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A National Wastewater Monitoring Program for a better understanding of public health: A case study using the Australian Census

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Abstract

Wastewater contains a large range of biological and chemical markers of human activity and exposures. Through systematic collection and analysis of these markers within wastewater samples it is possible to measure the public health of whole populations. The analysis of effluent and biosolids can also be used to understand the release of chemicals from wastewater treatment plants into the environment. Wastewater analysis and comparison with catchment specific data (e.g. demographics) however remains largely unexplored. This manuscript describes a national wastewater monitoring study that combines influent, effluent and biosolids sampling with the Australian Census. An archiving program allows estimation of per capita exposure to and consumption of chemicals, public health information, as well as per capita release of chemicals into the environment. The paper discusses the study concept, critical steps in setting up a coordinated national approach and key logistical and other considerations with a focus on lessons learnt and future applications. The unique combination of archived samples, analytical data and associated census-derived population data will provide a baseline dataset that has wide and potentially increasing applications across many disciplines that include public health, epidemiology, criminology, toxicology and sociology.

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ABSTRACT

Wastewater contains a large range of biological and chemical markers of human activity and exposures. Through systematic collection and analysis of these markers within wastewater samples it is possible to measure the public health of whole populations. The analysis of effluent and biosolids can also be used to understand the release of chemicals from wastewater treatment plants into the environment. Wastewater analysis and comparison with catchment specific data (e.g. demographics) however remains largely unexplored. This manuscript describes a national wastewater monitoring study that combines influent, effluent and biosolids sampling with the Australian Census. An archiving program allows estimation of per capita exposure to and consumption of chemicals, public health information, as well as per capita release of chemicals into the environment. The paper discusses the study concept, critical steps in setting up a coordinated national approach and key logistical and other considerations with a focus on lessons learnt and future applications. The unique combination of archived samples, analytical data and associated census-derived population data will provide a baseline dataset that has wide and potentially increasing applications across many disciplines that include public health, epidemiology, criminology, toxicology and sociology.

1. Introduction

Systematic collection and analysis of wastewater has become an effective tool for estimating population health status. This is achieved through the quantitative assessment of a population's use and exposure to chemicals, including a range of illicit drugs, alcohol, tobacco, pharmaceuticals and specific foods (Zuccato et al., 2005; van Nuijs et al., 2011; Lai et al., 2011; Ort et al., 2014; Thomas and Reid, 2011; Burgard et al., 2013), as well as the measurement of markers of public health status, such as oxidative stress (Ryu et al., 2016) or allergic reactions (Choi et al., 2018). Wastewater analysis can also be used to understand the release of chemicals from wastewater treatment plants (WWTPs). This remains a key environmental issue that also poses a human health risk from the range of organic chemicals that may remain in treated

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effluent. These chemicals include compounds that may affect reproductive function (Tan et al., 2007; Kidd et al., 2007; Schwarzenbach et al., 2006), selected antibiotics that may lead to antibiotic resistance (Watkinson et al., 2007), a large range of personal care products including UV filters and fragrances, and numerous chemicals used in industry (Wang and Khan, 2014).

Additionally, solids produced as part of the wastewater treatment process (referred to here as 'biosolids') are a sink for many chemicals that are subject to international treaties because of their implications for human health and the environment, such as trace metals (Smith, 2009), pesticides (Clarke et al., 2010), and various flame-retardants (Gallen et al., 2016). Biosolids use in agriculture and horticulture allows toxicants to re-enter the human food chain from the waste stream. Long-term, coordinated national programs of WWTP sampling therefore offer a unique set of samples to gain fundamental insights into a wide range of human and environmental health concerns at the catchment population level.

Multi-national long term monitoring programs analysing wastewater have been established over the last decade. For example, the European-based international collaboration, Sewage Analysis Core Group Europe (SCORE), uses annual wastewater analyses from 21 countries to compare trends in the consumption of illicit substances (Ort et al., 2014). Cross-sectional multisite and single site longitudinal studies investigating illicit drug usage have also been running in Australia since 2009 (Lai et al., 2016a; Lai et al., 2018; Tscharke et al., 2016).

Reliable per capita chemical consumption or exposure data are required to enable meaningful comparisons between jurisdictions and over time. Likewise, it is also relevant to assess the environmental release of chemicals on a per capita basis. Thus, it is important to have accurate data on WWTP catchment population sizes, i.e. the number of people contributing to a given wastewater sample or effluent discharge. WWTPs estimate and report catchment populations using a variety of approaches that vary in accuracy (O'Brien et al., 2014). Synchronising wastewater sampling with a national census, however, allows accurate estimation of the population size contributing to sampled wastewater, and thus more reliable per capita consumption, exposure and release data (O'Brien et al., 2014).

Census-collected ancillary data can also provide current and trend information on catchment population demographics and socioeconomics. This information, for example, can characterise the age profile, average income or employment type of each catchment population. This can be used to help explain differences in observed levels of chemical consumption, exposure and release, as well as the health of the population, between catchments.

