Impact of Roadside Ditch Dredging on Bacterial Communities and Biological Contamination of a Tidal Creek

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Abstract

Tidal creek networks form the primary hydrologic link between estuaries and land-based activities on barrier islands. A possible impact from the excavation of drainage ditch systems on bacterial communities and biological contamination was studied in the water column and sediments of headwater, mid-stream, and mouth sites of the intertidal Oakdale Creek on Sapelo Island, GA. Community analysis was performed using the MiSeq Illumina platform and revealed that dredging was the cause of a significant rise in Proteobacteria, especially γ-proteobacteria. Targeted biological contaminants included fecal indicator bacteria, *Enterococcus* spp. (*Entero-1*), pathogens, *Shigella* spp. (*ipaH*), and *Salmonella* spp (*invA*), virulence associated genes (VG's) of pathogenic *E. coli* (*eaeA*, *hlyD*, *stx*1, *stx*2, and *set1B*), integrons (*intI*1, *intI*2), and tetracycline resistance genes (TRGs). Incidence and gene concentrations of *Shigella* spp., *eaeA* and *set1B*, and of TRGs increased 3-20 folds after the onset of dredging, and followed the

dredging schedule. Principal Component Analysis suggested possible common carriers for *Shigella* spp., some TRGs, and the pathogenic *E.coli eaeA* gene. At the site of dredging, all of the above contaminants were detected at high concentrations. We concluded that excavation of roadside ditches caused significant changes in bacterial composition and a rise in incidence and concentrations of biological contaminants in the creek. The authors suggest a different approach for the maintenance of this material be explored.

Keywords: Dredging, Tidal Creeks, Bacterial composition, Shigella, Virulence Genes, Antibiotic resistance

Abbreviations: ARG- Antibiotic resistance genes, EAEC- Enteroaggressive *E. coli*, EHEC- Enterohaemoragic *E. coli*, EIEC- Enteroinvasive *E. coli*, EPEC- Enteropathogenic *E. coli*, ETEC- Enterotoxigenic *E. coli*, OTU- Operational Taxonomic Unit, PCA- Principal Component Analysis, PCoA- Principal Coordinate Analysis, PCR- Polymerase Chain Reaction, qPCR – Quantitative Polymerase Chain Reaction, TDS- Total Dissolved Solids, TRGs- Tetracycline resistance genes, UPEC- Uropathogenic *E. coli,* VGs- Virulence genes

1. Introduction

Estuaries of the South Eastern United States are a dynamic environment of shallow tidal creeks and salt marshes characterized by expansive low relief intertidal regions with broad fluctuations in physical water parameters and water quality (Wiegert et al. 1990). Tidal creek networks form the primary hydrologic link between estuaries and land-based activities on barrier islands, and the water quality in these creeks depends on and reflects these activities (Sanger et al. 2008). In particular, biological contamination of tidal creeks was shown to be significantly higher in urban and suburban areas than in forested areas (Sanger et al. 2008). Due to the complexity of coastal development activities in urban and suburban barrier islands, it is not always easy to identify impacts of these activities on bacterial composition and biological contamination of tidal creeks. Less complex environments of relatively undeveloped barrier islands may provide an insight into the relationships among land-based activities, bacterial composition, and biological contamination of coastal waters.

Surprisingly, bacterial community composition of tidal creeks and saltmarshes is far less known than even that of the deep ocean. A previous study of two saltmarshes on Sapelo Island (GA) discovered no seasonal trends in temporal dynamics of three marine γ-proteobacteria taxa (Hardwick et al. 2003). Our most recent study revealed spatial diversity and temporal consistency of bacterial communities in the headwater, midstream, saltmarsh, and mouth of intertidal Oakdale Creek (Sapelo Island, GA) (Barkovskii et al. 2015). This and a previous study (Barkovskii et al. 2012) demonstrated the presence of tetracycline resistance (TRGs) and virulence (VGs) genes in tidal creeks with a higher incidence observed nearby the only settlement on the island of roughly fifty residents.

55 One of the major and repetitive landward disturbances on low-populated barrier islands is
56 excavation of roadside drainage ditches. The main purpose of roadside ditches is to protect the
57 integrity of the road. Ditches along rural roads are often vegetated. The vegetation slows down
58 water flow allowing a portion to infiltrate into the soil causing some of the debris and pollutants
59 to settle out. Furthermore, sediments and associated pollutants also become trapped in the mesh
60 of plant roots that act as a filter for larger particles. To protect the roads and travelling public, the
61 hydraulic capacity of these ditches needs to be maintained via periodic removal of the above
62 materials. During excavation, the above materials are removed and stored on the roadside, and
63 thus are consequently washed out to tidal creeks during rainfall.
64 We hypothesized that excavation of roadside ditches causes major shifts in bacterial
65 community structure of tidal creeks and by doing so deteriorates the overall water quality. In this
66 study we analyzed spatial and temporal changes in bacterial communities by monitoring
67 incidence and abundance of biological contaminants in Oakdale Creek before, during, and after
68 dredging. Targeted biological contaminants included *Enterococcus* spp. that is specified by US
69 EPA as the primary fecal indicator for marine environments (U.S. EPA, 2000) and used for
70 monitoring water quality in estuaries (Ortega et al. 2009); *Salmonella* spp. that has been
71 commonly reported as a pathogen in rural watersheds and estuaries in South East USA (Haley et
72 al. 2009; Vereen et al. 2013); *Shigella* spp. reported as a leading causal agent of enteric diseases
73 in Southern States (Chang et al. 2009); virulence genes (VGs) associated with EHEC, EPEC,
74 EAEC, EIEC, ExPEC, UPEC and other pathogenic *E. coli* that were reported as reliable
75 indicators of water quality (Masters et al. 2011); TRGs and integrase genes *intI*1 and *intI*2 that
76 were previously detected in coastal waters around Sapelo Island (Barkovskii et al. 2012;
77 Barkovskii et al. 2010), and in Oakdale Creek (Barkovskii et al. 2015).

78

79 **2. Materials and Methods**

80 **2.1 Field Sites.**

81 The tidal creek, Oakdale Creek, within the Georgia Sapelo Island National Estuarine
82 Research Reserve was selected for this study. Oakdale Creek receives runoff from a watershed
83 comprised primarily of forested area (90% of its land mass) with elements of agriculture and
84 rural development within the remaining 10%. Hog Hammock, a small community of roughly 50
85 residents, is located in the lower headwater of this creek's watershed.

