

Comparative Analysis of Structure and Composition of Bacterial Communities in Wastewater Final Effluents and Receiving Stream

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The spread of antibiotic resistant bacteria is a significant concern for public health, and it is well established that aquatic ecosystems constitute reservoirs of antibiotic-resistant bacteria. In this work, we investigated the impact of antibiotic resistant bacteria from final effluents of a sewage discharge on the selection of antibiotic resistance in natural bacterial communities in sites downstream the treatment plant.

Samples were collected from the final effluents of the wastewater treatment plant and from two sites along the receiving stream. Unique clones from 16S rRNA gene libraries constructed using metagenomic DNA were sequenced and used to derive the composition and structure of the three microbial communities. Furthermore, bacteria were isolated from the same samples using culture-dependent techniques, which allowed to link antibiotic resistance to particular bacterial species. Clone library data showed that the three communities were all dominated by Betaproteobacteria, which constituted approximately two thirds of the final effluent community. The diversity and abundance of other bacterial phylotypes varied across sites. Bacterial diversity included 9 phylotypes in the final effluent, 15 and 13 phylotypes respectively in the sites downstream the discharge. The analysis of the isolates from the final effluent showed the presence of *Bacillus*, *Enterobacter*, *Acinetobacter*, and *Staphylococcus* species, while samples collected downstream the plant were characterized by species belonging to the genera *Brevibacterium*, *Chryseobacterium*, *Aeromonas* and *Delftia*. All isolates resulted resistant to amoxicillin, and most displayed resistance to multiple antibiotics. In conclusion, differences were noted in the community composition across the sites of sampling. However, the culture-dependent approach showed similarities in resistance to antibiotics in all the isolates, suggesting the possibility of lateral transfer of resistance genes from wastewater effluents to freshwater bacteria.

Embryonic exposure to MTBE, ETBE and TAME in zebrafish leads to chemical specific lesions and altered expression of angiogenesis related genes

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Morphological and molecular endpoints were examined in the zebrafish following exposure to methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE) and tert-amyl methyl ether (TAME). MTBE has been previously shown to disrupt normal vascular development. We hypothesized that two structurally related chemicals, ETBE and TAME, would also disrupt microcapillary formation and that lesions would correlate to components of the vascular endothelial growth factor (VEGF) pathway. MTBE, ETBE

and TAME at 0.625-10mM have a similar capacity to disrupt angiogenesis with a dose dependent increase in lesion occurrence and severity. The primary lesions observed included pericardial edema, cranial hemorrhages and abnormal ISVs. Heart rate, cranial facial abnormalities and delayed development were observed with ETBE and TAME, but not MTBE. MTBE appears to target the developing vasculature, while ETBE and TAME target multiple organ systems. Stage specific exposures were carried out to determine the developmental period sensitive to MTBE microvascular disruption. Embryos were treated from 0-15, 0-24, 0-30 and 0-48 hours post fertilization (hpf) or 15-120, 24-120, 30-120, or 48-120hpf. Embryos treated until 15hpf or after 30hpf did not exhibit lesions, while embryos treated from 15-30hpf had a significant increase in vascular lesions ($p \leq 0.05$). Genes associated with vascular development were analyzed by qPCR at 24hpf to determine the affect of 5mM MTBE, ETBE and TAME. The data indicate that MTBE, ETBE and TAME differentially affect genes associated with the VEGF pathway. MTBE significantly decreased VEGF isoforms a and c (0.5 and 0.3 fold respectively), Wnt3a (0.4 fold), VE-Cadherin5 (0.3 fold) and β actin (0.4 fold). ETBE increased VEGFa (1.4 fold), while decreasing Hif1 α (0.5 fold), MMP9 (0.7 fold), VE-Cadherin5 (0.5 fold) and β actin (0.5 fold). TAME decreased VEGFc (0.6 fold), VEGFR2 (0.5 fold), MMP 2 and 9 (0.3 fold each), Wnt3a (0.4 fold), VE-Cadherin5 (0.5 fold) and β actin (0.3 fold). These data demonstrate that modification of MTBE by one methyl group to produce ETBE or TAME greatly affects toxicity to the developing embryo at both morphological and molecular levels. MTBE's targeted effect on developing vasculature is explained by the decrease of both VEGF isoforms during the critical period (15-30hpf), in contrast to ETBE and TAME, which each affect only one VEGF isoform, but have a greater effect on genes involved in general development (Wnt3a, MMPs, β actin).