Although many countries maintain a census, the Australian Census is unique due to (i) an extremely high compliance rate (net undercount rates of the Australian 2011 and 2016 censuses were 1.7% and 1.0% respectively (ABS, 2018)) and (ii) the geo-referenced information on the presence of people on one particular day (census). The latter is a pre-requisite to intersect the physical catchment boundaries of WWTPs with administrative regions from the census at the highest accuracy level (i.e. an individual mesh block. These blocks comprise between 30 and 60 dwellings, and 99.9% have populations < 500 people so approximately 358,000 mesh blocks cover the Australian population without gaps or overlaps) (ABS, 2017).

Census data also contain other useful metrics which can help with the interpretation of wastewater analysis data. For example, in addition to population numbers, the Australian Census data contains many demographic and socioeconomic variables which are also available at this high level of resolution. The data made publically available through the Australian Bureau of Statistics (ABS) are not limited to the population on census day (*de facto*), but also include the usual residents (*de jure*). The difference between *de facto* and *de jure* populations can provide information on the population movement in the catchment. Additionally, questions regarding the person's workplace address may provide insight into intra-day population movement between catchments. Furthermore, in Australia, a large percentage of the population is covered by relatively few WWTPs (e.g. the largest five cover > 30% and largest twenty cover > 50% of the population). In addition, in most states two to five WWTPs capture more than half of the state population's wastewater. These factors make a national study logistically feasible and represent significant advances towards delivering complete, high resolution estimates of chemical exposure and release in Australia.

Here, we describe (i) a national wastewater monitoring study that connects influent, effluent and biosolids sampling with the Australian Census and (ii) an archiving program that allows estimation of per capita exposure to and consumption of chemicals, public health information, as well as per capita release of chemicals into the environment. The study concept, critical steps in setting up a coordinated national approach and key logistical and other considerations are discussed, with a focus on lessons learnt and future applications. Furthermore, with this paper we invite collaboration with research teams across all disciplines where samples can be provided to address specific research questions.

2. Study concept

A coordinated national campaign involving 110 WWTPs was undertaken to collect daily influent, effluent and biosolids samples for a week centred on the 2016 Australian Census date (9 August 2016). This study was unique to Australia and resulted in wastewater coverage of 70% of the Australian population (> 16 million people). Whilst some WWTPs were recruited opportunistically, the recruitment design ensured that WWTPs were included with a range of different catchment population sizes from all states and territories, covering both metropolitan and regional population centres. The high overall coverage and a high participation rate from non-urban communities resulted in a sample collection that was highly representative of the Australian population. The number of participating sites was further augmented by piloting a new passive sampling approach at WWTPs that lacked autosampling equipment.

2.1. Sampling

All sampling was undertaken by WWTP personnel at the participating sites using comprehensive sampling kits, i.e. collection containers, preservatives, personal protective equipment (PPE), return delivery form and detailed sampling instructions, provided by the study team. Questionnaire responses from the WWTPs were systematically collected over the census sampling period detailing the type of sampling equipment, daily sampling activities, daily flow rates, and the WWTP processes and infrastructure. Both detailed sampling instructions and questionnaire responses were critical to ensure high quality, reliable samples were collected. Following successful 2016 sample collections and significant interest from the participating WWTPs, almost all WWTPs agreed to participate in two subsequent anniversary sample collections in August 2017 and 2018.

2.2. Meta-data

The study was designed to collect a significant amount of ancillary information, including for the WWTPs (from questionnaire responses) and the catchment populations (national census-collected data) (Fig. 1). The daily influent flow data for each WWTP and the census-derived catchment population numbers were required to determine accurate estimations of per capita data, e.g. per capita chemical consumption, exposure and release or health status. The per capita data together with additional data collections, e.g. treatment processes, population demographics and socioeconomics (Fig. 1), facilitate a wide range of informational outputs from the study and represent high resolution

