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Use of N stable isotope and microbial analyses to define wastewater influence in Mobile Bay, AL

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Abstract

We assessed short-term ecological and potential human health effects of wastewater treatment plant (WTP) effluent by measuring δ^{15}N‰ and microbial concentrations in oysters and suspended particulate matter (SPM). We also tested male-specific bacteriophage (MSB) as an alternative to fecal coliforms, to assess potential influence of wastewater contamination on shellfish. WTP effluent did not affect oyster growth or survival, but SPM and oysters acquired wastewater-specific δ^{15}N‰. δ^{15}N values were depleted near the WTP, typical of low-level processed wastewater. Fecal coliform and MSB concentrations were higher in samples taken closest to the WTP, and MSB values were significantly correlated with δ^{15}N‰ in oyster tissues. Overall, oysters demonstrated relatively rapid integration and accumulation of wastewater-specific δ^{15}N‰ and indicator microorganisms compared to water samples. These data suggest oysters were superior sentinels compared to water, and MSB was a more reliable indicator of wastewater influence on shellfish than fecal coliforms.

Keywords: Fecal coliforms; Male-specific bacteriophage; MSB; Eastern oysters; Virus; Bacteria; Growth

1. Introduction

Wastewater treatment plants (WTP) and related sewage overflows account for more than 30% of all shellfish area closures in the US and are a primary source of viral and bacterial pollution in estuarine environments (Alexander, 1998; Calci et al., 1998; Shieh et al., 2003). Shellfishing areas near WTPs are typically closed to fishing because of increased concentrations of fecal coliform bacteria that may indicate human health risk (Calci et al., 1998; Burkhardt et al., 2000; US Food and Drug Administration, 2005). Despite these attempts to minimize harvest of wastewater-contaminated shellfish, millions of shellfish-borne viral illnesses are estimated to occur each year in the US (Mead et al., 1999; Butt and Sanders, 2004). These data and other recent studies suggest fecal coliforms are not reliable indicator organisms of sanitary quality (LaBelle et al., 1980; Griffin et al., 2001). Hence, some shellfish area closures may be inaccurate, not sufficiently protecting public health or unnecessarily restricting harvest by watermen (LaBelle et al., 1980; Rippey, 1994; Alexander, 1998; Griffin et al., 2001).

Recent studies have investigated the effectiveness of a new suite of viral pathogen indicator organisms, male-specific bacteriophages (MSB) (Doré and Lees, 1995; Lucena et al., 1996; Griffin et al., 2001). MSB has emerged as a promising indicator organism, presumably because it has similar physiochemical properties to human enteric viruses, hepatitis A virus and norovirus, which are of greatest concern to shellfish consumers (Doré and Lees, 1995; Doré et al., 2003; Calci et al., 1998; Burkhardt et al., 2000). In contrast, the mode of infection, survival, and sequestration of bacterial indicators, such as fecal coliforms, in estuarine

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waters and shellfish tissues are quite different from the viruses of concern (Burkhardt et al., 2000; Doré and Lees, 1995). Hence, MSB may prove more reliable than fecal coliforms as an indicator to better assess the risk of wastewater-borne viral contamination of shellfish.

Wastewater treatment plant effluents are a source of nutrients, including nitrogen (N), which has prompted eutrophication and alteration of marine ecosystems around the world (Bowen and Valiela, 2001; Khan and Ansari, 2005). Increased N loads from WTPs can increase shellfish growth by increasing production of algae that shellfish feed upon (Granéli and Sundbäck, 1985; Carmichael et al., 2004a,b), but over-enrichment may reduce shellfish survival through increased hypoxia (Cloern, 2001; Gray et al., 2002). The combination of N enrichment and low dissolved oxygen (DO) may affect microbial growth, further influencing microbial contamination of shellfish (Burkhardt and Calci, 2000; De Zwann and Babarro, 2001; Babarro and de Zwaan, 2002). To better define wastewater-related effects on ecosystem and human health, we must be able to clearly link effects on fisheries and pathogen concentrations to wastewater sources using biologically meaningful measures (Peterson et al., 1994; Alexander, 1998; NSTC, 2007).

