadA2. Successful amplification required new primer set. Set up and run PCR for tetB/IPC, tetL/tetM and tetO/tetW PCR assays for all 42 samples. Include negative and positive controls as well as the IPC for the tetB plate. Each target set required one 96-well plate.

16 April 2019

Set up and run PCR for ampC/vanA, ermB/mecA, blaSHV/blaPSE and floR/aadA2 PCR assays for all 42 samples. Included negative and positive controls.

17 April 2019

Set up and run PCR for tetG/tetQ PCR assays for all 42 samples. Included negative and positive controls.

Results

Wet season

Florida Keys sample sets

The 24 Florida Keys samples were composed of two 12 sample sets (one pretreatment and one post-treatment with amoxicillin) with each set containing 4 sediment, 4 coral mucus and 4 water column samples (see the following spreadsheet – greyed area is pretreatment samples, no color is post-treatment samples). The tetB resistance target was detected in all of the water and coral mucus samples but not in the pre-treatment sediments. None of the other antibiotic resistance gene targets were detected in this pre-treatment sample set. Following treatment of the diseased corals with amoxicillin (2-weeks post-treatment) the same number and sample types were collected. In this sample set the tetB target was again detected but in only one of the mucus samples. In addition, the blaSHV target which imparts resistance to amoxicillin was detected in one water column and one coral mucus sample.

Table 4. Florida Keys PCR data

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Tube #	Sample type	Sample site	tetB	tetL	tetM	tetO	tetW	ampC	vanA	ermB	mecA	blaSHV	blaPSE	floR	aadA2	tetG	tetQ
Keys 1	mucus - pret	2	(++)														
2	water - pretr	1	(++)														
3	sediment - p	2															
4	water - pret	1	(++)														
5	water - pretr	2	(++)														
6	water - pret	2	(++)														
7	sediment - p	1															
8	mucus - pre	1	(++)														
9	mucus - pre	1	(++)														
10	sediment - p	2															
11	sediment - p	1															
12	mucus - pret	2	(++)														
13	sediment - p	2															
14	sediment - p	2															
15	water - post	2															
16	sediment - p	1															
17	sediment - p	1															
18	mucus - pos	2	(++)														
19	mucus - post	2															
20	water - post	1															
21	water - post	2															
22	water - post	1										(++)					
23	mucus - post	1										(++)					
24	mucus - post	1															

Blank = negative reaction, (+/+) = positive reaction.

Southeast Florida sample set

The Southeast Florida wet season sample set was composed of 6 wastewater samples (samples highlighted in yellow in the following spreadsheet) collected at three different wastewater treatment plants, their respective outfalls, and 32 sediment samples collected at those outfalls and in an array and along transects centered on the Hollywood outfall. Nine of the antibiotic resistance genes were detected in the sample set. The most prevalent antibiotic resistance genes detected in the samples were tetW and aadA2 at 68.4 and 60.5%, respectively. Seven (tetB, tetW, ampC, vanA, ermB, mecA and tetQ) of the fifteen antibiotic resistance genes were detected in the wastewater samples and six (tetO, tetW, ampC, vanA, mecA and aadA2) were detected in the sediment samples. tetB, ermB and tetQ were only detected in the wastewater samples and tetO and aadA2 were only detected in the sediment samples. tetL, tetM, blaSHV, blaPSE, floR and tetG were not detected in any of these samples. A following heatmap illustrates the occurrence of the different numbers of antibiotic resistance genes detected at each site.