86 Unpaved West Autobahn Road crosses the settlement and Oakdale Creek, and connects this
87 settlement to the Southwest end of the Island within 150-300 m from the creeks bank. Roadside
88 ditches were excavated for their routine maintenance over a total length of 3.6 km. Main
89 dredging occurred between April, 1st and May, 5th of 2014 for a total 70 hours with some
90 sporadic events in July and August. The dredged roadway did not reach the Oakdale Creek
91 mouth region (Figure S1) and thus this area remained least affected by the excavation. There is
92 no available data on the total mass of the excavated materials.

93 Water and sediments of the Oakdale Creek were collected at three stations (Figure S1). Station
94 1 was located in the headwater region (31°25'43.6"N 81°16'21.5"W) of the creek immediately
95 downstream of the Hog Hammock community and received runoffs directly from this
96 community and the main dredged roadways. Station 2 was located mid-stream (31°24'36.9"N
97 81°17'22.6"W) and was also impacted by dredging. Station 3 was located in the mouth of the
98 creek (31°24'28.5"N 81°17'37.2"W) that was the most remote from the community and dredged
99 sites; therefore, this station was expected to receive a lower and delayed impact from dredging.
100 Barkovskii et al. (2015) reported much lower TRG and VG incidence frequency in the Oakdale

Creek mouth compared to the Hog Hammock runoff outfall. Roadside ditches were sampled in three landward stations (Figure S1). Station 4 (31°25'34.9"N 81°16'08.8"W) was located in the closest proximity to Oakdale Creek, Station 5 (31°25'33.5"N 81°16'04.7"W) was located between the Hog Hammock community and Oakdale Creek, and Station 6 (31°25'31.8"N 81°16'05.7"W) was located directly outside the community. Sediments and water samples were collected directly from the above ditches.

2.2 Sample collection and preparation.

Water column and sediment samples were collected six times between March and August of 2014. Roadside ditches were sampled before, during, and after dredging as well as from the upstream dredged locations, site 4, 5, and 6, after dredging had ceased. At each sampling event salinity, temperature, conductivity, turbidity, oxygen concentration, total dissolved solids, oxidation-reduction potential, temperature, and potential water density were analyzed using a Horiba U-52G Multiparameter Water Quality Meter (Horiba, Kyoto, Japan). All samples were collected in triplicates. Water samples were taken at 0.25 m below the surface using a 4000 Subsurface Grab Bottle Sample (Ben Meadows, Janesville, WI). Sediment samples were collected from the top 15 cm of sediment layer with a 15lb SST Dredge (Ben Meadows, Janesville, WI). Each sample was aseptically transferred into 1L sterile bottles or sterile Whirl-Pak® bags and immediately stored on ice until their extraction within 24 h.

Water samples were filtered using a custom-built water filtration system. Particulate matter was collected on 0.22 µm nitrocellulose filters (Millipore, Billerica, MA). Community DNA from water samples was extracted using the PowerWater DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). DNA from sediment samples was extracted using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). DNA was quantified using a NanoDrop

ND-2000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE) and isolated DNA was stored at -20º C until processed.

2.3 Analysis of bacterial composition.

Bacterial composition was evaluated using the MiSeq Illumina Sequencing System (San Diego, CA) performed by Research and Testing Laboratory (Lubbock, TX). DNA extracted from three replicates for each sample was pooled together before the analysis. The 16S assay 28F/519R targeting V1-3 regions was used for DNA amplification. All the PCR products included into further analysis were within 250-500 bp, forward and reverse reads were taken in FASTQ format and merged together using the PEAR Illumina paired-end read merger (Zhang et al. 2014). Failed sequence reads and those that had low quality tags were excluded from the analysis. Clustering was performed at a 4% divergence using the USEARCH application (Edgar 2010). Rarefaction was performed with QIIME at up to 20000 sequences resulted in Species Richness higher than 3000. To classify the large number of clusters into OTUs, the UPARSE OTU selection algorithm was used (Edgar 2013). The USEARCH global search algorithm along with a python program was used to determine the actual taxonomic assignment for each read (Bokulich et al. 2015). Once confidence values were assigned for each sequence, an RDP formatted output file was generated to be used for final analysis. Clusters that contained <2 members (singleton clusters) were not added to the output file, and thus were removed from the data set.

Bacterial diversity was examined from two perspectives. First, overall richness (i.e., number of distinct organisms present within the microbiome) was expressed as the number of operational taxonomic units (OTUs), and was quantified using the Chao1 richness estimator ($S_{chao1} = S_{obs} + n_1(n_1-1)/(n_2+1)$), where n_i is the number of OTUs with abundance i. Second, overall diversity

147 that was determined by both richness and evenness (i.e., the distribution of abundance among
148 distinct taxa) and expressed as Shannon Diversity. Shannon diversity (H') was calculated using:
149 $H' = -\sum_{i=1}^{R} piln(pi)$, where R is richness and p_i is a relative abundance of i^{th}.

150 Microbiome divergences among samples were illustrated using Principal Coordinates Analysis
151 (PCoA). PCoA presented total multivariate changes among samples using only two independent
152 axes, and was based on inter-sample distances. Distances among samples were calculated in two
153 distinct ways. First, changes in the phylogenetic composition of communities were considered by
154 using the unweighted UniFrac distance measure. Second, changes in relative abundances of
155 constituent OTUs were considered by using a Bray-Curtis distance measure. UniFrac distances
156 were calculated using QIIME, and all other analyses were conducted in R using the vegan and
157 labdsv packages (R Development Core Team 2011). Respectively, PCoA plots reflected either
158 changes in the composition of samples (unweighted UniFrac distance measures) or changes in
159 relative abundance as well as changes in composition (Bray-Curtis distance measure). The
160 amounts of total variability accounted for by each axis are indicated in the axis labels.