The Potential Impact of the Asian Isopod, *Synidotea laticauda*, on the Delaware Bay, USA

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The non-indigenous isopod *Synidotea laticauda* was first documented in Delaware Bay in 1999 and recent data indicates extremely high seasonal abundances (Bushek and Boyd 2006). These observations suggest a potentially strong impact on the local ecosystem. To better understand the extent of any impact and the potential for further spread we need to know how *S. laticauda* is distributed in Delaware Bay and how its niche characteristics are likely to influence further establishment. Because its arrival to the Northeast coast is relatively recent, there is a general lack of scientific knowledge about the impact this isopod may have on local ecosystems. This study is the first to address issues relating to the presence of *S. laticauda* in Delaware Bay, and was conducted during its establishment rather than after the fact.

Specific Objectives and Hypotheses

1. Catalogue the distribution and abundance of *S. laticauda* with respect to environmental parameters of the isopod
2. Determine environmental tolerances to temperature and salinity as a mechanism for identifying potential limits to its aquatic distribution

3. Identify potential food resources for *S. laticauda* in Delaware Bay
4. Identify potential predators of *S. laticauda* in Delaware Bay

Synidotea laticauda was documented along portions of both the New Jersey and Delaware coastlines of Delaware Bay. However, they were only present in areas where the salinity was between 2 and 20 psu and were generally associated with anthropogenic structures, particularly marinas. Isopods were not observed along the Atlantic coast of New Jersey. At the present time it is unlikely that the northern range of *S. laticauda* in the bay will expand into freshwater portions of the estuary. Temperature-salinity challenges found that isopods died quickly in the freshwater treatments. However, isopods were able to survive in salinities of 30 and 35 psu. This tolerance may allow isopods to expand their range farther south into portions of the bay where higher salinities are prevalent. Temperature-salinity trials also found that *S. laticauda* were capable of surviving in water temperatures typical for Delaware Bay. Although lethargic, isopods experienced very little mortality at 5°C; however, high mortality (> 65 %) was experienced above 30°C. The normal upper temperature limit for Delaware Bay is 28°C (Sharp 1988) and appears to be close to upper limit for this isopod, but is not likely to be limiting, although isopods trapped in tidal pools and shallow waters during summer may not survive the increased temperatures these areas experience.

Decreased reproductive capacity of killifish (*Fundulus heteroclitus*) from Newark Bay, NJ, was correlated with decreased estrogen sensitivity and vitellogenin production

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Previous work had demonstrated that female killifish (*Fundulus heteroclitus*) collected from the chemically impacted Newark Bay, NJ exhibited decreased gonadal weight, delayed gonadal development, and decreased hepatic vitellogenin (VTG) and gonadal aromatase expression. Our current hypothesis was that delayed oocyte development resulted in low fecundity and was due to decreased sensitivity of the VTG pathway to 17 β -estradiol (E2) in comparison to a reference population (Tuckerton, NJ). Histological analysis of female gonads showed an increased percentage of pre-vitellogenic follicles (43% at Tuckerton, 66% at Newark Bay) and a decreased percentage at the mature stage (25% at Tuckerton, 4% at Newark Bay). These results demonstrated that while many primary oocytes formed in Newark Bay females, few developed beyond the stage requiring VTG. A fecundity study was therefore aimed to directly measure the reproductive capacity of Newark Bay females. Killifish were strip spawned at the peak of reproduction and Newark Bay females produced fewer mature eggs (11 embryos/female) compared to Tuckerton (140 embryos/female). Average embryo weight and yolk volume from Newark Bay were significantly decreased by 15% and 25%, respectively, indicating impaired yolk formation. This was likely due to significantly lower levels of VTG expression and as a result of this we investigated the sensitivity of the VTG pathway to E2. Adult killifish were injected with graded doses of E2 and both genders from Newark Bay expressed circulating VTG protein at significantly lower levels compared to Tuckerton killifish. Newark Bay males had concentrations (0.01 to 10 ng E2/g) where VTG levels were significantly less responsive (1-53% of Tuckerton response). Newark