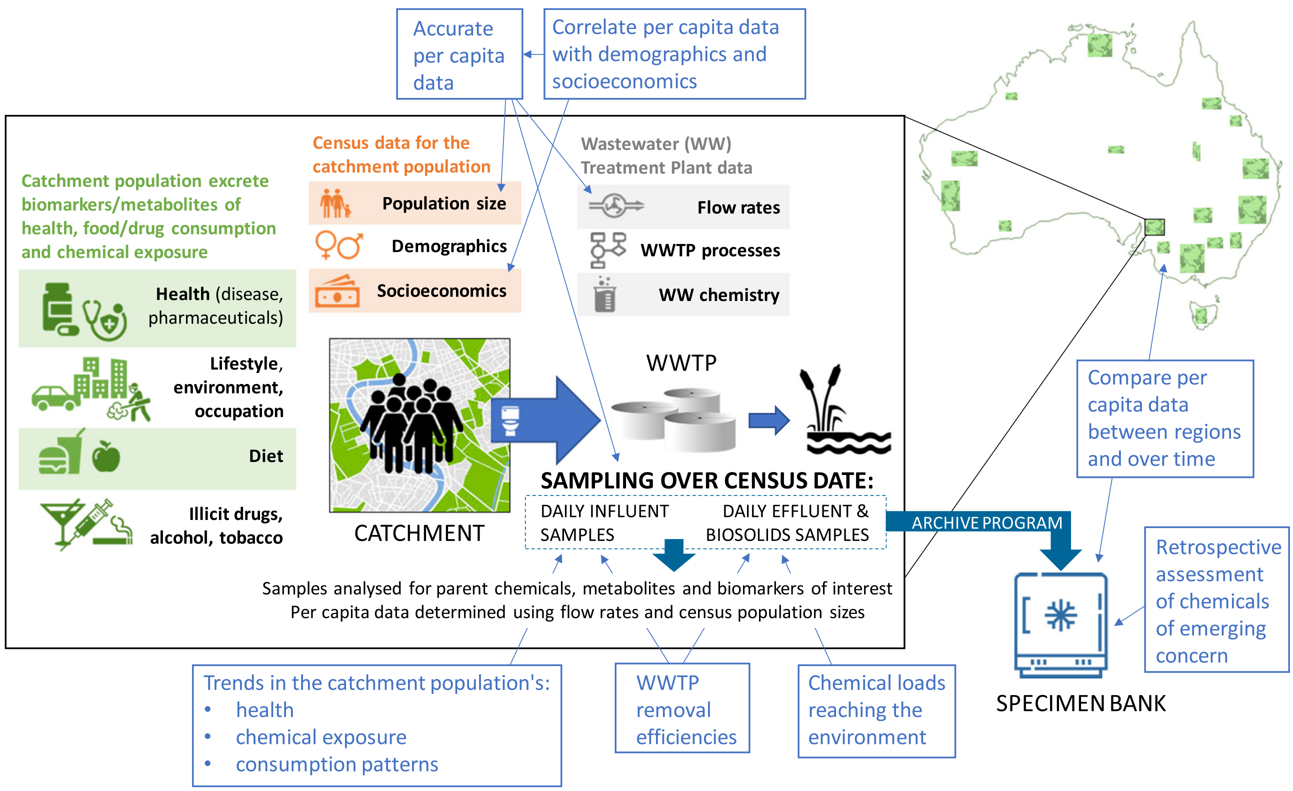

Fig. 1. Concept of a national wastewater monitoring program and its potential applications. For each WWTP catchment, the data and samples collected are indicated, together with examples of the information that can be reported from this study design (outer blue boxes). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

baseline data for future applications.

2.3. Anticipated outputs

The anticipated outputs from the study include temporal and spatial trends in biomarkers for specific health endpoints, chemical exposure and chemical consumption; WWTP chemical removal efficiency (comparing paired, time-resolved influent and effluent samples); prediction of chemical loads in aquatic, agricultural and other receiving environments; and correlation of per capita data with population demographics and socioeconomic data across all catchments to improve understanding of factors potentially driving observed consumption/exposure/release patterns (Fig. 1).

2.4. Coverage

Extensive national coverage (i.e. 70% of the Australian population) and inclusion of a range of different WWTPs and catchment characteristics were key aims of the study concept. The high population coverage allows results from the participating sites to be extrapolated to generate a national picture of the Australian population's health and chemical exposure/consumption patterns. Furthermore, the range of different WWTPs recruited to the study ensured coverage of, for example, different treatment processes and catchment demographics. This allows for investigation of factors that may drive differences in observed chemical data between individual WWTPs or different regions. For example, differences in observed chemical removal efficiencies between WWTPs may be associated with different treatment processes; or variations in estimated pharmaceutical usage between catchments may be explained by the average catchment population age.

2.5. Archive

A further key aspect of the study was the parallel archiving program (Fig. 1). Archived census samples represent a future resource that can be interrogated over time as analytical tools continue to improve. Such a joint sampling and archiving program thus provides the basis for accurate assessment of temporal and spatial trends. Importantly, it allows retrospective analysis for a wide range of as yet unknown chemicals of emerging concern and other target parameters. The unique combination of archived samples and associated census-derived population data has wide and potentially increasing application across many disciplines, including public health, epidemiology, criminology, law enforcement, toxicology and sociology.

3. Study logistics and quality assurance/quality control (QA/QC)

Five key stages are involved in a coordinated national wastewater sampling program timed to coincide with a national census (Fig. 2). Whilst there will be region-specific considerations, the study approach described here is considered broadly relevant to most jurisdictions and generalisations can be made with respect to logistics and lessons learnt.