161 **2.4 Detection of biological contaminants.**

162 Sixteen TRGs were monitored- (A), A(P), (B), (C), (D), (E), (G), (K), (L), (M), (O), (Q), (S),
163 (T), (W), and (X). The gene's presence was determined by PCR amplification from conservative
164 fragments using previously described primers and PCR conditions (Aminov et al. 2001; Ng et al.
165 2001) (Table S1). All the reported genes were detected in all three replicates collected at each
166 station. PCR reactions were performed with a total volume of 25µL containing 3-5 ng of
167 template DNA, 200 µmol L^{-1} dNTP, 15 mmol L^{-1} $MgCl_2$, 500 nmol L^{-1} primer sets, 1X PCR
168 buffer, and 0.625 U of Taq polymerase (Promega). The TRG temperature conditions were as
169 follows: initial denaturation at 94^{o}C for 5 min followed by 25 cycles of a 60 sec denaturation

170 step at 94º C, annealing at 55ºC (or specified annealing temperature) for 60 sec, elongation at
171 72ºC for 90 sec, and a final elongation step at 72ºC for 6 min.

172 *Salmonella* spp. (Ferretti et al. 2001), *Shigella* spp. (Vargas et al. 1999), and *Enterococcus* spp.
173 (Ryu et al. 2013) were detected and monitored with *invA*, *ipaH*, and *Entero-1* genes respectively.
174 Five virulence genes (VGs) associated with *Shigella* spp., and pathogenic *E. coli* (*eaeA*, *stx1*,
175 *stx2* (Sharma et al. 1999), *hlyD* (Rodriguez-Siek et al. 2005), and *set1B* (Vargas et al. 1999)) as
176 well as two integrase genes (*intI1* and intI2) (Goldstein et al. 2001) were also included in the
177 study (Table S2).

178 PCR reactions were run in the Thermal Cycler HB PXE02 (Thermo Scientific, Odessa, TX).
179 To insure negative results were not due to the presence of PCR inhibitors in samples, a control
180 amplification of 16S rDNA was performed (Courtois et al. 2001) prior to testing for biological
181 contaminants. To insure observed products were targeted amplicons and not a result from non-
182 specific amplification of unrelated gene fragments, all PCR runs included positive controls, a
183 blank, and a negative control. *E. coli* ATCC 11775 was used as a negative control in all the
184 experiments except for quantification of the 16S rRNA gene for which a sample of yeast DNA
185 was used. No false positive reactions were observed. Obtained products (amplicons) were
186 visualized in 1.0 % agarose gel and compared to corresponding amplicons from reference
187 plasmids and to a DNA ladder. The gels were documented on the Biochemi Bio Imaging system
188 with Lab Works 4.6 software package. The above measures allowed to minimize false positive
189 reactions and to validate PCR results.

190 In order to quantify representative genes, further qPCR analysis was performed using three
191 TRGs; *tet*A(P), (B), and (C) that represented TRGs with a high to low incidence frequency rates,
192 as well as 10 genes that represented virulence factors, integrons, pathogen and the fecal indicator

193 *Enterococcu*s spp. QPCR was performed using SYBR green fluorescent dye and the MJ
194 MiniOpticon™ thermocycler and Bio-Rad CFX manager system (Bio-Rad, Hercules, CA). Each
195 25 µL reaction contained: 12.5 µL of iQ 2X SYBR® Green Supermix (Bio-Rad, Hercules, CA),
196 20 ng (~1 µL) of template DNA, 200 nM (1.25 µL) of each FW/RV primers, and 10 µL of
197 diH_2O. All reactions were performed in triplicates, and the average copy numbers were
198 calculated. The primers and conditions used for quantification of TRGs are shown in Table S1.
199 The primers used for quantification of VGs, pathogens, and *Enterococcu*s spp. are shown in
200 Table S2. The qPCR conditions for these genes, their functions, and associated pathogens are
201 shown in Tables S3 and S4. The fidelity of primers was validated via analysis of the products'
202 melting temperatures and the absence of additional non-specific peaks along the melt curve. The
203 lowest reliable quantifiable concentration was observed in the order of 10^2 copies/µL. The
204 obtained copy numbers were corrected to the dilution factor and the mass/volume of 1g or 1mL
205 of the original sample.

206 **2.5 Biostatistics.**

207 TRGs, VGs, pathogens, and fecal indicator incidence frequencies were defined as the total
208 number of events when corresponding genes were detected. Pearson and Spearman Rank
209 correlations were used to evaluate the extent to which the incidence frequencies and abundances
210 of the above biological contaminants correlated to water quality parameters as described in
211 previous studies (Barkovskii et al. 2015; Barkovskii et al. 2012), statistical significance values
212 were determined, and p values ≤ 0.05 were considered as significant. Using the rgl package,
213 three-dimensional Principal Component Analysis (PCA) was used to explore relationships
214 between the presence and concentrations of TRGs, VGs, *Enterococcus* spp., and pathogens in
215 Oakdale Creek (R Development Core Team 2011).

3. Results

3.1 Bacterial populations and their dynamics.

OTU numbers in both the water column and sediments of Oakdale Creek varied temporally and spatially before, during, and after dredging. Overall, sediments revealed higher richness than the water column, with the highest at 4500 OTUs detected in mouth sediments. The lowest richness at 500 OTUs was observed in Hog Hammock headwater (data not shown). Shannon diversity was likewise higher in sediments. Whereas midstream and mouth sediments demonstrated consistently high Shannon diversity, its value varied between 3 and 7 in headwater sediments, and to a lesser extent in the water column of all three stations (Figure S2). Uweighted UniFrac distance measures provided a better insight on temporal compositional changes in bacterial communities (Figure S3). Bacterial community structure in the sediment and water column of Oakdale Creek mouth (where dredging did not occur) remained essentially unchanged between March and August revealing a lack of seasonal or dredging-related trends. Midstream sediment bacterial composition also remained relatively unchanged overall however the bacterial community structure of the midstream water column revealed shifts for March and May when communities were clustered together. In the headwater water column (and to a lesser extent in sediments), bacterial communities exhibited a similar pattern of shifts and clustering. Remarkably, tighter clustering in the headwater region was again observed between March and May in bacterial communities collected right before the onset of excavation and after it had essentially ceased. The opposite was found for the bacterial communities collected in this region during excavation indicating variations in community dynamics coincided with dredging. The most dramatic temporal changes were observed in bacterial communities of the water column.