Table 4. Southeast wet season PCR data

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Tube #	Sample type	Sample site	tetB	tetL	tetM	tetO	tetW	ampC	vanA	ermB	mecA	blaSHV	blaPSE	floR	aadA2	tetG	tetQ
Broward 1	water	Broward Nor	(+/+)														(+/+)
2	water	Broward North WWTP outfall pipe - H2O															
3	water	Hollywood W	(+/+)					(+/+)	(+/+)	(+/+)	(+/+)						(+/+)
4	water	Hollywood WWTP outfall pipe - H2O					(+/+)			(+/+)							(+/+)
5	water	Miami Dade	(+/+)							(+/+)							(+/+)
6	water	Miami Dade	(+/+)							(+/+)							(+/+)
7	sediment	Broward North WWTP outfall pipe - sed					(+/+)								(+/+)		
8	sediment	Hollywood outfall - S (transect) sed100					(+/+)				(+/+)						
9	sediment	Hollywood outfall - S (transect) sed200					(+/+)								(+/+)		
10	sediment	Hollywood outfall - S (transect) sed400					(+/+)								(+/+)		
11	sediment	Hollywood outfall - S (transect) sed800					(+/+)								(+/+)		
12	sediment	Hollywood WWTP outfall pipe - sed					(+/+)								(+/+)		
13	sediment	Hollywood outfall - E (rosette) sed25									(+/+)						
14	sediment	Hollywood outfall - E (rosette) sed50															
15	sediment	Hollywood outfall - SE (rosette) sed25															
16	sediment	Hollywood outfall - SE (rosette) sed50															
17	sediment	Hollywood outfall - N (rosette) sed25															
18	sediment	Hollywood outfall - N (rosette) sed50															
19	sediment	Hollywood outfall - NE (rosette) sed25					(+/+)		(+/+)		(+/+)				(+/+)		
20	sediment	Hollywood outfall - NE (rosette) sed50					(+/+)	(+/+)							(+/+)		
21	sediment	Hollywood outfall - NW (rosette) sed25				(+/+)	(+/+)	(+/+)			(+/+)				(+/+)		
22	sediment	Hollywood outfall - NW (rosette) sed50					(+/+)	(+/+)							(+/+)		
23	sediment	Hollywood outfall - W (rosette) sed25					(+/+)	(+/+)							(+/+)		
24	sediment	Hollywood outfall - W (rosette) sed50					(+/+)	(+/+)									
25	sediment	Hollywood outfall - SW (rosette) sed25				(+/+)	(+/+)	(+/+)							(+/+)		
26	sediment	Hollywood outfall - SW (rosette) sed50					(+/+)								(+/+)		
27	sediment	Hollywood outfall - S (rosette) sed25				(+/+)	(+/+)								(+/+)		
28	sediment	Hollywood outfall - S (rosette) sed50				(+/+)	(+/+)				(+/+)				(+/+)		
29	sediment	Hollywood outfall - W (transect) sed100				(+/+)	(+/+)	(+/+)							(+/+)		
30	sediment	Hollywood outfall - W (transect) sed200					(+/+)				(+/+)						
31	sediment	Hollywood outfall - W (transect) sed400				(+/+)	(+/+)	(+/+)			(+/+)				(+/+)		
32	sediment	Hollywood outfall - W (transect) sed800				(+/+)		(+/+)							(+/+)		
33	sediment	Hollywood outfall - W (transect) sed1600				(+/+)	(+/+)								(+/+)		
34	sediment	Hollywood outfall - N (transect) sed100				(+/+)	(+/+)								(+/+)		
35	sediment	Hollywood outfall - N (transect) sed200					(+/+)	(+/+)							(+/+)		
36	sediment	Hollywood outfall - N (transect) sed400					(+/+)	(+/+)							(+/+)		
37	sediment	Hollywood outfall - N (transect) sed800					(+/+)								(+/+)		
38	sediment	Miami Dade North WWTP outfall pipe - sed					(+/+)	(+/+)							(+/+)		
percent positive			10.5	0	0	23.7	68.4	34.2	5.2	10.5	21.1	0	0	0	60.5	0	13.2

Blank = negative reaction, (+/+) = positive reaction.