Bay females had significantly lower levels of VTG induction at concentrations of 0.01, 0.1, 1.0 and 100 ng E2/g (11, 21, 39 and 75% of Tuckerton response, respectively). This right-shifted dose-response curve for VTG induction indicates a decreased responsiveness to E2 and impaired estrogen signaling. Doses at which the Newark Bay killifish have a significantly lower VTG expression are physiologically relevant to levels during female reproduction (0.2-2 ng E2/mL plasma). Therefore, we conclude that the decreased sensitivity of the vitellogenin pathway to E2 results in levels of circulating vitellogenin that are lower than normal in the Newark Bay female population and is the likely cause of delayed follicular development and low fecundity.

Interspecific variation in behavioral aversion of sympatric temperate zone amphibians to road deicers

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Contamination of vernal pools and other bodies of water by winter road deicers has been shown to impact larval amphibian survival, but adult responses to deicer contamination are less well-known. We tested adults of four sympatric amphibian species from the northeastern United States using choice trials to determine whether they showed aversion to road deicers (primarily NaCl, but also CaCl₂ and other alternatives), and if so, at what threshold concentration. Individuals of two obligate vernal pool-breeding species, wood frogs (*Lithobates sylvaticus*) and spotted salamanders (*Ambystoma maculatum*), and two non-obligate vernal pool breeders, green frogs (*Lithobates clamitans*) and eastern newts (*Notophthalmus viridescens*), were placed in a test chamber containing aged tap water on one side and a test solution on the other. Location and behaviors were recorded during a 10-minute trial. Each animal was tested at several concentrations (0 to 500 mM) of NaCl or an alternative deicer in a repeated measures design, with at least three days of recovery between trials. EC₅₀ values (threshold concentration at which half of the animals showed aversion, defined as spending less than 5 min in the test solution) were calculated from logistic curve fits. Species differences in threshold concentrations were observed; in particular, wood frogs showed no aversion to any NaCl concentration tested. Animals showed greater aversion to other cations (CaCl₂ and MgCl₂ solutions) than to sodium. Species with higher behavioral thresholds may attempt to breed in habitats unsuitable for successful egg and larval development. These results will help predict future impacts of increased habitat salinity on amphibian communities.

Potential Role for Bacteria in Arsenic Release to Groundwater

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There are both anthropogenic and geological sources of arsenic to the environment of New Jersey [1,4,6]. The shale bedrock of the Lockatong and Passaic Formations contains arsenic at concentrations up to 240 mg/kg, and groundwater from these formations contains arsenic levels of up to 215 µg/L [6]. Deep sediments within the Coastal Plain have average arsenic concentrations of 15-23 mg/kg [2,3]

We hypothesize that microbes play an active role in the mobilization of arsenic from bedrock and subsurface sediments. We have examined the potential impact of microbial activity on the mobilization of arsenic from subsurface sediments into the groundwater at a site on Crosswicks Creek in southern New Jersey. Site bed sediment contains arsenic at 25 mg/kg. Groundwater beneath the streambed contains arsenic at concentrations of 60 µg/L, while the site surface water has an arsenic concentration of 0.55 µg/L. DNA extracted from site sediment and groundwater was used to characterize the microbial community of the site. The 16s small subunit rRNA gene was amplified, cloned and sequenced to determine the phylogenetic relationships and diversity of the site microbial community. The arsenic respiratory reductase gene, *arrA*, was used as a biomarker for arsenic reducing bacteria [5]. The *arrA* gene was successfully amplified from groundwater, indicating the presence of arsenic reducing microorganisms. Microcosms developed from site sediment and groundwater show active microbial reduction of arsenate. Additionally, the *arrA* gene was amplified from these microcosms, further supporting the presence of arsenic reducing microorganisms that facilitate arsenic release.