The initial planning stage was the most critical phase of the study and ideally should commence at least six months before sampling. In this stage, we defined core strategies for WWTP selection and recruitment, sampling, transport and sample management and archiving, together with the associated quality assurance/quality control (QA/QC) approaches. Also in this early phase, we developed drafts of the questionnaires that were a vital part of the information collection.

The logistics of a large scale project pose many QA/QC challenges to ensure reliable samples are collected and preserved. These challenges were anticipated and planned for as far as possible through consultation with the study research team and collaborators who have previously undertaken similar, albeit smaller scale, projects (for examples see: (van

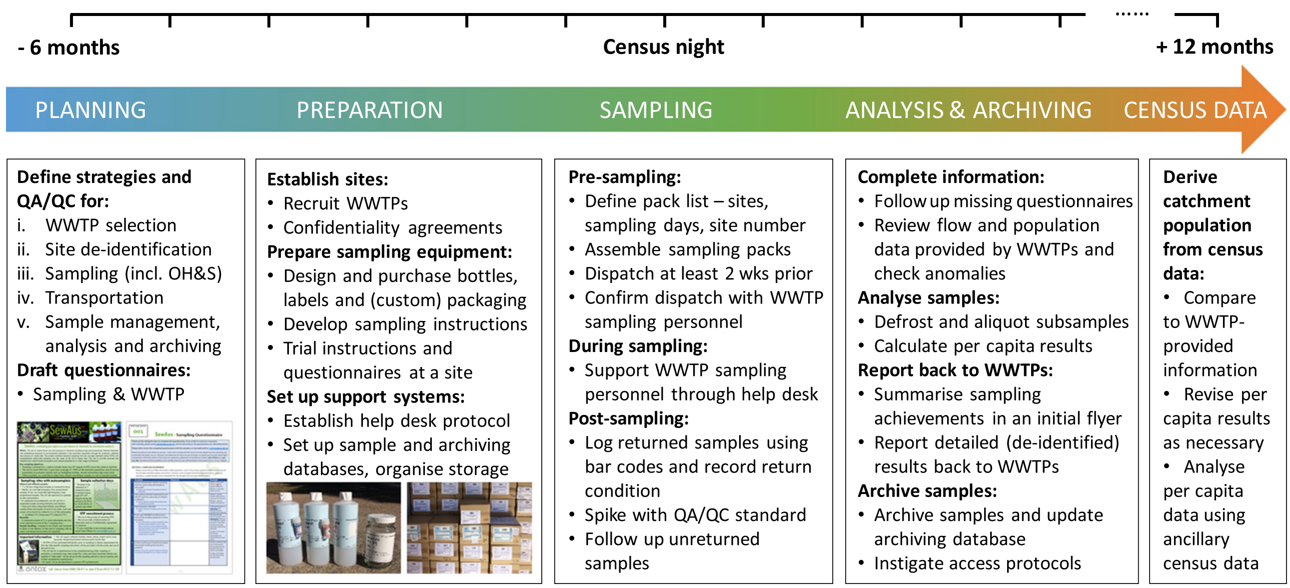

Fig. 2. Key stages and associated tasks in a national wastewater sampling program linked to a national census event.

Nuijs et al., 2018; Lai et al., 2016b; O'Brien et al., 2014)). QA/QC considerations are discussed under the relevant sections: sampling (Section 3.2), transport (Section 3.3), questionnaires (Section 3.4), sample management (Section 3.5) and sample archiving and access (Section 3.6).

3.1. WWTP selection and recruitment

The overarching objective for WWTP recruitment was to target the maximum possible number of WWTPs to represent multiple locations, sizes, demographics and other factors, within the cost and logistical constraints of a large national study. Within this broader objective, other considerations and recruitment approaches were also identified, including commercial considerations, as well as opportunistic and specific interest approaches (Table 1).

Given a high number of relatively small WWTPs of interest to the study in regional areas of Australia, it was important to consider the reliability and confidentiality of data generated from WWTPs which service (very) small communities, some of which might be vulnerable to stigmatisation if identified in public output. Sampling uncertainties due to low and intermittent flow associated with small catchment populations need to be balanced against the benefits of generating new data for sites of interest. Studies involving small populations require risk mitigation strategies to meet ethical considerations (Prichard et al., 2014; Prichard et al., 2016). Sixteen sites with a population below 10,000 people were included in the study and two with a population below 1000. To protect the anonymity of these sites, we followed protocols approved by an accredited human research ethics committee: identifying data on population size was withheld and only population ranges were reported; data from the two smallest sites were aggregated; and all researchers avoided media or public commentary on the location of the sites.

Regardless of the value of a site to the study, the overriding consideration for inclusion of a WWTP was our confidence that the site would adhere to the quality controls necessary to ensure reliable sample collection. Fostering a good understanding and relationship

Table 1
Criteria underlying the WWTP recruitment strategy (i.e. which WWTPs to target for participation).