Figure 1. Wet season heatmap (not to scale)

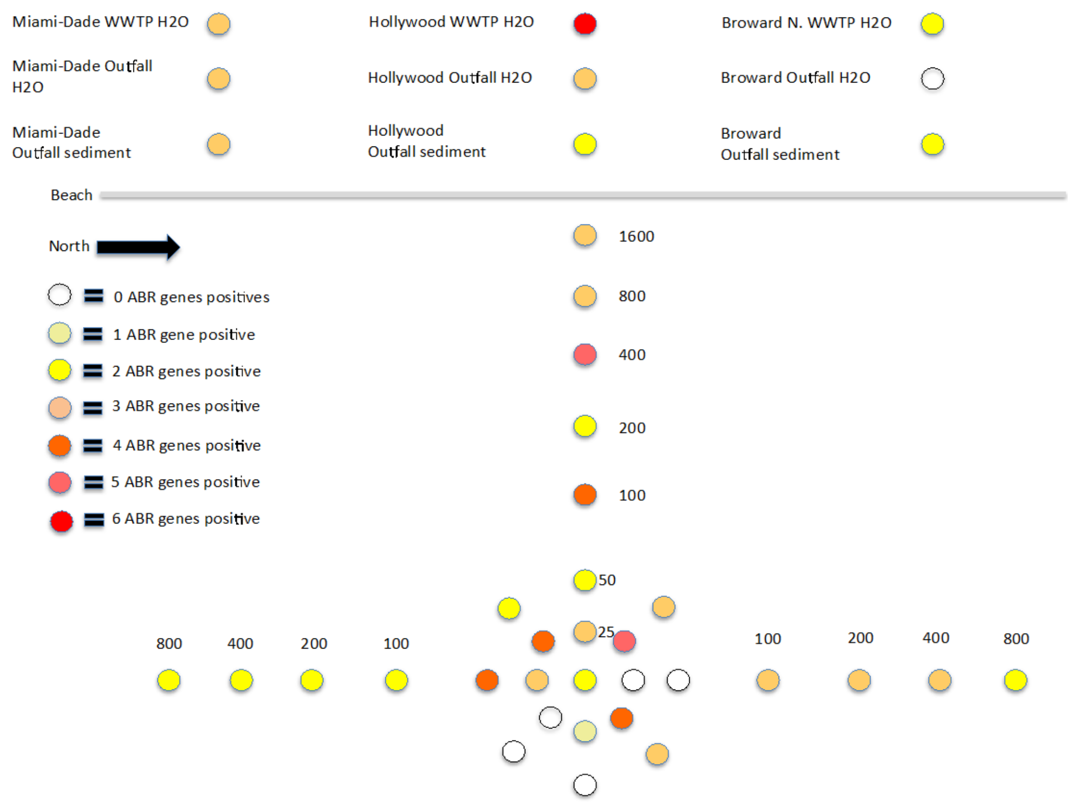
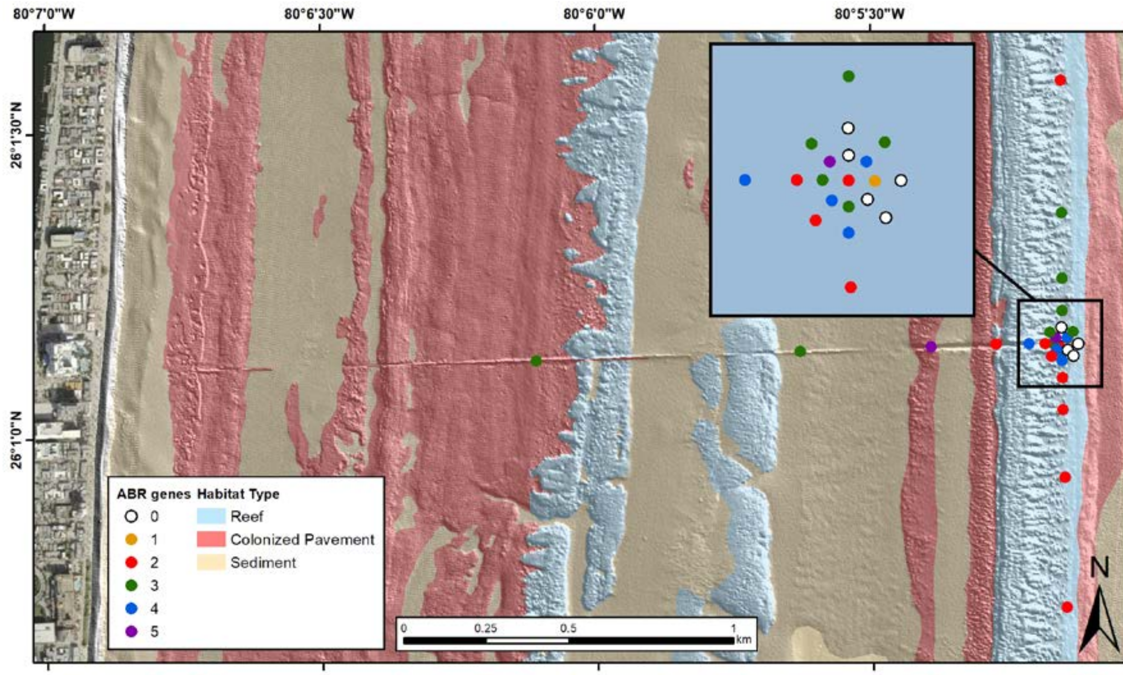


