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Stimulation of fecal bacteria in ambient waters by experimental inputs of organic and inorganic phosphorus

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ABSTRACT

Fecal microbial pollution of recreational and shellfishing waters is a major human health and economic issue. Microbial pollution sourced from stormwater runoff is especially widespread, and strongly associated with urbanization. However, non-point source nutrient pollution is also problematic, and may come from sources different from fecal-derived pollution (i.e. fertilization of farm fields, lawns and gardens, and ornamental urban areas). Fecal bacteria require nutrients; thus the impact of such nutrient loading on survival and abundance of fecal coliform bacteria in ambient waters was experimentally investigated in a constructed wetland in coastal North Carolina, USA. A series of nutrient-addition bioassays testing impacts of inorganic and organic nitrogen and phosphorus demonstrated that additions of neither organic nor inorganic nitrogen stimulated fecal coliform bacteria. However, phosphorus additions provided significant stimulation of fecal coliform growth at times; on other occasions such additions did not. Dilution bioassays combined with nutrient additions were subsequently devised to assess potential impacts of microzooplankton grazing on the target fecal bacteria populations. Results demonstrated grazing to be a significant bacterial reduction factor in 63% of tests, potentially obscuring nutrient effects. Thus, combining dilution experiments with nutrient addition bioassays yielded simultaneous information on microzooplankton grazing rates on fecal bacteria, fecal bacterial growth rates, and nutrient limitation. Overall, when tested against a non-amended control, additions of either organic or inorganic phosphorus significantly stimulated fecal coliform bacterial growth on 50% of occasions tested, with organic phosphorus generally providing greater stimulation. The finding of significant phosphorus stimulation of fecal bacteria indicates that extraneous nutrient loading can, at times, augment the impacts of fecal microbial pollution of shellfishing and human contact waters.

1. Introduction

Coastal areas are highly important ecologically and economically due to their biodiversity, critical biotic habitats, human recreational opportunities and seafood production. These attributes also make coastal areas attractive for human development. Urbanization has led to decreased water quality in coastal regions due to ecosystem impacts from nutrient

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loading to waterways, and human health issues from microbial pollution. Microbial pollution comes from inputs of fecal bacteria, viruses and protozoans; the fecal coliform bacteria count (FC) is the most commonly used indicator of microbial pathogen abundance for freshwater and shellfish standards.

Fecal coliform bacteria are a group of organisms originating in the intestinal tract of humans and other animals (Murr et al., 1995). *Esherichia*, usually *Escherichia coli*, comprises about 90% of the fecal coliforms. Fecal microbes enter ambient waterways through point and nonpoint sources. Point sources include industrial and municipal wastewater treatment plant effluents and failing septic systems (Chigbu et al., 2004; Cahoon et al., 2006; Lewis et al., 2007). Nonpoint sources include wildlife manure, pet manure in developed areas, and runoff from farm animal feedlots (Chigbu et al., 2004; Mallin et al., 2000; Whitlock et al., 2002; Mallin and Cahoon, 2003; Holland et al., 2004). Fecal coliform bacteria are not well adapted to the aquatic environment, and most bacteria die within hours to days of entering the aquatic environment, (Evison, 1988) although in cases of sewage spills or runoff large numbers may persist in sediments (Mallin et al., 2007; Fries et al., 2008). Decline of water column counts depends on several abiotic factors; in freshwater systems, temperature and solar radiation are the two of the most important factors responsible for the decline in bacteria in the water column (Murr et al., 1995).

Biotic factors that decrease fecal counts in water are predation by microzooplankton and competition (Chigbu et al., 2005). Microzooplankton communities include protozoa and small metazoa, and play a significant role in connecting the microbial food web to higher trophic levels. Due to their small size microzooplankton feed on smaller sized particles including bacteria, and make that biomass available to higher trophic levels (Landry and Hasset, 1982; Vaque et al., 1992). Field experiments in a New Zealand study showed that microzooplankton communities, mainly heterotrophic flagellates and ciliates, could exert a grazing pressure on bacterial communities (James et al., 1996). Bacterial growth rates were not lower than in other places, however, suggesting that other factors such as substrate availability may be an important controlling factor in bacterial growth rates (James et al., 1996). The role of microzooplankton grazing upon fecal bacteria has been understudied, but a few studies have suggested that grazing is a major means of fecal bacteria removal (Enzinger and Cooper, 1976; Menon et al., 2003).

