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Research Paper

Steroid estrogens and estrogenic activity are ubiquitous in dairy farm watersheds regardless of effluent management practices

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ABSTRACT

Steroid estrogens contamination has been linked to adverse effects on exposed aquatic biota. Steroid estrogens are excreted by all mammals and are found in most agricultural wastes including dairy manure and dairy shed effluent (DSE). Some previous studies have demonstrated elevated levels of free and conjugated estrogenic steroids in DSE and this source has increased as New Zealand has experienced rapid expansion and intensification of dairy farming. This research used an approach incorporating analytical chemistry and bioassays to evaluate the levels of estrogenic activity in environmental samples from representative dairy watersheds with differing DSE management practices: either land-applied or discharged to water. The results demonstrated that estrogenic activity and steroid estrogens were prevalent in the waterways within all the studied dairy watersheds. Estrone was the predominant steroid measured in watershed waters because of its presence in dairy cow wastes and as a degradate of the main dairy cow estrogen, 17α-estradiol. Measurable estrogenic activity (17β-estradiol equivalents, EEq) was found at low levels in 83% of the stream samples (highest 1.44 ng L^{-1} EEq) and 75% of the groundwater samples ($\leq$0.15 ng L^{-1} EEq). While estrogenic activity was generally $<$ 1 ng L^{-1}, one (of 10) stream with measurable estrone, 17α- and 17ß-estradiol had activity of 1.4 ng L^{-1}, a level potentially harmful to aquatic biota. Comparable steroid estrogen concentrations and estrogenic activity were found whether DSE was spray irrigated on farm paddocks or directly discharged into waterways. This suggests that direct access of cattle to streams, the direct input of DSE into waterways and runoff from land application all require more intervention and effective management.

1. Introduction

Contamination of streams and rivers with steroid estrogens at very low but biologically active concentrations, has been linked to adverse effects on the endocrine systems of exposed biota, particularly freshwater fish (Sumpter, 2005). This was first demonstrated in streams downstream of wastewater treatment plants (Purdom et al., 1994; Desbrow et al., 1998). However, steroid estrogens are excreted by all mammals including livestock and can be present in agricultural manures and wastewaters at concentrations of 40–7000 ng L^{-1} (Raman et al., 2004; Sarmah et al., 2006; Gadd et al., 2010b; Noguera-Oviedo and Aga, 2016). These concentrations are up to 100-fold higher than in municipal wastewater effluents (1–80 ng L^{-1} (Luo et al., 2014)) and 1000-fold higher than concentrations recommended to protect wildlife from endocrine disruption (Caldwell et al., 2012). Consequently, estrogenic steroids have been identified in streams and ponds receiving discharges of treated agricultural effluents (Kolodziej et al., 2004), in runoff from fields following land application of animal waste (Nichols et al., 1998; Finlay-Moore et al., 2000), in streams draining fields where livestock are grazed (Matthiessen et al., 2006; Kolodziej and Sedlak, 2007) and in groundwater within intensively farmed agricultural systems (Kolodziej et al., 2004). Estrogenic activity has been measured in many streams and rivers within agricultural areas (Vethaak et al., 2002; Stuer-Lauridsen et al., 2005; Matthiessen et al., 2006; Noguera-Oviedo and Aga, 2016) though Alvarez et al. (2013) reported only low concentrations in watersheds dominated by dairy farming.

In New Zealand, the number of dairy cattle increased by nearly a million (25% increase) from 1997/98 to 2007/08, and nearly tripled in

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the Canterbury region, growing from 275,000 to 755,000 heads (Dairy NZ, 2014). This expansion and intensification has led to increasing concern regarding the potential impacts of intensive dairy farming on the health of freshwater ecosystems (MacLeod and Moller, 2006). Our previous research had demonstrated the concentration of steroid estrogens and their conjugates varied between 40 and 2000 ng L^{-1} in dairy shed effluents (DSE: the urine, faeces and washdown water collecting during milking operations), their incomplete removal in the typical pond treatment processes applied in New Zealand, and their presence at < 15–1400 ng L^{-1} in effluents being discharged to waterways (Gadd et al., 2010a,b). Despite this, there has been no investigation of steroid estrogens and estrogenic activity in watersheds within New Zealand where dairy farming is the dominant agricultural land use and likely to be the greatest source of these potent contaminants. To address this absence of information we undertook an investigation using a combination of trace chemical analysis and the E-Screen bioassay to determine the concentration of steroid estrogens and estrogenic activity in waterways within intensive dairy farming watersheds in New Zealand.

In New Zealand dairy cattle graze outdoors on paddocks, with the bulk of their excreta deposited directly to land with no treatment, though this is distributed over the grazing paddocks. However, 10–20% of the excreta from dairy cows is collected at the dairy milking shed, and managed as DSE. At the time of this study different methods were employed between different geographic regions for the disposal of DSE. In the North Island Waikato region DSE was predominantly treated in oxidation ponds before discharge into nearby waterways. In the South Island Canterbury region DSE was collected in sumps (small concrete pits) from where it was directly applied to the surface of farm paddocks by spray irrigation. We hypothesised that the steroid estrogen concentrations would be higher in streams of the Waikato Region, due to direct discharges of treated DSE to waterways during baseflow. The aims of this study were to:

1) characterise the concentration of estrogenic steroids in streams and groundwater within New Zealand watersheds predominated by intensive dairying activities, and,
2) determine if the concentration of estrogenic steroids and estrogenic activity in waterways within intensive dairy farming watersheds could be related to DSE disposal and management practices.