Figure 2. Wet season map of sampling locations and habitat type



Dry season

The dry season sample set was composed of 10 wastewater samples (samples highlighted in yellow in the following spreadsheet) collected at three different wastewater treatment plants, their respective outfalls, and 32 sediment samples collected at those outfalls and in an array and along transects centered on the Hollywood outfall. Ten of the antibiotic resistance genes were detected in the sample set. The most prevalent antibiotic resistance genes detected in the samples were ermB and tetW at 35.7 and 31.0%, respectively. Ten (tetB, tetM, tetO, tetW, ampC, vanA, ermB, mecA, blaSHV and tetQ) of the fifteen antibiotic resistance genes were detected in the wastewater samples and four (tetW, ampC, vanA and ermB) were detected in the sediment samples. tetB, tetM, tetO, mecA, blaSHV and tetQ were only detected in the wastewater samples and all of the four antibiotic resistance genes found in the sediment samples were also detected in the wastewater samples. tetL, blaPSE, floR and tetG were not detected in any of these samples. A following heatmap illustrates the occurrence of the different numbers of antibiotic resistance genes detected at each site.

Table 5. Dry season PCR data

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample type	Sample site	tetB	tetL	tetM	tetO	tetW	ampC	vanA	ermB	mecA	blaSHV	blaPSE	floR	aadA2	tetG	tetQ	USGS lab sample number	
water	Broward North WWTP - effluent	(+/+)				(+/+)	(+/+)		(+/+)							(+/+)	36	
water	Broward North WWTP outfall pipe - H2O	(+/+)				(+/+)	(+/+)		(+/+)	(+/+)						(+/+)	14	
water	Hollywood WWTP - influent	(+/+)		(+/+)	(+/+)	(+/+)			(+/+)	(+/+)						(+/+)	38	
water	Hollywood WWTP - effluent					(+/+)	(+/+)	(+/+)	(+/+)								37	
water	Hollywood WWTP outfall pipe - H2O	(+/+)			(+/+)	(+/+)	(+/+)		(+/+)	(+/+)	(+/+)					(+/+)	16	
water	Miami Dade North WWTP - influent - high	(+/+)		(+/+)	(+/+)	(+/+)	(+/+)	(+/+)	(+/+)	(+/+)	(+/+)					(+/+)	40	
water	Miami Dade North WWTP - influent - medium	(+/+)			(+/+)	(+/+)	(+/+)	(+/+)	(+/+)	(+/+)						(+/+)	41	
water	Miami Dade North WWTP - influent - low	(+/+)			(+/+)	(+/+)	(+/+)	(+/+)	(+/+)	(+/+)						(+/+)	42	
water	Miami Dade North WWTP - effluent	(+/+)				(+/+)			(+/+)	(+/+)						(+/+)	39	
water	Miami Dade North WWTP outfall pipe - H2O	(+/+)			(+/+)	(+/+)	(+/+)		(+/+)	(+/+)	(+/+)					(+/+)	2	
sediment	Broward North WWTP outfall pipe - sed					(+/+)			(+/+)								13	