We have identified major bacterial groups responsible for the observed temporal shifts. (Figure 1). After a slight decline in ɤ-proteobacteria populations at the onset of dredging, their proportion sharply increased in April through July from 3% to 40% and 10% to 25% at Station 1 and 2 respectively and afterward sharply dropped to 4% and 13%. In the mouth, corresponding changes were much less pronounced. β-Proteobacteria, δ-proteobacteria and unidentified proteobacteria, were also on the rise in these environments between March and April and ceased by the end of May or July. Other major changes in the headwater and midstream water columns involved the fall of an unknown *Burkholderiales* group that represented 40% of the overall bacterial population of the headwater area preceding excavation, and decreased below 5% during the dredging. After the dredging, this group returned to a proportion of around 10%.

Rise of ɤ-proteobacteria between April and July was also observed in the headwater sediment

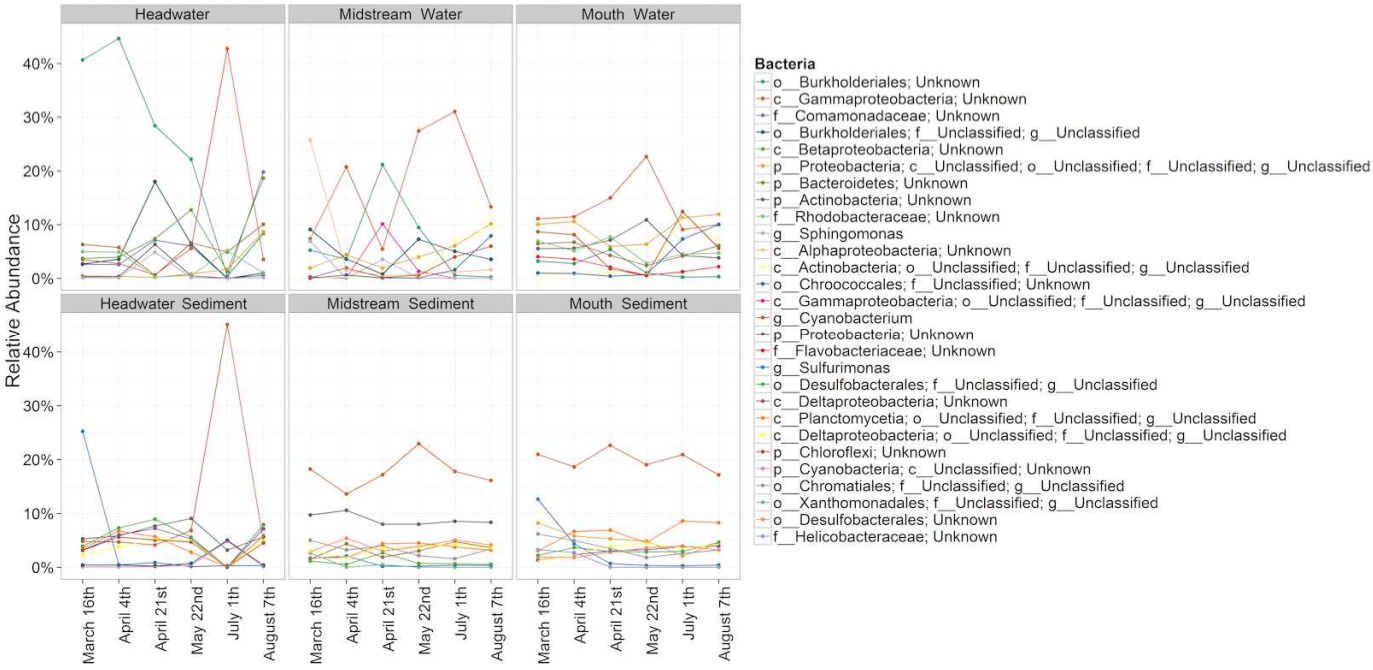

Figure 1. Bacterial composition dynamics in Oakdale Creek tributaries. Upper green line corresponds to *Burkholderiales*, the next yellow-orange line corresponds to γ-proteobacteria, and light blue line corresponds to *Sulfurimonas*. Letters K,P,C,O,F,G,S represent phylogenetic rank. Dredging started on April 1st between the first and second sampling event.

and coincided with an increase in the abundance of α- and β-proteobacteria. Even if the observed increase could be partially due to variations in PCR amplification during sequencing, its primary occurrence in headwater and midstream sediments coincided with much more pronounced rise of gammaproteobacteria in the water columns of the above environments. These changes were less pronounced in midstream sediment and not observed in the mouth. One unknown group of *Sulfurimonas* spp. decreased in all the sediments between March and the end of April.

Summarizing temporal changes that occurred during the dredging, a substantial rise was observed in various proteobacteria populations followed with their decrease to pre-excavation levels. A sharp decrease of *Burkholderiales* populations followed with their recovery, however the *Sulfurimona*s population never recovered. There was no major correlation to any water parameter among the observed bacterial groups except for *Burkholderiales* and *Chrococcales.* The abundance of the former strongly and negatively correlated to the total dissolved solids and salinity while the abundance of the latter demonstrated the opposite trend (Table S5).

3.2 Occurrence of biological contaminants.

All targeted genes with exception of *stx2*, *tet*(K), *tet*(S), and *tet*(T) were observed in the water column and sediments, each with different individual incidence frequencies. After the onset of dredging, their cumulative incidence frequencies (presence or absence) in the water increased 2-3 fold, remained at this level in April, descended in May, and picked up again in July and August, particularly at the headwater and midstream sites (Figure 2a). The highest incidence immediately after the onset of dredging was observed in both the water column and sediment at Station 1, the closest to where excavation of roadside ditches had begun. In addition to the dredging, there was also an apparent correlation between the cumulative incidence frequency and the rainfall that likely amplified the impact of dredging due to wash out of both dredged and

273 stored materials. In sediments, the overall increase in incidence directly after dredging was also
274 documented. Unlike the water column, there was no apparent correlation to the rainfall (Figure
275 2b).