Criteria	Example
Rural status and geographical spread	All states and territories were targeted, with coverage across all five ABS area codes – major city, inner regional, outer regional, remote and very remote
Population size	Largest 15 WWTPs in Australia all individually targeted (collectively service 54% of the national population) WWTPs of different size included to achieve a range of different catchment population demographics/socioeconomics and treatment processes Some very small WWTPs were included in the study to involve catchments important for remote and very remote regional health assessments
Logistical issues	Availability of autosampling equipment Availability of WWTP personnel to undertake sampling Accessibility of influent/effluent streams to deploy passive samplers
Cost considerations	Some sites rely on external contractors to collect samples (for their own regulatory testing), e.g. remote WWTPs and/or WWTPs with few on-site personnel, resulting in additional sampling costs to the study
Confidentiality/commercial considerations	WWTPs run by local councils were generally open to participation and were often highly committed to the social aspects of the study, whereas privately managed WWTPs (i.e. not managed by local government) often declined, even when confidentiality agreements were available
Sites of specific interest	Sites of specific interest to the multidisciplinary study team and research partners were individually approached, e.g. those in areas with suspected unusual drug or other chemical usage or industries, or regions not picked up in drug surveys, catchments with specific population demographics or close to highly sensitive receiving environments
Opportunistic approaches	Several recruitments were achieved through advertisements in newsletters via state or regional bodies that WWTPs are affiliated with As awareness of the study increased (e.g. through distribution of flyers and word of mouth), some WWTPs approached the study team requesting to be involved

with the WWTPs was essential to achieve this. Effective communication was a critical consideration, particularly to ensure that the study objectives were clearly understood, that WWTPs felt engaged and vested in the process and that issues related to site confidentiality were adequately addressed. Engagement with WWTPs was accomplished through:

- Clearly communicating the aims of the project and the role that WWTPs play in the study (delivered via an initial emailed flyer and follow-up phone call(s))
- Ensuring that all WWTPs were de-identified in the study outputs
- Clear and timely communication of sampling logistics (i.e. what we needed of them)
- Provision of a comprehensive sampling kit (see Section 3.2), i.e. sampling containers, PPE (e.g. gloves), pen for completing questionnaire, packing tape to reseal box for return transport and prepaid return courier slips – thus ensuring the WWTP personnel were fully equipped to carry out the sampling
- Logical, well presented instructions sent with sampling kits and available online. These were trialled in advance
- A help phone line for questions during sampling
- Feedback to the WWTPs after sampling. Initially a flyer was distributed outlining how many sites participated, the total number of samples collected etc. After analysis for the first suite of compounds (illicit drugs), a full, de-identified report was sent to each site, detailing data for their WWTP and comparisons with all other sites

Discussions with WWTP personnel indicated that an understanding of what their efforts achieved was critical to keep participating sites invested in the project. When a public release of the first tranche of study results was planned, feedback to the WWTPs was timed so that each WWTP received their report ahead of the media release. Together with good organisation and communication, this was a key factor in the willingness of up to 90% of sites to participate in the two subsequent years' sampling activities. As a result, longitudinal data for 100 WWTPs around the country were obtained.

The practicalities of recruiting sites involved developing effective information packs (initially a one page flyer followed by a more detailed sampling information sheet), contacting all WWTPs individually and recording WWTP information in a sampling database (see Section 3.5 for database details). Most approaches were initiated through an email with the flyer attached, followed by a phone call, but cold calling was also surprisingly effective. Contact numbers were, however, in many cases not publically available and getting access to the appropriate person to approve the involvement of the WWTP in the study could be time-consuming. Overall, the optimum approach was to contact the senior management team of the WWTP to get buy-in for the study objectives and then discuss sampling logistics with the operational team.

Confidentiality agreements were often a condition of participation. In line with ethical guidelines developed for wastewater analysis studies (Prichard et al., 2016), de-identification of WWTP names and other identifying features was offered to all participating WWTPs. Confidentiality was also formalised at the request of individual sites through either a confidentiality agreement or a memorandum of understanding (MOU). Usually the WWTP legal team accepted template agreements provided by the university but in some cases, WWTP-specific agreements were prepared. Whilst confidentiality agreements are legally binding, MOUs are not; however, the research team entered into all agreements with the intention of non-disclosure unless legally required to by a court of law. Sub-agreements with researchers outside of the core study team who utilised the data and/or samples were put in place to ensure non-disclosure of WWTP details.

The overall high population coverage for the WWTPs recruited to the study (70% of the national population) resulted from high coverage (55–99%) in six of the eight participating Australian states and territories. The two jurisdictions with low population coverage (< 30%) have largely de-centralised, regional populations, and so would require many more participating sites to yield higher percentage coverage. This should be considered in future collections where project aims require similar population coverage across each participating region. Furthermore, having a greater number of WWTPs means that there is greater potential for localised analyses.