sediment	Hollywood outfall - S (transect) sed100								(+/+)								6	
sediment	Hollywood outfall - S (transect) sed200								(+/+)								5	
sediment	Hollywood outfall - S (transect) sed400																4	
sediment	Hollywood outfall - S (transect) sed800																3	
sediment	Hollywood WWTP outfall pipe - sed					(+/+)			(+/+)								15	
sediment	Hollywood outfall - E (rosette) sed25																17	
sediment	Hollywood outfall - E (rosette) sed50																18	
sediment	Hollywood outfall - SE (rosette) sed25																19	
sediment	Hollywood outfall - SE (rosette) sed50																20	
sediment	Hollywood outfall - N (rosette) sed25																23	
sediment	Hollywood outfall - N (rosette) sed50																24	
sediment	Hollywood outfall - NE (rosette) sed25																31	
sediment	Hollywood outfall - NE (rosette) sed50																32	
sediment	Hollywood outfall - NW (rosette) sed25																25	
sediment	Hollywood outfall - NW (rosette) sed50																26	
sediment	Hollywood outfall - W (rosette) sed25																29	
sediment	Hollywood outfall - W (rosette) sed50																30	
sediment	Hollywood outfall - SW (rosette) sed25																27	
sediment	Hollywood outfall - SW (rosette) sed50																28	
sediment	Hollywood outfall - S (rosette) sed25																21	
sediment	Hollywood outfall - S (rosette) sed50								(+/+)								22	
sediment	Hollywood outfall - W (transect) sed100																33	
sediment	Hollywood outfall - W (transect) sed200																34	
sediment	Hollywood outfall - W (transect) sed400						(+/+)	(+/+)									35	
sediment	Hollywood outfall - W (transect) sed800																11	
sediment	Hollywood outfall - W (transect) sed1600																12	
sediment	Hollywood outfall - N (transect) sed100																8	
sediment	Hollywood outfall - N (transect) sed200																7	
sediment	Hollywood outfall - N (transect) sed400																9	
sediment	Hollywood outfall - N (transect) sed800																10	
sediment	Miami Dade North WWTP outfall pipe - sed					(+/+)			(+/+)								1	
percent positive		21.4	0	4.8	14.3	31	21.4	11.9	35.7	19	2.4	0	0	0	0	21.4		
	IPC (control) at 27 cycles to an Rn of 0.1, all IPC samples except sample 33(29 cycles) at 28cycles = minimal assay inhibition																	

Blank = negative reaction, (+/+) = positive reaction.

Figure 3. Dry season heatmap (not to scale)