Stable isotope analysis (SIA) is a powerful tool capable of tracing material through ecosystems and trophic webs (Peterson and Fry, 1987; Fry, 2006). Use of SIA to trace the flow of wastewater-derived N through watersheds and into the tissues of organisms, including shellfish, is well established (McClelland et al., 1997; Carmichael et al., 2004a,b; Tucker et al., 1999; Savage, 2005; Piola et al., 2005; Martinetto et al., 2006). These linkages are possible because N stable isotope ratios are fractionated with biological and physical processing. Hence, N from raw or directly discharged sewage is relatively light compared to N conveyed through groundwater from septic systems or adjacent WTPs (Tucker et al., 1999; Carmichael et al., 2004a; Savage, 2005). Accordingly, the $\delta^{15}N$ values of producers and consumers in receiving estuaries shift, with appropriate fractionation, to reflect the influence of a given wastewater source (McClelland et al., 1997; Carmichael et al., 2004a; Savage, 2005).

In this study, we assessed potential short-term ecological and human health effects of WTP effluent in Mobile Bay, Alabama, relative to distance from a major WTP. We defined ecological effects by measuring growth and survival of transplanted sentinel commercially important bivalves (*Crassostrea virginica*) and comparing these data to dissolved inorganic N and chlorophyll *a* (chl *a*) concentrations, along with changes in DO, temperature, and salinity in water samples at each site. We defined the spatial extent of wastewater influence across study sites by measuring N stable isotope ratios in oysters and SPM at each site. This approach, in turn, allowed us to link biological changes in oysters and estuarine attributes to wastewater-derived N. We then compared concentrations of fecal coliforms and MSB to N stable isotope ratios to determine whether these indicators were correlated with wastewater exposure.

2. Methods

2.1. Field transplants

We transplanted hatchery-reared oysters (50–70 mm) at four sites distally located 0.07, 0.50, 2.18 and 5.68 km south of the Clifton C. Williams WTP outfall in Mobile Bay in Alabama (Fig. 1). Sampling locations were chosen to best capture variation in dilution due to hydrology and level

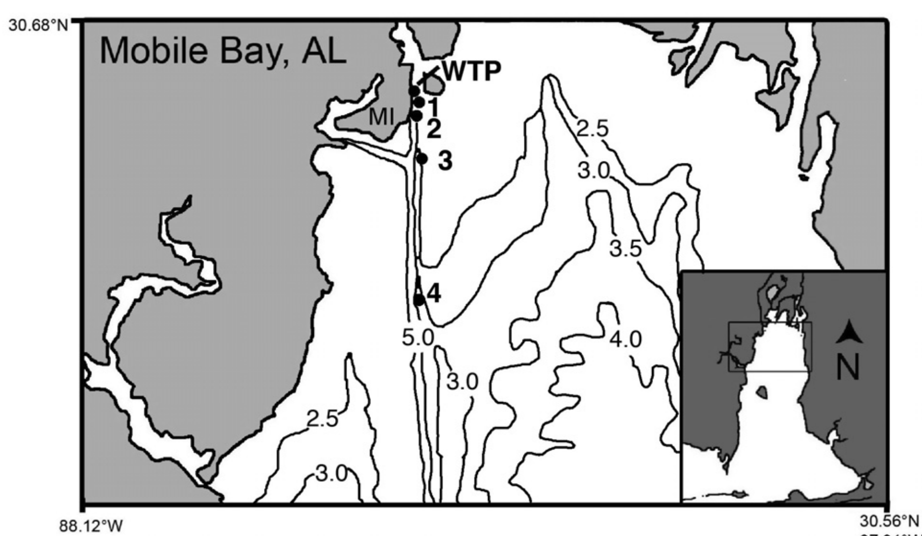

Fig. 1. Sampling sites at four locations in Mobile Bay, AL, relative to the effluent discharge site (WTP) from the Clifton C. Williams Wastewater Treatment Plant on McDuffie Island (MI). Sites 1, 2, 3, and 4 were located 0.07, 0.50, 2.18 and 5.68 km from the outfall, respectively. Contours show mean water depth in meters (Chen et al., 2005).