3.2. Sampling protocols for influent, effluent and biosolids

Technical considerations for wastewater analysis practitioners regarding sampling protocols are discussed in detail in this section. Wherever possible, we collected daily samples (24 h composites collected using an autosampler on the highest available sampling frequency operating in flow proportional mode as recommended by Ort et al. (2010) of influent and effluent. By collecting and archiving daily samples, we had the option of obtaining time resolved data (e.g. to elucidate intra-weekly patterns in exposure or consumption) or pool subsamples to determine weekly averaged concentrations. WWTPs were asked to provide seven consecutive days of samples to include the night of the census (allowing at least one day of sampling before or after census night).

An important consideration was to make the process as streamlined as possible; for example, for sites that were not staffed at weekends, weekend composites were requested or alternatively, if this was not feasible, only weekday samples were collected. Biosolids, i.e. the final treated solid waste that leaves the facility, collected on a specific day were assumed, at most sites, to be a homogenous mix of biosolids inputs over the preceding days, weeks or even months (depending on the process for processing biosolids at the WWTP). As such, a single day's sample was collected from any day over the sampling week. Although the biosolids samples themselves may not be reflective of the "*de facto*" population on census day, they are still likely to represent the "*de jure*" population of the catchment and are of interest as they typically re-enter the environment via agricultural practices. The logistics and QA/QC aspects of the sampling protocol are outlined in Table 2.

Wastewater sample preservation prior to archiving was of paramount importance if this sample set was to provide baseline data for future analyses of chemicals which have not yet been identified. Steps were accordingly taken to limit the samples' biological activity and ensure that the chemical stability and biological activity of wastewater did not lead to degradation of selected analytes (i.e. chemicals being targeted for analysis) between sample collection and analysis.

As outlined by Baker and Kasprzyk-Hordern (2011), no single preservative or technique can ensure stability of all analytes. However, adding a biocide or reducing temperature, pH and/or particulate matter all influence stability of specific chemicals. After reviewing the available literature (mainly limited to certain pharmaceuticals and illicit drugs (Baker and Kasprzyk-Hordern, 2011; Castiglioni et al., 2013; McCall et al., 2016)), we adopted a multi-tiered approach to preserve as many analytes as possible in the samples.

Guidelines provided to WWTP operators instructed them to operate autosamplers at < 4 °C during sample collection. After shaking the daily composite samples to increase homogeneity, three separate 400 mL aliquots were collected into 500 mL HDPE pre-cleaned bottles (Table 2). One aliquot was left unpreserved, one had 4 mL of 2 M hydrochloric acid (HCl) added to it to acidify it to ~pH 2 and the third had $2.0\,g\,L^{-1}$ of the biocide, sodium metabisulphite ($Na_2S_2O_5$), added (Fig. 3). At this concentration of sodium metabisulphite, however, matrix interference specific to noroxycodone was subsequently observed and after undertaking testing, the preservative concentration was decreased to $0.5\,g\,L^{-1}$ for subsequent collections. After collection, samples were frozen immediately onsite prior to shipping frozen to the lab.

As for biosolids, there are limited data on preservation techniques to reduce biodegradation, particularly in regards to stability of

Table 2
Study logistics and QA/QC approach for sampling influent, effluent and biosolids at WWTPs.

Process	Detail
Sample type	Daily 24 h composite samples of influent and effluent, ideally flow proportional samples collected at < 15 min intervals Daily biosolid sample collected from the processing belt, when this was not possible an individual biosolid sample was collected from stockpiles (sampling points and frequency were recorded in the sampling questionnaire)
Sample volumes	3 × 400 mL for each of influent and effluent, per day (collected in 500 mL bottles to allow for expansion of the liquid when freezing) 300 mL of biosolids (collected in a 375 mL glass jar)
Sample preservation	For each 24 h composite influent or effluent sample, three bottles were collected: 1. unpreserved 2. acidified by addition of 4 mL of 2 M hydrochloric acid to 400 mL sample 3. preserved by addition of 4 mL of 1 M sodium metabisulphite to 400 mL sample
Sample handling	Each daily composite sample was shaken to mix completely and three daily sample bottles filled. The relevant preservative vial (attached to the sampling bottle) was added immediately and the sample stored in the freezer until transport
Sampling support	To ensure sampling protocols were clearly communicated and queries could be answered: • site-specific sampling instructions were prepared (covering the type of sampling occurring at that site). All instructions were trialled at a local WWTP site before release • a help phone line was established during the census sampling period to ensure operators could get questions answered in 'real time' as sampling was occurring. The phone number was also written on each bottle/jar to encourage sample collectors to immediately ask questions if unsure.
Sample QA/QC	All sampling containers were pre-cleaned with methanol and then rinsed with MilliQ water For each batch of bottles received from the supplier, blanks were cleaned as per the sample bottles and filled with MilliQ water. Blanks are handled and stored as per sample bottles and analysed with each batch of samples (i.e. bottles from the same supplier batch) All bottles had pre-printed, colour-coded labels (indicating influent or effluent and type of preservative) with the site number and day written on them A fill line was printed on the label to indicate 400 mL volume on the 500 mL bottles Preservatives (HCl and $Na_2S_2O_5$) were added to 5 mL screw top vials labelled with appropriate safety warnings and colour coded to match the sampling bottle labels. The vials were taped to the side of the relevant sampling bottle. This ensured the correct preservative was added to the correct bottle immediately after sampling (note that adding the preservatives to the sampling bottle prior to dispatch to the site was not an option as some WWTP procedures for sample collection require WWTP personnel to wash out the bottle first with wastewater before collection thus discarding the preservative) Samples were stored frozen prior to return shipping to the lab