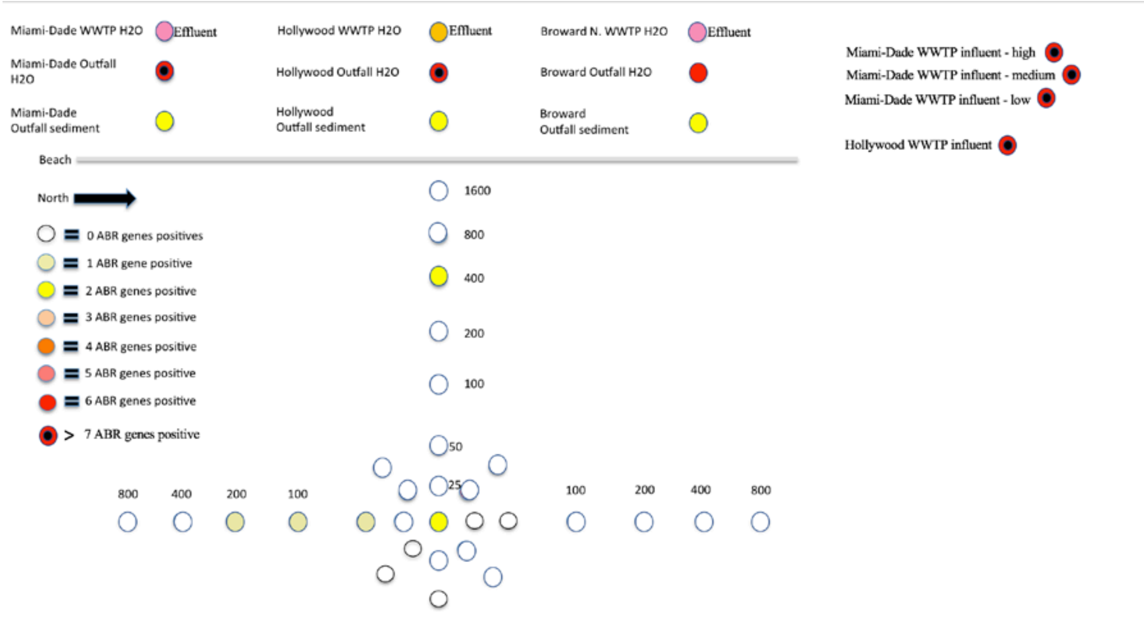
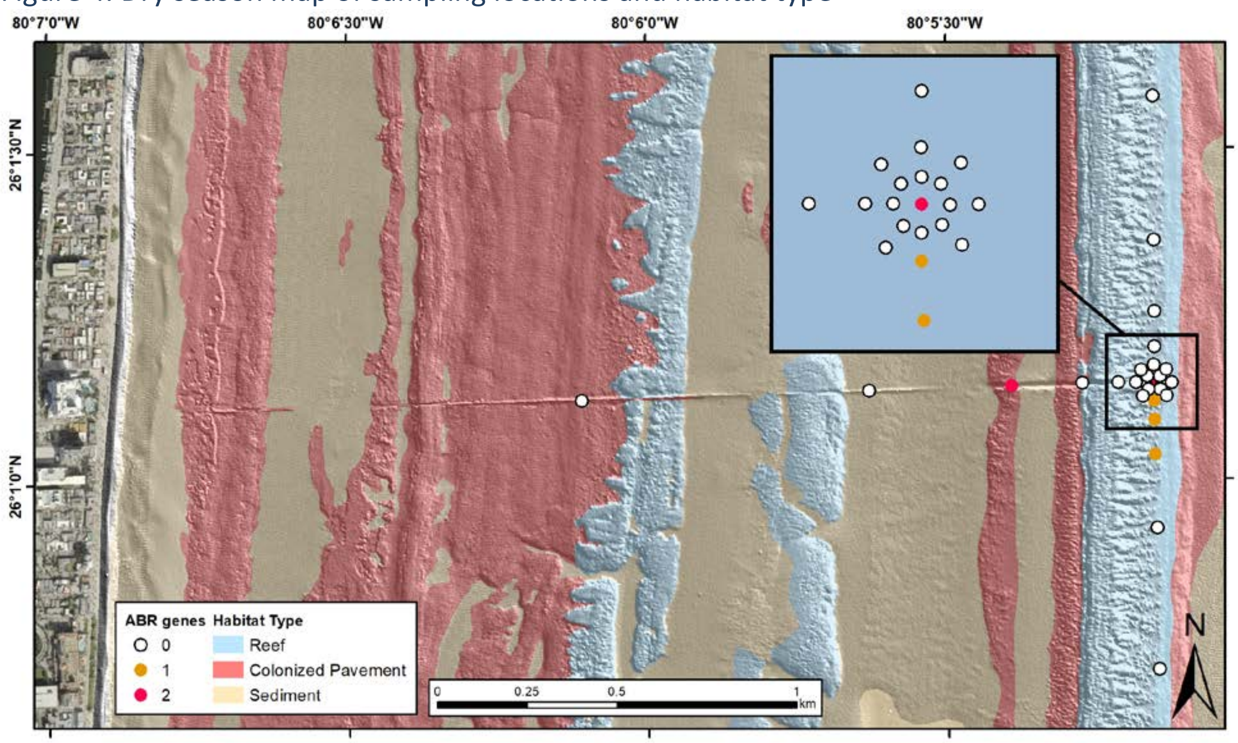


