



Effects of watershed densities of animal feeding operations on nutrient concentrations and estrogenic activity in agricultural streams

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ABSTRACT

Application of manures from animal feeding operations (AFOs) as fertilizer on agricultural land can introduce nutrients and hormones (e.g. estrogens) to streams. A landscape-scale study was conducted in the Shenandoah River watershed (Virginia, USA) in order to assess the relationship between densities of AFOs in watersheds of agricultural streams and in-stream nutrient concentrations and estrogenic activity. The effect of wastewater treatment plants (WWTPs) on nutrients and estrogenic activity was also evaluated. During periods of high and low flow, dissolved inorganic nitrogen (DIN) and orthophosphate (PO₄-P) concentrations were analyzed and estrogens/estrogenic compounds were extracted and quantified as 17β-estradiol equivalents (E2Eq) using a bio-luminescent yeast estrogen screen. Estrogenic activity was measurable in the majority of collected samples, and 20% had E2Eq concentrations > 1 ng/L. Relatively high concentrations of DIN (> 1000 µg/L) were also frequently detected. During all sampling periods, there were strong relationships between watershed densities of AFOs and in-stream concentrations of DIN ($R^2 = 0.56\text{--}0.81$) and E2Eq ($R^2 = 0.39\text{--}0.75$). Relationships between watershed densities of AFOs and PO₄-P were weaker, but were also significant ($R^2 = 0.27\text{--}0.57$). When combined with the effect of watershed AFO density, streams receiving WWTP effluent had higher concentrations of PO₄-P than streams without WWTP discharges, and PO₄-P was the only analyte with a consistent relationship to WWTPs. The results of this study suggest that as the watershed density of AFOs increases, there is a proportional increase in the potential for nonpoint source pollution of agricultural streams and their receiving waters by nutrients, particularly DIN, and compounds that can cause endocrine disruption in aquatic organisms.

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1. Introduction

Livestock wastes contain high concentrations of nutrients and steroid estrogens (Hanselman et al., 2003; Johnson et al., 2006; Mallin and Cahoon, 2003; USDA, 1992). These compounds may enter surface waters through runoff or leachate from agricultural land that has received applications of manure from animal feeding operations (AFOs) as fertilizer (Finlay-Moore et al., 2000; Kjaer et al., 2007; Matthiessen et al., 2006; Shore et al., 1995). Surface water contamination by nutrients and hormones in animal waste can also occur when grazing animals deposit waste into or directly adjacent to bodies of water (Kolodziej and Sedlak, 2007). The negative effects of excess nutrients in surface waters are well documented (e.g. Boesch et al., 2001). Several studies have shown that estrogens can disrupt endocrine system function of aquatic organisms at concentrations less than 10 ng/L (Young et al., 2004). In regions with high densities of AFOs, there is increasing concern that high rates of manure application on local agricultural

land will lead to eutrophication of surface waters and potential endocrine-related effects in aquatic biota (Kellog et al., 2000; Mallin and Cahoon, 2003; Yonkos et al., 2010).

Relationships between AFOs, nutrients, estrogens, and streams require further assessment in order to manage livestock waste effectively and protect the health of surface waters in agricultural landscapes. A recent study of Iowa rivers in watersheds receiving wastes from AFOs showed a strong relationship between nitrate concentrations and watershed densities of animal units within the AFOs (Weldon and Hornbuckle, 2006), but hormone concentrations were not evaluated. Most studies of estrogens or estrogenic activity in livestock wastes have focused on the potential for aquatic contamination, through measurement of concentrations in storage facilities (Hutchins et al., 2007; Raman et al., 2004) or concentrations in runoff and leachate from manure-treated fields (Finlay-Moore et al., 2000; Kjaer et al., 2007; Nichols et al., 1997). In-stream assessments have generally been conducted in headwater streams adjacent to individual feedlots or farms receiving waste applications (Matthiessen et al., 2006; Shore et al., 1995). These studies isolated the effects of animal wastes on concentrations of estrogens in streams, but did not assess the cumulative downstream effects of animal wastes that may occur in streams with larger drainage areas.