compounds across multiple chemical classes. Even reviews on emerging organic contaminants in biosolids haven't investigated the role of preservation on the stability of chemicals within the collected samples (Clarke and Smith, 2011). Autoclave, formaldehyde and freezing have all been proposed as biosolid preservation techniques. An autoclave is however not common on-site at WWTPs and not appropriate for heat-labile compounds. Formaldehyde when trialled as a preservative for hormones within swine manure (which we deem to be similar to biosolids) caused partitioning issues between the liquid and solid phases (Combalbert et al., 2010). Additionally there are also issues regarding human exposure to formaldehyde such as short-term health effects and it is listed as a carcinogen by the International Agency for Research on Cancer and formaldehyde can be expected to be reactive towards analytes such as amphetamine as reactions between amines and aldehydes/ketones are known to produce imines. Therefore, even if formaldehyde were an effective biosolid preservative it would pose what we would deem an unacceptable risk to the biosolids sample collectors. As such we froze the biosolids immediately after collection to reduce bioactivity and archived them at −20 °C. Future research should investigate the role of preservatives on analyte stability within archived wastewater and biosolids samples. Further details on preservatives are given in Table 2.

Sampling containers, labelling and transport packaging were custom-designed to meet the study sampling requirements (e.g. sample volumes, addition of preservatives after sampling), ensure unique identification of individual samples (over 4000 samples were archived after sampling), meet occupational health and safety requirements (e.g. responsibility to provide suitable protective gloves), minimise transport

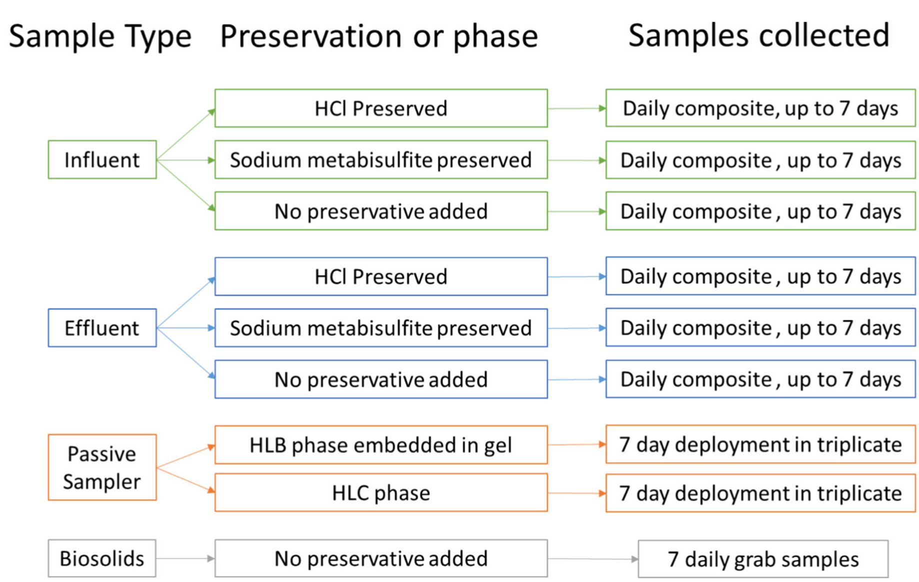

Fig. 3. Summary of samples and preservation techniques used.

Table 3
Study logistics and QA/QC approach for transportation.

Process	Detail
Packaging	Cardboard boxes lined with 2 cm polystyrene for insulation were custom ordered to fit 8 × 500 mL bottles The size and weight of the box was optimised to ensure courier costs were minimised Local occupational health and safety requirements (handling biological materials) necessitated the samples being bagged in zip lock bags within the transport box. This was communicated clearly in the instructions
Sampling kit dispatch to WWTPs	The logistics of sending out numerous (> 600) sampling kits within a short timeframe were discussed and agreed with a national courier company well in advance but issues still occurred. Optimising this step is critical to ensure WWTP engagement is not compromised Addressing and booking boxes to ship to > 100 locations was time consuming and needed careful planning
Return transport	Pre-paid courier slips were included in sample kits which encouraged prompt return of the samples. WWTP personnel were required to book sample pick up Samples were couriered back only at the beginning of a work week to ensure samples were received and could be archived before the weekend (thus avoiding samples sitting at room temperature for extended periods and defrosting)

costs and ensure that samples could be shipped frozen back to the laboratory (see also Section 3.3 for transport considerations).