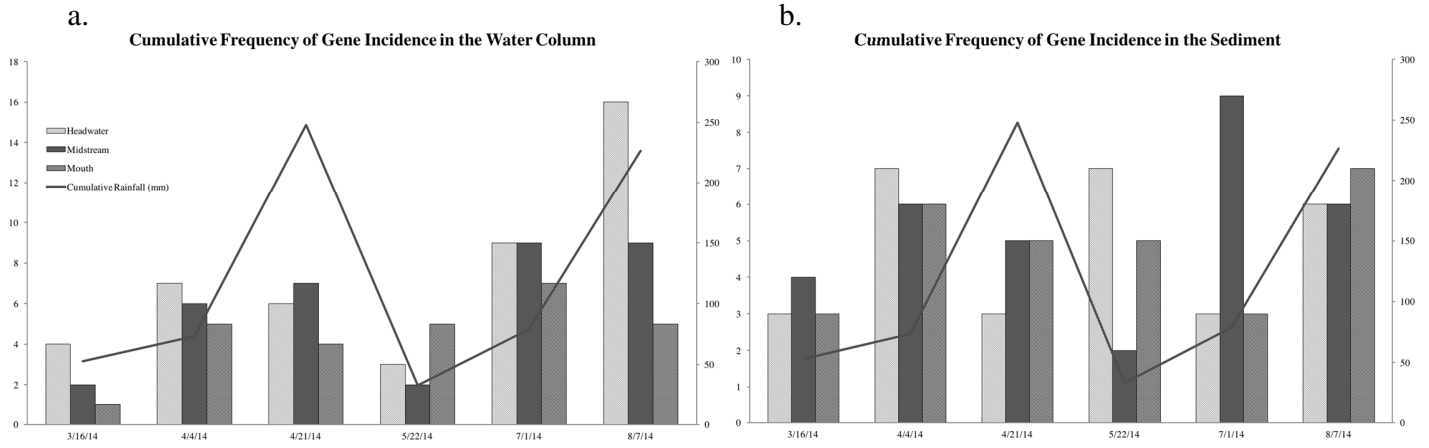

Figure 2. Temporal changes in the occurrence of biological contaminants. The dredging started on April 1st between the first and second sampling event. Notice that Y scales are different for (a) and (b). The left Y-axis shows the incidence frequencies of genes detected using PCR while the right Y-axis shows the average rainfall in mm

276 **3.3 Quantification of biological contaminants.**

277 The pathogen genes for *Salmonella* spp., *Shigella* spp., as well as for the fecal indicator
278 bacteria *Enterococcus* spp., and the most commonly observed VGs, *eaeA set1B,* and *stx1,* were
279 quantified at all stations (Figure 3). After the onset of excavation, *Shigella* spp. appeared in the
280 Station 1 water column, and *set1B* and *eaeA* concentrations increased approximately one order of
281 magnitude. At the same time, *Shigella* spp. also appeared in the Station 2 water column, and
282 concentrations of the above two VGs and s*tx1* increased one order of magnitude. Concentration
283 dynamics of VGs correlated to the dynamic of the 16S rRNA gene at Station 1 but not Station 2
284 (Fig. 3a and b). No dredging-related changes were observed in the water column of Station 3
285 (Fig. 3c). During the dredging, *Shigella* spp. and *eaeA* concentrations also increased in sediments
286 at Stations 2 and 3 but not Station 1 (Fig. 3d-f).

287 Overall, concentrations of *Shigell*a spp., *set1B*, and *eaeA* were in the range of 10^5-10^9/ml in the
288 water column and 10^8-10^{12}/g in the sediment. Copies of *Salmonella* spp. and *Enterococcus* spp.
289 specific genes fluctuated within the same range with no particular relationship to the dredging
290 schedule. The lowest concentrations of targeted genes were detected in the water column in late
291 May revealing the same trend as their incidence frequencies, and also corresponding to the
292 lowest amount of rainfall.

293 Three TRGs with high (*tet*(C)), average (*tet*(B)), and low (*tet*A(*P*)) incidences were also
294 quantified (Figure 3). In the water column at Station 1 and 2, t*et*(B) and *tet*(C) either appeared
295 after the onset of excavation, or their concentrations increased. The *tet*(B) dynamics followed
296 that of the 16S rRNA gene, and its concentration similarly increased at Station 3. *Tet*A(*P*) was
297 the only gene which was negatively affected by the dredging, in particular at Stations 1 and 3
298 where this gene disappeared after the onset of excavation and appeared again only after the
299 dredging had mostly ceased in May and July respectively. In sediments, *tet*A(*P*) disappeared at
300 Station 1 after the onset of dredging and reappeared in May, thus exhibiting the trend observed in
301 the water column. TRG concentrations have been observed between 10^3-10^7/ml of water and 10^6-
302 10^8 /g of sediments, therefore at lower levels than VGs, pathogens, and fecal indicators in
303 corresponding environments. Except *tet*A(*P*), their lowest concentrations have been detected in
304 water columns of Stations 1 and 2 in late May.

305 Interestingly, PCA revealed high uniformity in the presence and concentrations of *Shigella*
306 spp. and TRG concentrations corresponded to the dredging schedule (Figure S4). In the water
307 column, relative concentrations of *Shigella* spp. and *tet*(B) tightly clustered (Figure S4a). In
308 sediments, abundances of *Shigella* spp., *Salmonella* spp., *eaeA*, and *tet*(B) were also closely
309 correlated to one another (Figure S4b).