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The relationship between livestock production and water quality is of particular interest in the Shenandoah River watershed (Virginia, USA). The 7600 km² watershed is 39% agricultural land, which receives manure from approximately 1200 AFOs and 300 farms that maintain grazing beef cattle (VADCR, 2010; VADEQ, 2006). Seasonal fish kills have occurred in the Shenandoah River since 2004, and resident smallmouth bass have impaired immune function (Ripley et al., 2008) and a high proportion of males with intersex compared to other basins (Blazer et al., 2007). Because estrogenic compounds can induce intersex (Hahlbeck et al., 2004; Lange et al., 2009) and affect the immune function of fishes (Iwanowicz and Ottinger, 2009; Robertson et al., 2009), assessment of their presence in the watershed and relationship to land use is warranted. Nutrient concentrations are of concern due to risk of local eutrophication and potential effects on the health of aquatic organisms (Camargo et al., 2005; Guillet and Edwards, 2005; Johnson et al., 2010). The relationship between nutrient concentrations and land use has management implications outside of the Shenandoah River watershed, as the Shenandoah discharges into the Potomac River, a major tributary of Chesapeake Bay. In order to adequately protect and restore the health of Chesapeake Bay, estimated required reductions in nitrogen and phosphorous loadings for the Shenandoah and Potomac Rivers are 44% and 29%, respectively (Commonwealth of Virginia, 2005).

Comprehensive assessment of the potential effect of AFOs on concentrations of estrogens and nutrients in streams within agricultural landscapes requires consideration of additional sources of these compounds. The Shenandoah River watershed has 81 municipal wastewater treatment plants (WWTPs); the majority (71) are minor facilities discharging less than 1 million gal of effluent per day. Effluent from WWTPs can be a significant source of natural and synthetic estrogens from human excretion, and synthetic xenoestrogens from household and industrial use, to surface waters (Lagana et al., 2004; Muller et al., 2008; Petrovic et al., 2002; Ying et al., 2008). Depending on the level of treatment, WWTPs can also contribute significant nutrient loads to receiving waters, and reducing these loads is a significant component of the strategy to protect and restore the health of Chesapeake Bay (Commonwealth of Virginia, 2005).

The main objective of this study was to evaluate relationships between watershed densities of AFOs and concentrations of nutrients and estrogenic activity in streams within the larger Shenandoah River watershed. Increasing in-stream concentrations of nutrients and estrogenic activity were expected with increasing watershed densities of AFOs due to the potential for increased manure application in the local area. A secondary objective was to examine the effect of WWTPs in combination with AFOs on nutrients and estrogenic activity. The presence of WWTPs was expected to further increase concentrations of nutrients and estrogenic activity in streams. Estrogenic activity was selected over measurement of individual compounds in order to assess the overall potential for biological activity of estrogens and estrogenic compounds in stream water, and to evaluate the utility of screening techniques in large-scale water quality monitoring programs.

2. Methods

2.1. Selection and characterization of study sites

Land use in the Shenandoah River watershed was characterized using a geographic information system (ArcGis 9.3, ESRI, Redlands, CA). Delineated 12-digit, 6th level Hydrologic Unit Code (HUC 6) subwatersheds, 40–160 km² in size, were used to quantify all land uses within drainage areas of Shenandoah River tributaries. Locations and numbers of animal units for AFOs (poultry, dairy, and beef), farms maintaining grazing beef cattle, and locations and permit information for WWTPs were obtained from Virginia state agencies. In Virginia, AFOs are defined as facilities that confine animals for at least 45 days and preclude the growth of vegetation, while concentrated AFOs maintain > 300 animals