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Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersey Pinelands

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The New Jersey Pinelands is an extensive pine forest that comprises the largest wilderness on the east coast of the United States. Small isolated wetlands 20 - 100 m diameter are present in persistent canopy openings throughout Pinelands region that may be related to periglacial conditions found in New Jersey during the Wisconsin glaciation. Improved understanding the formation of these wetlands may have implications for improving our knowledge of the paleoclimate and for understanding water movement through soils in the pinelands. Though several previous studies have attempted to explain their origin, the results of previous investigations were inconclusive and this vegetation patterning is not well understood. For this study, my hypothesis was that variations in the subsurface geology control vegetation patterning, and thus explain the presence of the wetlands. The objectives were to use near-surface geophysical methods such as ground penetrating radar and electromagnetic induction methods along with direct sampling to identify geologic anomalies that control these otherwise unexplained canopy openings and relate the geophysical results to regional water resource models. Results indicate that layers of clay are present below the wetlands that likely retard water infiltration. The spatial extent of three of these layers has been estimated using geophysical methods.

Green Remediation of Tetracyclines in Soil-Water Systems

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Veterinary antibiotics are used in large amounts in concentrated animal feeding operations (CAFOs) to treat disease and protect the health of animals, and as feed supplements to improve growth rate, thereby allowing animals to be brought to market much faster and at lower cost. VAs and other compounds of wastewater origin have been observed throughout the U.S. including New Jersey in surface waters mainly impacted by urban activities. The presence of VAs in aquatic and terrestrial environments is of concern because even at ng/L levels, these molecules are biologically active and can affect critical development stages and endocrine systems of aquatic and terrestrial organisms. Among the VAs commonly used in the livestock industry is the tetracycline group of antibiotics (TCs) (tetracycline (TTC), oxytetracycline (OTC), and chlorotetracycline (CTC)), which ranks second in production and usage worldwide. Pharmacokinetic literature shows that absorption of TCs in the gut of animals is incomplete, and substantial amounts (70-80%) are excreted in urine and feces. In the reported batch sorption study, we examined the effect of solution chemistry (pH (3-11), ionic strength (0.01-0.5M KCl), sorbate: sorbent ratio (SSR) (25-200 g L⁻¹), and reaction kinetics (0.16-96 h) on oxytetracycline (OTC) sorption by a waste byproduct of the drinking-water treatment process, namely, aluminum-based drinking-water treatment residuals (Al-WTR) as a function of initial OTC concentration (0.05-2.0 mM OTC L⁻¹) under aerobic conditions. The effect of a competing ligand, phosphate on OTC sorption was also evaluated. Degradation of OTC was investigated using solid-phase extraction and liquid chromatography–tandem mass spectrometry (LC-MS). Sorption of OTC on Al-WTR increased with increasing pH up to pH 7, above which it started decreasing in the range of 8 to 11. A concentration of 50 g L⁻¹ was deemed as optimum SSR for Al-WTR where more than 95% of the OTC was sorbed and equilibrium was reached in 2 h regardless of the initial OTC concentration. The reaction kinetics data best fit second order model ($r^2=0.99$). Sorption isotherms were plotted to evaluate the effect of ionic strength on OTC sorption. Best data fits were obtained using the linearized form of the Langmuir isotherm ($r^2=0.99$). No significant effect ($p>0.05$) of ionic strength on sorption of OTC was observed between 0.1 and 1 mM. However, at higher initial concentration of OTC (>1 and <2 mM), ionic strength dependence of OTC sorption was observed. Sorption of OTC by Al-WTR decreased in the presence of P(V), also exhibiting strong pH dependence. The LC-MS data showed the presence of 4-epioxytetracycline, α -apo-oxytetracycline, and β -apo-oxytetracycline. The above compounds were only present in trace concentrations relative to the parent OTC. The current research is the first step in our endeavor to ultimately develop a “green” remediation technique to immobilize and stabilize TCs in manure and manure-amended soils. The research will also provide a cost-effective medium for TCs immobilization in wastewater treatment facilities.

Restored oyster reef habitat use by the American Eel (*Anguilla rostrata*) in the Lower Delaware Bay

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A preliminary small-scale oyster restoration project was funded in summer 2006 through a Rutgers University Research Council grant, and demonstrated the potential for creating oyster habitat in the intertidal zone of the lower Delaware Bay. Preliminary minnow trap sampling data showed an increase in macrofauna abundance associated with the shell bag reefs compared to adjacent control sand areas. Of the 27 total fish caught on the shell bag reefs, the American eel (*Anguilla rostrata*) accounts for 25% of this total, while no eels were caught on the adjacent sand areas. Interestingly, the total length of *A. rostrata* increased from 35cm to 55 cm during the June through October sampling season. The preliminary catch and length data from the Cape Shore reefs reported above corresponds with the predicted migration time of *A. rostrata*. These findings imply that oyster reef habitat use by American eels in the lower Delaware Bay is important for successful migrations.