2. Materials and methods

2.1. Sampling sites

2.1.1. Primary dairying watersheds

Samples were collected in four primary watersheds as detailed in Fig. 1. We selected watersheds with predominantly dairy land use but differing effluent disposal methods to investigate their effect on in-stream concentrations as summarised in Table 1.

We completed an initial assessment of the Toenepi Stream in the Waikato region, as the agricultural activity within this watershed was exclusively dairy farming and it was being studied by several different groups as a Best Practice Dairy Catchment (Wilcock et al., 2006). Samples were collected at two locations in the Toenepi stream, and at single sites within its two tributaries. These stream samples were collected in August 2006 (milking season start, late winter, mean temp. approx. 20 °C) and May 2007 (milking season end, late autumn, mean temp. approx. 22 °C). Our previous investigation of free estrogen concentrations in DSE at the start and end of the dairy cow milking season had demonstrated there was no significant seasonal changes on their concentration (Gadd et al., 2010b)

This initial assessment was followed by an assessment of 3 watersheds in Canterbury, in which the sole agricultural activity was dairy farming and effluent disposal was land application of DSE, but with differing water irrigation methods on the farms (Table 1). The Waikuku Stream watershed (no irrigation, 3 sampling sites) and Pahau River (sprinkler irrigation, 2 sites) samples were collected once during March and April 2007, with an additional sample collected from the Pahau River in March 2008. Dairy farms within the Waikakahi Stream watershed used a surface irrigation (flood irrigation) system known as border-dyke, where a network of irrigation canals border the farm paddocks and essentially saturate the soil within each paddock.

This irrigation method has higher potential than sprinkler irrigation to transport estrogens from effluents applied to land into the waterways via surface runoff.

Sampling in the Waikakahi watershed was conducted during late summer (March 2008), coinciding with the peak period of irrigation. Stream flows within this watershed are artificially raised during summer as the irrigation water is supplied from a neighbouring watershed and this method of irrigation has low water application efficiency. Samples were collected from three locations along the length of the Waikakahi Stream and from five groundwater wells located in paddocks within the border-dyke irrigation zones, where groundwater depths varied from 1.2–7.3 m below ground level.

2.1.2. Survey of 12 streams

Water samples were collected from single sites in 12 streams located within intensive dairying watersheds in the wider central Waikato Region (Fig. S1 provides details on the location of the sampling sites). These 12 streams were distributed region-wide and were selected on the basis they were in areas dominated by dairy farming activities. The sampled streams included those receiving point source discharges of treated DSE, and others with few or no discharges of DSE directly into water, but with numerous discharges of DSE to land within the watershed via spray irrigation. The stream water samples were obtained during summer base flow conditions in January 2008 when there was less dilution of estrogenic steroids in DSE discharged into waterways. For each of these streams, the number of discharges of DSE directly into water, and farm properties on which DSE was spray irrigated onto land in the watershed, was obtained from the local regulatory authority. While dairy farming was not the exclusive agricultural activity within the watersheds the sampled streams bisected it was the predominant agricultural land use within each watershed (details for each stream are provided in the Sup Table 1s).

2.2. Sample collection, filtration, and extraction

Stream water samples, obtained as grab samples, were collected from below the water surface as close to the middle of the stream as possible by wading into each stream. The collection of groundwater samples was initiated after ground water wells were purged with at least three well volumes (based on well dimensions, water depth and measuring the volume of water purged) and once conductivity and pH measurements had stabilised in extracted groundwater. Water samples were collected in solvent-rinsed 4 L amber glass bottles with Teflon-lined lids, immediately preserved by the addition of sulfuric acid (to pH ≤ 2) and transported on ice to the laboratory. Sample processing began immediately on return to the laboratory and sample extraction was completed within 36 h.

Four litres of stream or groundwater sample was filtered through glass fibre filters (GF/C, pore size 1.2 mm Whatman) then extracted (2 L each) for i) chemical analysis of steroid estrogens (estrone (E_1), 17α-estradiol and 17β-estradiol (E_2)), and ii) estrogenic activity using the E-Screen assay. Samples destined for chemical analysis were spiked with surrogate standards (100 ng each E_1-d_4, 17β-E_2-d_4, E_1-3S-d_4 and 17β-E_2-3S-d_4 except for spring 2006 sampling in the Toenepi watershed, 250 ng 17β-E_2-d_4 only). All samples were extracted using 500 mg 6 mL Oasis HLB solid-phase extraction cartridges (preconditioned with 10 mL of methanol followed by 10 mL of MQ water) at a flow rate of 5–10 mL min^{-1}. The extraction procedure resulted in a concentration factor of 20,000× for estrogenic steroids prior to