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Chemical, biological, and DNA markers for tracing slaughterhouse effluent

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ABSTRACT

Agricultural practices, if not managed correctly, can have a negative impact on receiving environments via waste disposal and discharge. In this study, a chicken slaughter facility on the rural outskirts of Sydney, Australia, has been identified as a possible source of persistent effluent discharge into a peri-urban catchment. Questions surrounding the facility's environmental management practices go back more than four decades. Despite there having never been a definitive determination of the facility's impact on local stream water quality, the New South Wales Environment Protection Authority (NSW EPA) has implemented numerous pollution reduction requirements to manage noise and water pollution at the slaughter facility. However, assessment of compliance remains complicated by potential additional sources of pollution in the catchment. To unravel this long-standing conundrum related to water pollution we apply a forensic, multiple lines of evidence approach to delineate the origin of the likely pollution source(s). Water samples collected between 2014 and 2016 from irrigation pipes and a watercourse exiting the slaughter facility had elevated concentrations of ammonia (max: 63,000 μg/L), nitrogen (max: 67,000 μg/L) and phosphorous (max: 39,000 μg/L), which were significantly higher than samples from adjacent streams that did not receive direct runoff from the facility. Arsenic, sometimes utilised in growth promoting compounds, was detected in water discharging from the facility up to ~ 4 times (max 3.84 μg/L) local background values (< 0.5 μg/L), with inorganic $As^{(\Sigma V+III)}$ being the dominant species. The spatial association of elevated water pollution to the facility could not unequivocally distinguish a source and consequently DNA analysis of a suspected pollution discharge event was undertaken. Analysis of catchment runoff from several local streams showed that only water sampled at the downstream boundary of the facility tested positive for chicken DNA, with traces of duck DNA being absent, which was a potential confounder given that wild ducks are present in the area. Further, PCR analysis showed that only the discharge water emanating from the slaughter facility tested positive for a generalized marker of anthropogenic pollution, the clinical class 1 integron-integrase gene. The environmental data collected over a three-year period demonstrates that the slaughter facility is indisputably the primary source of water-borne pollution in the catchment. Moreover, application of DNA and PCR for confirming pollution sources demonstrates its potential for application by regulators in fingerprinting pollution sources.

1. Introduction

When industrial and agricultural operations are situated alongside residential land, conflicts arise from the different needs and expectations of land users (Fowler and Shi, 2016, James and O'Neill, 2016, Pribadi and Pauleit, 2016, Wei et al., 2016). Animal husbandry and slaughter facilities are frequently the subject of community complaints and environmental investigations by regulatory authorities, with claims of pollution often vigorously rebutted (e.g. Bienkowski, 2015 and Heaney et al., 2015). Poultry processing facilities are frequently investigated as they produce significant volumes of contaminated waste water that can adversely impact the adjacent environment (Gan and Hu, 2016).

To implement more effective and costly pollution management, the primary hurdle is concurrence from industry that practices need to change. Typically, this requires sufficient proof that the target pollutants are linked to industrial or agricultural sources. In this regard, it can be difficult to establish definitive evidence, which is particularly important when major changes to a polluter's operations are required to bring it in line with licence arrangements or public expectations. This

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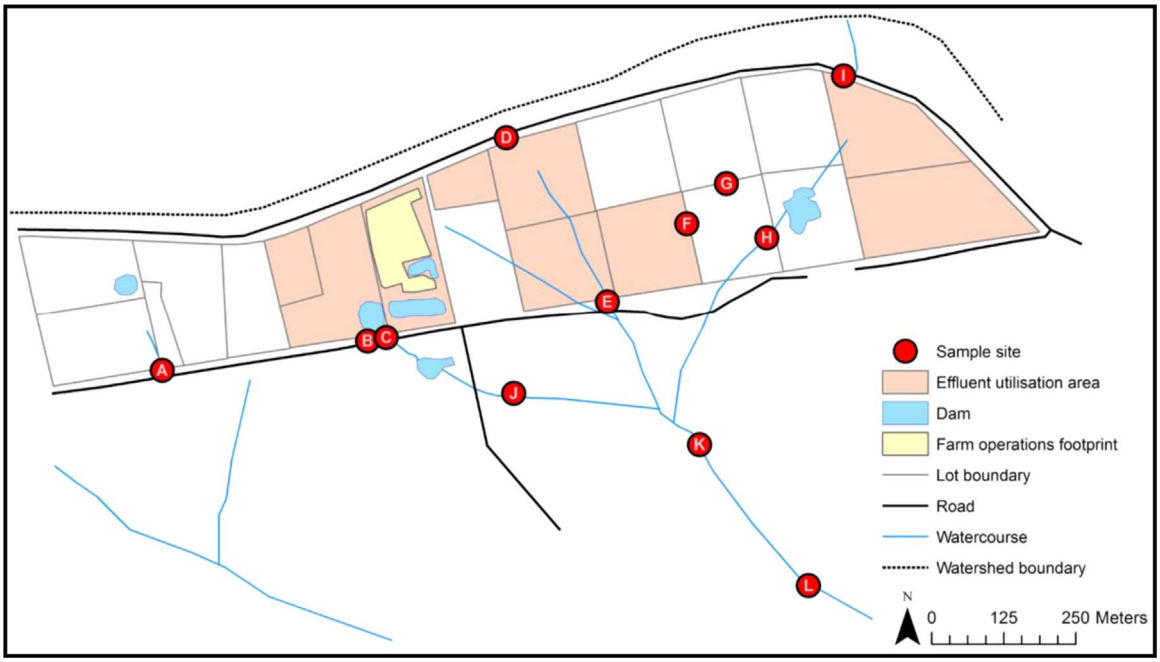