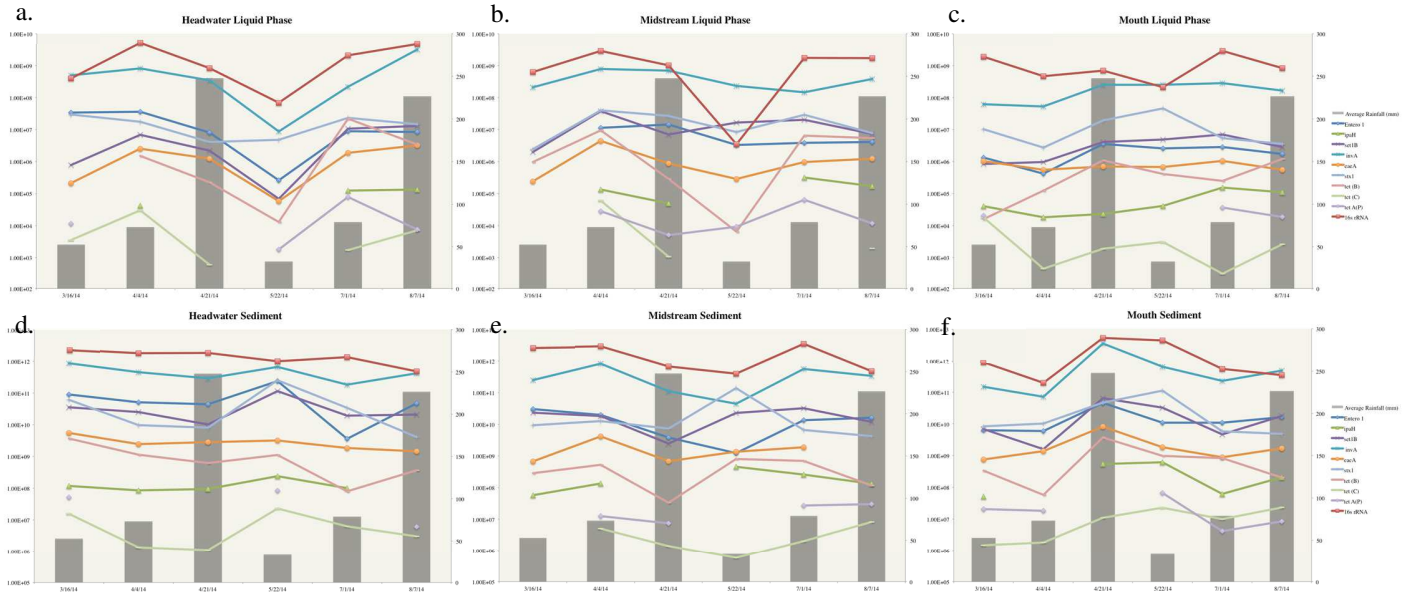

Figure 3. Concentration dynamics of biological contaminants in Oakdale Creek. The dredging started on April 1st between the first and second sampling event. The standard deviations varied between 2% and 20% and are not included into the Figure to allow for better clarity. Gene concentrations in copies per mL (for the liquid phase) and copies per gram (in sediment) are shown on the left Y- axis while average monthly rainfall in mm is shown on the right Y- axis.

3.4 Biological contaminants in dredged materials.

An increase in incidence frequencies and concentrations of biological contaminants in Oakdale Creek coincided with excavation of roadside drainage ditches and suggested the causal relationship between the two. Analysis of water and sediments taken directly from the previously excavated ditches revealed the presence of the same biological contaminants in these materials at concentrations comparable to or exceeding those in Oakdale Creek. The profiles and concentrations of VGs, pathogens, and fecal indicator were remarkably similar between the landward stations with concentrations of individual biological contaminants varying between 10^5-10^8/ml of water and 10^8-10^{12}/g of sediments (Figure 4). There was no such uniformity for TRGs, however the most commonly observed genes were *tet*(B) and *tet*(C) that were also the most often detected in Oakdale Creek after the onset of dredging. Concentrations of TRGs in

321 dredged materials were between 10^3-10^5/mL of water and 10^5-10^7/g of sediments. The third
322 TRG, *tet*A(*P*) was detected only in sediments and only at one landward station.

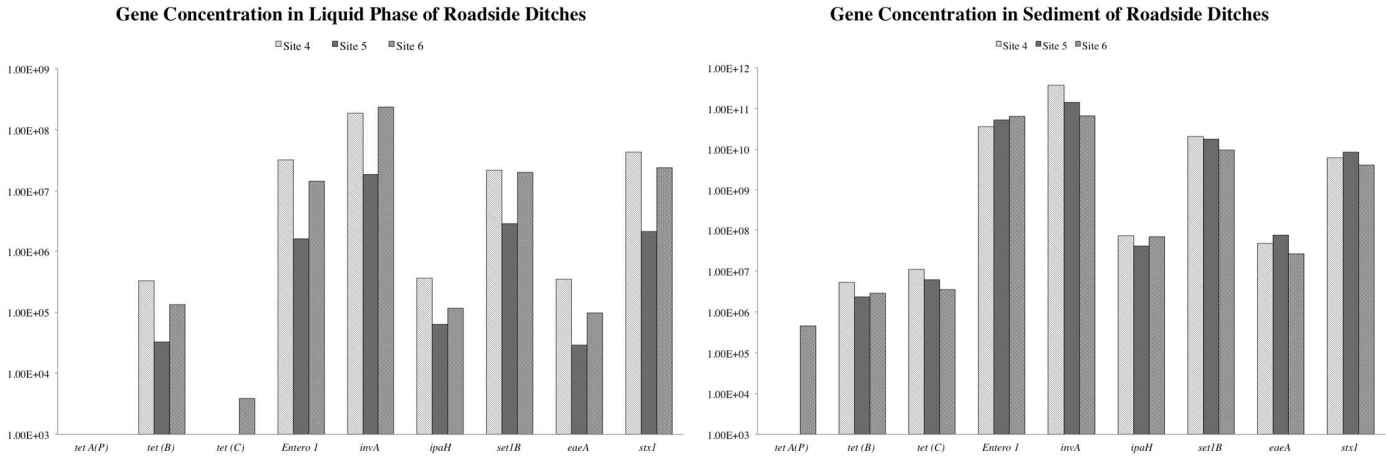

Figure 4. Quantification of VG's, Pathogens, and Fecal Indicator Bacteria sampled directly from roadside ditches. Copies per mL (in the liquid phase) and copes per gram (in sediments) are found on the left Y-axis.

323

324 **4. Discussion**

325 **4.1 Bacterial populations and their dynamics.**

326 There are numerous publications reporting the impact of shoreline land uses on bacterial
327 contamination of coastal waters. In these publications, bacterial contamination is mostly
328 reported as counts of fecal indicators that are proxies to waterborne pathogens, mostly of
329 *Shigella* spp., *Salmonella* spp. and other enterobacteria (Sanger et al. 2008; Kirby-Smith and
330 White, 2006). Biological contamination however is not limited to the above but includes a wider
331 spectrum of bacteria and genes impeding environmental and human health. Assessment of
332 coastal development as related to bacterial quality of coastal waters should also include its
333 impacts on indigenous bacterial communities as was recently shown by Van Rossum et al. 2015
334 in order to correlate river microbiomes to land use and water quality. When comparing the
335 abundance of functional genes between high- and low-quality water samples collected from an

agricultural area, the latter had a higher abundance of genes related to nutrient metabolism and bacteriophage groups possibly reflecting an increase in agricultural runoff.