The present study was designed to test the hypothesis that yellow phase American eels, *Anguilla rostrata*, utilize oyster reefs and oyster aquaculture racks in the intertidal zone of lower Delaware Bay during their migration season. The objectives were as follows: 1) Provide novel documentation on the ecological importance of restored oyster beds as habitat for yellow phase *Anguilla rostrata* in the lower Delaware Bay, 2) Determine abundance and length-weight relationship of *A. rostrata* associated with shell restoration and aquaculture sites, 3) Determine the relationship between period of maximum abundance of *A. rostrata* and their migration season, and 4) Determine if *A. rostrata* are resident or transient species associated with restoration sites and aquaculture racks through mark-recapture efforts.

Two pathways for the stimulation of mercury methylation by syntrophic interactions

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In methylmercury-contaminated freshwater ecosystems where sulfate reducing bacteria (SRB) are the dominant Hg methylators, sulfate is a key limiting factor for methylation. Under sulfate limitation, syntrophic associations stimulate the growth of both methanogens and SRB, suggesting that this association may also enhance microbial Hg methylation. This possibility was supported by the detection of 16S rRNA and *dsr* genes that were most similar to those of *Syntrophobacter* spp. in *Sphagnum* moss enrichments which stimulated Hg methylation. We tested the role of syntrophy in Hg methylation by setting up incubations of propionate-utilizing *S. wolinii*, *S. sulfatireducens*, and *S. fumaroxidans* with *Methanospirillum hungatei* or with Hg methylating SRB, strains *Desulfovibrio desulfuricans* ND 132 and *Desulfovibrio africanus* DSM 2603 which cannot grow with propionate. *S. wolinii* had low level of Hg methylation activity (0.29%/day) when grown with propionate (15.6 mM) and sulfate

(19.7 mM) while the other two *Syntrophobacter* spp. did not methylate Hg. This activity was also demonstrated (0.20 %/day) when *S. wolinii* was grown with *M. hungatei* in cocultures in the absence of sulfate. When strains ND132 and DSM 2603 were grown with *M. hungatii* in a sulfate-free lactate medium, methylation was stimulated by 2 to 4 fold (3.5 %/day) and 7 to 24 fold (4.2 %/day) after four days of incubation, respectively, as compared to their activity without the methanogen. Thus, the syntrophy of *M. hungatei* with a weak Hg methylator (*D. africanus*) more significantly enhanced methylation as compared with a strong methylator (ND 132). Surprisingly, significantly higher potential methylation rates after four days of incubation were observed in the cocultures of *S. sulfatireducens* DSM 16706 with strains ND 132 (5.1 %/day) and DSM 2603 (0.4 %/day) in a propionate (15.6 mM) medium with 3.94 mM sulfate in comparison with rates of the *Desulfovibrio* sp. growing alone. Cell counts of the methylating strains in co-cultures with strain DSM 16706, as determined by flow cytometry cell sorting, increased (from 5.4×10^5 to 2.7×10^6 cells ml⁻¹ for ND 132, and from 7.8×10^5 to 2.6×10^6 cells ml⁻¹ for DSM 2603) and sulfate concentrations decreased (from 4.0 to 1.9 mM for ND 132, and 4.0 to 2.6 mM for DSM 2603) while biomass and sulfate concentrations of the methylating strains growing alone remained unchanged in absence of the syntroph. The results suggest that the *S. sulfatireducens* by oxidizing propionate provided hydrogen and carbon source essential for growth of the methylating *Desulfovibrio* spp. With increasing sulfate in propionate/sulfate media from 0.39 to 19.71 mM, methylation (%) of cocultures of *S. sulfatireducens* with strain ND132 decreased while that with strain DSM2603 increased even though methylation by both of *Desulfovibrio* spp. was enhanced in the cocultures in comparison with the two strains growing alone. Altogether the data suggest that Hg methylation by SRB may be stimulated by syntrophy with methanogens in low sulfate environments and by syntrophy with *Syntrophobacter* spp. in environments with limited availability of oxidizable organic matter.