Fig. 1. Map of the study area located in the rural outskirts of northern Sydney illustrating sample sites and sample codes. Map based upon the 2016 NSW Department of Land and Property Information Globe software land parcel and lot boundary map data. Samples within the discharge catchment of the facility (sites B, C, E, F, G), sites that receive no water from the facility (A, D, H and I) and those that receive a mixed source of water including some water from the facility (J, K and L).

hurdle can be overcome in two main ways: (a) using a weight of evidence derived from a suite of biological, chemical and physical environmental measures and their spatial-temporal associations with respect to a possible polluter; (b) the production of evidentiary material to show an unequivocal causal link between indices of pollution and known activities at a site.

In addressing the challenges of identifying the source of pollution, this current study sets out the application of a forensic, multiple lines of evidence approach to identify the source of environmental pollution that has been previously linked, but never proven, to a livestock (chicken) slaughtering and processing facility (the facility) located in the rural the outskirts of northern Sydney, New South Wales, Australia. The facility adjoins semi-rural residential allotments (~ 5 ha) and light agricultural activities. It is situated at the top of a small headwater catchment with runoff passing through several adjoining properties before entering an ecologically sensitive national park (Fig. 1).

The facility and its operations are licenced under a New South Wales Environment Protection Authority (NSW EPA) environment protection licence (NSW EPA, 2016b). The NSW EPA is an independent statutory authority whose role, inter alia, is to 'work with the community, business, industry and government to maintain a balance between protecting the environment, managing competing demands on the environment and supporting sustainable growth' (NSW EPA, 2016a). Like most EPAs, one of its key roles as set out by the *Protection of the Environment Operations Act 1997* (NSW) (POEO Act), is to manage and regulate pollution via environment protection licences (NSW EPA, 2016a). Community feedback about environmental problems, often through the NSW EPA's Environment Line, is used to help inform it in executing compliance with respect to environmental protection licence requirements (NSW EPA, 2013; EPA, 2015). The facility's environmental licence permits irrigation of its effluent water at a set number of adjoining allotments (Fig. 1) at a rate such that does not result in off-site discharge.

The environmental impact and management practices of the facility examined here have been the subject of debate including controversial discussion in the NSW Parliament and local media (e.g. Cordina, 2000; Gallacher, 2000; Hornsby Advocate, 1998, 2000a, b; Howard, 2000; Inshaw, 2000, 2001; Rhiannon, 2000, Ward, 2000a, 2000b). The facility has received numerous non-compliance notifications over the period of its operating licence (EPA, 2015). It appears that the NSW EPA has not had sufficient evidentiary material to unequivocally confirm the link between the facility activities as the source of water pollution and the consequent deleterious geochemical and biological impacts on the adjoining environment.

This study uses a range of conventional (e.g. faecal coliform analysis, total phosphorus and nitrogen) and novel (e.g. arsenic speciation and chicken DNA detection) environmental markers to decipher the source(s) of the environmental pollution around the facility. In doing so, this study examines the utility of applying a suite of conventional and alternative environmental markers for resolving environmental pollution problems.

2. Methods and approach

The field setting for this study is complex in that there are a number of separate watercourses and an above-ground pipe network that conveys discharge from the facility to domestic lots as well as to lots listed on the slaughter facility's licence as its effluent utilisation zone (Fig. 1). A targeted sampling strategy was used to: (a) sample suspected run-off from the facility; (b) assess the quality of water piped to domestic lots and the effluent utilisation zone; (c) sample discharge in adjacent drainage pathways not receiving run-off from the facility (Fig. 1).

2.1. Sampling

This study was conducted over a three-year period and incorporated multiple sampling times and locations. Samples were collected from sites (streams and plumbed irrigation supplies) within the discharge catchment of the facility (sites B,C, E, F, G; Fig. 1), sites that receive no water from the facility (A, D, H and I; Fig. 1) and those that receive a mixed source of water including some water from the facility (J, K and L; Fig. 1).

Sampling was targeted and in part, inherently opportunistic because it required capturing suspected facility discharges. Samples were taken only from flowing water. Preliminary *in situ* water quality sampling and