To date there is a lack of information on bacterial community structure in tidal creeks. Our previous study demonstrated the prevalence of marine species and freshwater bacteria in the mouth and headwater of Oakdale Creek respectively (Barkovskii et al. 2015). The general bacterial composition remained largely unchanged between June and October of 2012. The current study revealed significant shifts in the bacterial composition of Oakdale Creek that occurred between March and August of 2014. The main changes observed were at headwater and midstream sites and involved various groups of proteobacteria, especially γ-proteobacteria.

Observed community shifts could be attributed to seasonal changes but taxonomic composition of coastal waters was previously reported as temporally stable, in particular as related to proteobacteria (Schauer et al. 2003). A previous study of temporal dynamics of three γ-proteobacteria taxa in two salt marshes on Sapelo Island (GA) demonstrated that variations in relative abundance of these three groups did not exhibit seasonal trends (Hardwick et al. 2003). This was also confirmed in the previous study (Barkovskii et al. 2015). An increase in proteobacteria abundance at stations where excavation occurred, and lack of noticeable changes in these populations in the mouth of the creek where no excavation was performed; lack of correlation between abundance of proteobacteria and water parameters; and the above considerations allowed for the attribution of excavated materials as the source of proteobacteria to the creek. To the extent of our knowledge, the impact of upland dredging on bacterial composition of coastal waters is reported here for the first time.

The *Burkholderiales* population significantly decreased during dredging. This decrease coincided not only with dredging schedule but also correlated with increased salinity and total

359 dissolved solids. In contrast to other proteobacteria, there is no information on seasonal
360 dynamics of *Burkholderiales* in coastal waters and tidal creeks. These bacteria are known for
361 their preference for freshwater and low-salinity environments (Newton et al. 2011). In fact, in
362 three coastal wetlands this group was reported to dominate the only site with the lowest salinity
363 (Matteo et al. 2013). Based on the above considerations, we attributed the decline of
364 *Burkholderiales* to be from an increased salinity at headwater and midstream stations.

365 **4.2 Occurrence and abundance of biological contaminants.**

366 All TRGs, *intI* genes, and some VGs and pathogens detected in this study have been
367 previously observed in the same creek (Barkovskii et al. 2012, Barkovskii et al. 2015). Their
368 cumulative incidence frequencies (all genes at three stations summarized together) in the water
369 column varied between one during the drought and five during a rainy season and exhibited a
370 positive correlation to rainfall and location (Barkovskii et al. 2015). Positive correlations
371 between the rainfall and fecal pollution of coastal waters has also been previously reported
372 (Coulliette et al. 2009).

373 For a similar set of genes, the incidence frequencies observed in the current study before the
374 onset of excavation were similar to those reported in our previous study (Barkovskii et al. 2015).
375 After two days of dredging cumulative incidences frequencies reached 18 in the water column
376 and 19 in sediments of the same stations, and never diminished except during a period of
377 unusually low rainfall. We attributed this sharp increase to the impact of dredging. To the
378 extent of our knowledge, the relationship between landward dredging and the incidence
379 frequency of biological contaminants in coastal waters is reported here for the first time.

380 One of the genes, which concentration increased during the dredging, *ipaH*, encodes the
381 *Shigella* spp. E3 ubiquitin ligase and is located on the main *Shigella* pathogenicity island

382 (Schroeder and Hilbi, 2008). This gene has also been found in *Salmonella* spp. and some other
383 bacterial pathogens (Ashida and Sasakawa, 2015). *EaeA* similarly increased during excavation,
384 and encodes the primary virulence factor intimin most commonly found among EHEC and
385 EPEC strains which facilitates their attachment and effacement to the intestinal epithelium
386 (Sharma et al. 1999).

387 *Shigella* spp. have been previously reported in Georgia waters, and in 2014 there were 1,069
388 *Shigella* spp. outbreaks in Georgia watersheds (CDC, 2015). Some correlations between the
389 *ipaH* gene concentrations and environmental conditions have also been observed at Great Lake
390 beaches (Oster et al. 2014). The IpaH family effectors have thus been suggested as versatile tools
391 for monitoring pathogenic bacteria in aquatic environments (Ashida and Sasakawa, 2015). Shiga
392 toxigenic *E. coli* have been also reported earlier as common pathogens in estuarine and brackish
393 waters, and in Australia 100% of those causing intestinal infections during the wet season were
394 shown to carry *eaeA* genes (Masters et al. 2011). In France, EPEC carrying the *eaeA* gene has
395 also been documented in shellfish-harvesting area and their watersheds (Balière et al. 2015). This
396 gene was previously observed in Oakdale Creek where oyster reefs are abundant (Barkovskii et
397 al. 2015).

398 The third gene in this group, *set1B*, encodes for *Shigella* enterotoxin 1, and was originally
399 described in *Shigella flexneri* and EAEC (Henderson et al. 1999). It has more recently also been
400 found in pathogenic *E.coli* such as EIEC and UPEC however not in other species of *Shigella*
401 (Niyogi et al. 2004; Soto et al. 2009; Telli et al. 2010). *Shigella flexneri* has been infrequently
402 reported for U.S. aquatic environments (Kramer et al. 1996) however our study has revealed an
403 unexpectedly high presence of *set1B* gene in the water column and sediments of Oakdale Creek.

We attribute this fact to the presence of EAEC, EIEC, UPEC, and possibly other pathogenic *E. coli* rather than *S. flexneri*.

Concentrations of *set1B* and the two other VGs in Oakdale Creek followed the dredging schedule. This is the first report of a correlation between excavation of roadside drainage ditches and the release of shiga toxigenic bacteria to coastal waters. Since both *Shigella* spp. and *Escherichia coli* belong to γ-proteobacteria, an increase in their concentrations during dredging could explain the changes observed in the Oakdale Creek bacterial communities. Interestingly, the dredging did not impact *stx1* bacterial carriers, or *Salmonella* spp. which concentrations in the water column and sediments did not increase after the onset of dredging. Similarly, there was no observed impact from dredging on concentrations of the fecal indicator bacteria *Enterococcus* spp. Sources of *Enterococcus* spp. to Oakdale Creek other than dredged materials can be implied. Due to their natural residence in sediments and on aquatic plants, these background concentrations could also be high enough to offset the impact of dredging (Byappanahalli et al. 2012). Interestingly, concentrations of Entero 1 and *stx1* in Oakdale Creek were similar to the concentration of the16S rRNA gene. The Entero 1 primer set used targets the 23S rRNA gene that usually correlates well to copy numbers of the 16S rRNA gene (Klappenbach et al. 2001), and *stx1* (as many other VGs) may present in extremely high copy numbers within the cell (Ichinohe et al. 2009), which explains the observed high concentrations of these genes.