Some other factors influencing bacterial survival include sedimentation, osmotic effects, and pH (Murr et al., 1995). Large quantities of fecal bacteria can settle into the sediments and remain viable for several weeks (Jeng et al., 2005; Fries et al., 2008); therefore disturbances that resuspend the sediments, such as storms or boat passage, can increase fecal coliform counts (Mallin et al., 2007; Toothman et al., 2009). Fecal coliforms can grow within the sediments, where they are sheltered from solar irradiance and predation and presumably find a nutrient-enhanced environment (Toothman et al., 2009).

Many of the tidal creek watersheds in the southeastern United States are sites of intense human development. Fecal coliform counts have been significantly and positively correlated to human population and urban/suburban development factors in tidal creek watersheds, primarily a result of loss of natural land cover and increased impervious surface leading to enhanced stormwater runoff (Mallin et al., 2000; Holland et al., 2004; Sanger et al., 2011). Loss of vegetation stemming from urbanization reduces the retention of nutrients (nitrogen (N) and phosphorus (P)) on riparian land and increases N and P loading to water bodies (Lewis et al., 2007; Sanger et al., 2011). Lawn, garden and agricultural fertilizers are a source of (non-sewage derived) nutrient loading to receiving water systems. Nutrients, especially nitrogen (N) and phosphorus (P), are biologically necessary for fecal bacteria survival (Kirchman, 1994). Increases in nutrient loading to streams can stimulate not only algal productivity but bacterial abundances, potentially including coliform bacteria sourced from leaking or overflowing sewers (McFeters and Stuart, 1972; Lewis et al., 2007). Lawn, garden and agricultural fertilizers increase stream P and N concentrations without adding fecal bacteria. Phosphate mining wastes would also increase P loading to aquatic systems without adding fecal bacteria.

Mallin et al. (2004) found that in blackwater streams and rivers natural field bacteria responded to P additions, suggesting a potential P limitation. Natural bacteria have a greater need for P as opposed to N, structurally as well as for ATP (Kirchman, 1994); experiments using non-fecal bacteria have shown stimulation from P additions but not N additions (Chrzanowski et al., 1995; Sundareshwar et al., 2003). Toothman et al. (2009) found that fecal bacteria in creek sediments are usually not N or P limited, but in areas where sediment P concentrations are low, experimental additions of P significantly stimulated fecal bacterial abundances. In a sewage effluent-enriched creek Surbeck et al. (2010) suggested a die-off threshold below 70 μg/L of soluble P for *E. coli* and enterococci. Therefore, P is predicted to increase the activity and biomass of bacteria, either directly or indirectly (Morris and Lewis, 1992). Though fecal coliform bacteria are not very well adapted for aquatic living, nutrient additions can increase the chance of survival and extend their survival time (Murr et al., 1995).

Due to the health hazards to humans, fecal bacteria are a major issue in recreational water systems as well as shellfishing areas, especially in drainages with high urbanization. The purpose of this study was to determine if the addition of nutrients, N and P, to water from field sites increased the survivability of the fecal coliform bacteria in fresh water samples. The hypotheses were:

- The addition of phosphorus will increase the survivability more than nitrogen, or phosphorus and nitrogen together in fresh water because of the high phosphorus requirements by bacteria.
- Organic nutrients will have more effect on survivability than inorganic nutrients because organic nutrients also provide a carbon substrate.

To accomplish these goals nutrient addition bioassays and dilution experiments were performed on water samples taken from systems impacted by human development and thus receiving fecal bacterial loading. Ancillary physical, chemical and biological data were collected concurrently with water collections to improve interpretation of the results.