Figure 4. Dry season map of sampling locations and habitat type



Conclusion

The Southeast Florida data illustrates that antibiotic resistance genes are readily detectable in the wastewater stream and in sediments close to and alongside the outfall and outfall pipe. The wet season data set illustrates widespread occurrence of multiple antibiotic resistance genes with the highest occurrences occurring in the wastewater stream and alongside the Hollywood outfall pipe and in close proximity to the outfall. The dry season data illustrates concentrated occurrence in the wastewater stream but less offshore occurrence relative to the wet season data. The offshore positive samples are associated along the outfall pipe, the outfall and along the southern transit. The prevalence flip between the seasons is interesting, with the wet season showing a lower prevalence in the wastewater stream and higher prevalence in the sediment samples and the opposite trend occurring in the dry season. The Florida Keys data set illustrates little background occurrence, but it does demonstrate acquisition of resistance following a specific (amoxicillin) exposure event.

Sewage associated wastewater is well known to carry numerous antibiotics due to public health use. Microbial communities under the influence or stress of antibiotic laden wastewater will acquire resistance. Microbial communities are known to share resistant genes within and across genera when there is an exposure source, even at a heightened metabolic cost (5, 6). These data illustrate that type of microbial response. The data indicate that there is a heightened prevalence of these genes in the wet season that may be due to factors such as seasonal water temperatures, seasonal wastewater discharge rates, seasonal antibiotic usage and coastal flow dynamics. Members in these coastal microbial communities may present risks to recreational water use and to the ecosystem itself. It should be acknowledged that there are very few scientific journal articles published on the topic of antibiotic resistant bacteria in coastal environments that would allow us to contrast the outfall related data reported here. The Florida Keys sample set may illustrate a more normal profile that would be expected given a lower human population and no local outfall influences. The Florida Keys data does however illustrate the possible consequences of releasing or using antibiotics in coastal marine and coral reef environments.

Recommendations

"*This work is a follow-on to the pilot study results from 14ESFUSAFDEP3519 completed between 10/2014 & 9/2016. This project is a continuation of the SE Florida Coral Reef Initiative (SEFCRI) Technical Advisory Committee's recently concluded Outfall Biomarker Pilot Project (LBSP 28/29 Phase I) and builds upon the results and outcomes of that initial work.*" (current FLDEP/USGS FUSA agreement for this project)

The LBSP 28/29 Phase 1 data indicated a degree of toxicity in samples collected near the outfall. This current project was undertaken to further investigate that observation and more specifically to demonstrate if genetic based changes could be observed due to association with a contaminant source.

I would recommend screening sites in the future where antibiotics may be utilized in coral disease trials. This particular approach of using antibiotic resistance genes as a marker for anthropogenic sources of pollution may also be very useful in addressing the extent of inlet influences into our coral reef communities in Southeast Florida. To contrast this current data set given the stark seasonal change, a four-season study may provide additional insight. Additionally, a heatmap of the Southeast Florida Coral Reef Ecosystem Conservation Area in peak and/or low prevalence season would be of global significance. It may also be helpful in regard to potential remediation pathways to analyze samples where wastewater is being released into the environment from a system that utilizes a more advanced level of sewage treatment (e.g., soils from the sprayfields utilized by the City of Tallahassee).

References:

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