Tetracycline resistance genes *tet*(B) and *tet*(C) have been previously reported as being well represented in waterborne *E. coli* isolates (Tao et al. 2010). In an earlier study it was reported that thirty-two percent of non-clinical Shiga-toxin producing *E. coli* contain one or more genes coding for tetracycline resistance (Mora et al. 2005), and thirty-one percent of non-clinical *E.*

427 *coli* isolates were reported as resistant to tetracycline with *tet*(B) being the most prominent
428 (Bryan et al. 2004). This current study followed *tet*(B) and *tet*(C) concentrations closely and
429 found that their carriers increased substantially after the onset of dredging. The former's
430 concentrations correlated closely to that of *Shigella* spp. and pathogenic *E. coli* as suggested by
431 the *ipaH* and *eaeA* genes throughout the study. This correlation suggests *Shigell*a spp. and
432 pathogenic *E. coli* as possible *tet*(B) carriers but more in depth research must be done to confirm
433 any definite links.

434 In contrast, *tetA*(P) was not detected in these isolates. The only reported host for this gene was
435 *Clostridium perfringens*, and no other hosts could be found in the literature (Sloan et al. 1994).
436 This gene was detected only once in Oakdale Creek during the previous study (Barkovskii et al.
437 2015) but has been earlier observed in the water column, sediments, and oysters from Doboy
438 Sound which is the receptacle for this creek (Barkovskii et al. 2010). The latter, and a low
439 presence of *tetA*(*P*) in roadside ditches suggested that Doboy Sound rather than dredged
440 materials was the source of this gene to Oakdale Creek.

441

442 **5. Conclusion**

443 This study revealed that excavation of roadside drainage ditches affects the bacterial
444 community structure of Oakdale Creek and was a significant source of biological contamination
445 to the creek. While maintenance dredging is necessary for proper functioning of theses ditches a
446 better approach to excavation and storage of dredged materials should be implemented to prevent
447 the negative impacts it has on coastal waters leading to undesired shifts in indigenous bacterial
448 community structure and increasing hazard to public and environmental health.

449 **Supporting Information:**

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459

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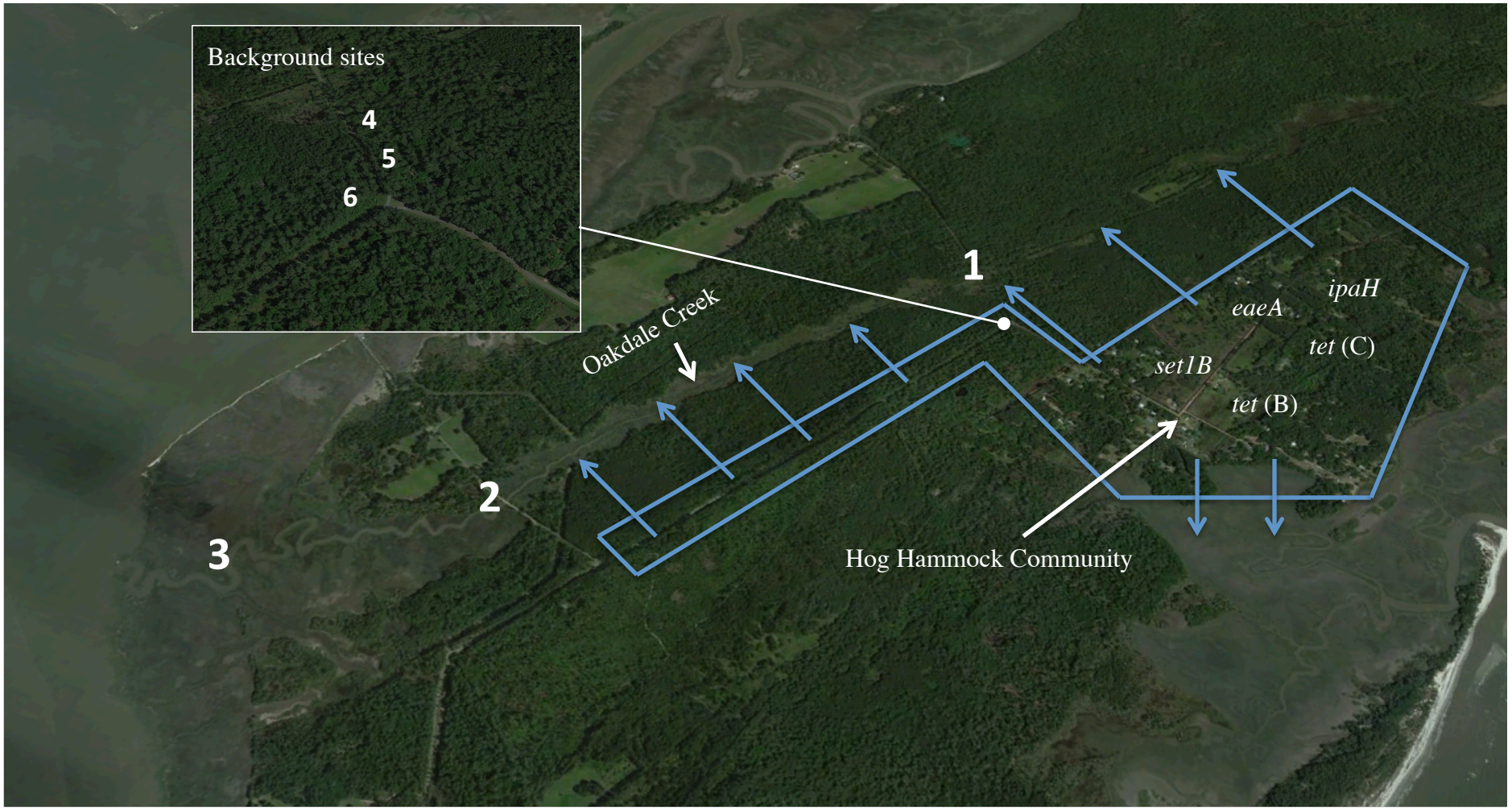
Background sites
4
5
6
1
2
3
Oakdale Creek
ipaH
eaeA
tet (C)
set1B
tet (B)
Hog Hammock Community