(cattle or swine). In the Shenandoah River watershed, the 680 poultry AFOs maintain 10,000–200,000 birds and all hold Virginia Pollution Abatement (VPA) permits. Only 21 of 430 dairy AFOs and three of 110 beef AFOs are concentrated operations and require VPA permits. For this study, the 278 farms maintaining grazing beef cattle were included in beef AFO calculations, because of a similar risk of contamination of surface water from manure of grazing animals (Kolodziej and Sedlak, 2007; Soupir et al., 2006). Many pastures in the area have noticeably high concentrations of feces, particularly during winter/early spring, and cattle are allowed access to streams for water during the summer in many areas (personal observation).

Eighteen sampling sites were selected to represent a gradient of influence from AFOs combined with presence/absence of WWTP discharges (Fig. 1). Sampling sites were located in 14 Shenandoah River tributaries; four tributaries drained multiple subwatersheds and an upstream sampling site was located in the primary subwatershed as well as a downstream site draining multiple subwatersheds. Eight sampling sites could not be located near the outlet of the delineated HUC 6 subwatershed due to limited tributary access, and the watershed area was recalculated using U.S. Geological Survey Digital Elevation Model (DEM) data (30 m resolution). The new delineation was used to quantify upstream land use variables. The number of each type of AFO and each type of animal in each watershed was converted to a density (number/1000 acres) and the total watershed density of AFOs (all types) was calculated for each sampling site. For poultry, dairy, and beef AFOs, the watershed density of animals was strongly correlated with the watershed density of each type of operation ($r = 0.96, 0.99$, and 0.88 , respectively), so only AFO density was used in data analysis. For each watershed with a WWTP discharge, the WWTP permit information was used to calculate the total permitted effluent discharge (in millions of gallons per day; MGD). Pasture/hay and cropland are primary application sites for manure from AFOs. Therefore, land cover data for the Shenandoah River watershed (30 m resolution) were obtained from the 2001 National Land Cover Database (Homer et al., 2007). Reclassification and areal tabulation were used to quantify the percentage of forest, developed land, pasture/hay, and cultivated crops upstream of each sampling site. Percentages of all other land use types (i.e. open water, barren land, etc.) were negligible (<1%) in each watershed of interest. For each study site, a total of nine land use variables

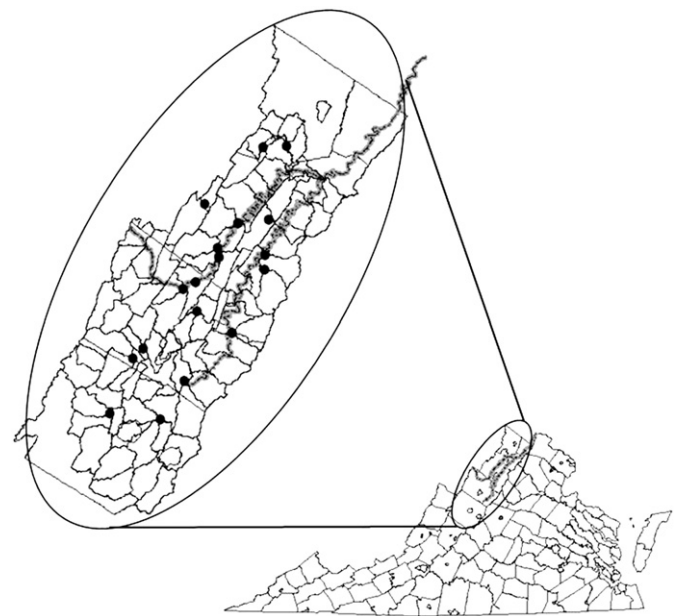


Fig. 1. Locations of the 18 study sites within the Shenandoah River watershed. The enlargement shows the 78 12-digit hydrologic unit code (HUC) subwatersheds of the Shenandoah River (indicated in grey) and the counties included in the entire watershed, relative to the state of Virginia.

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