Discussions with individual WWTPs prior to sampling were crucial to understand the approach to sampling at each site. Where our study's sampling instructions conflicted with usual practise within the WWTP, local practise would generally be followed unless specifically requested otherwise. For example, some WWTPs that run autosamplers for multiple days to collect samples for internal testing purposes keep daily samples within the refrigerated sampler rather than transferring them daily to a freezer. Alterative arrangements were required to remove the samples from the autosampler each day to ensure samples were frozen immediately.

WWTP operations and equipment varied considerably between sites so sites were contacted individually prior to sampling and asked to complete a short survey on available equipment and expected timing of sampling. Although not initially anticipated, many smaller sites routinely operated autosampling equipment. For those that did not, passive samplers were considered (see Section 3.2.1). A small number of larger sites were not able to change their 'routine' sampling schedule to fit with the study requirements, necessitating work-arounds at these sites. Overall, however, the commitment and willingness of all participating WWTPs to undertake sampling was unprecedented.

3.2.1. Passive sampling

WWTPs without auto-sampling equipment provided a unique motivation to develop and deploy passive samplers to estimate the time-weighted-average concentration of chemicals. A pilot passive sampling approach, and its validation, were designed for this study as little research has been done at WWTP sites to date (Baz-Lomba et al., 2017). Benefits of passive sampling technologies include the simplification of sampling and analytical process by providing a time-integrated sample with typically low detection limits and in situ enrichment of chemicals (Harman et al., 2012). Within a wastewater study context, this could mean a more accurate representation of chemical exposure, particularly at remote locations or where site access is limited and/or at sites in which autosampling equipment is unavailable.

Details on the passive sampler design and quality control/quality assurance are given in SI Section 1. The greatest challenge with the passive sampler deployment was to ensure they remained submerged in the wastewater stream for the duration of the sampling. Particularly for influent, the water level in the influent inlet channel can change significantly as upstream pump stations are (de)activated. Through pre-trials, the optimised set up was a 5 m rope weighted at one end with a 0.5 m length of chain link (approximate weight 0.5 kg) (SI, Fig. S-1). The sampler and passive flow monitors (PFMs) were attached above the weight to maintain a height of approximately 30 cm above the channel base. The sampling instructions indicated that passive samplers should be deployed downstream of the inlet screens (which remove solids in the waste stream) to reduce the risk of debris attaching to the samplers.

To maximise the chances of successful deployment, the entire passive sampling system (i.e. samplers, PFMs, and chains pre-attached to deployment ropes) was shipped to sites pre-assembled with a clear and succinct guide containing deployment procedures and an illustration of the optimal positioning in inlet/outlet channels. The deployment QA/QC protocol required all labels, stickers and ropes to be colour coded, similar to the wastewater sampling procedures described above, and clearly labelled with site numbers and "influent" (i.e. green ropes/labels) and "effluent" (i.e. blue ropes and labels). WWTP personnel were required only to tie off the rope in a suitable position and remove the transit housings from the samplers and PFMs before submerging in the wastewater. The intact passive sampling system was returned to the lab (with the passive samplers placed back in their original transit housings) so that the condition of the system at the end of the deployment could be observed and carefully recorded. Of the 80 site deployments, only one set of passive samplers were deployed incorrectly to our knowledge, i.e. transit housings were not removed from the samplers during deployment.

3.3. Transportation

How samples are handled after collection can pose a risk to sample integrity for any wastewater project. These risks are amplified in a large scale national project. The key objective for the transportation was to ensure that samples remained frozen during transit to minimise analyte degradation. Portable coolers were prohibitively expensive and insulated transport boxes were custom designed (Table 3) to be effective for a 24–48 h transport journey (samples still contained ice on receipt after 48 h). Air space once the box was packed was designed to be kept to a minimum to maintain low internal temperature.

For all samples, the status of the sample on arrival was carefully recorded. If we suspected that samples had been compromised, quality control processes could be implemented to ensure that sample transport or storage had not impacted the condition of the sample; i.e. markers such as paracetamol, which have been shown to have a nationally uniform consumption and therefore predictable levels in influent, could be used to identify instances of significant degradation (O'Brien et al., 2017). Except for one box lost by the courier company and two broken glass jars with biosolids samples, all samples collected were returned intact to the lab.

3.4. Information requirements: sampling and WWTP questionnaires

Two questionnaires (see SI, Sections S-2 and S-3) were designed to:

i) record detailed information on the sampling activities (completed by the operator doing the sampling), and
ii) describe the WWTP processes, infrastructure and catchment (e.g. industry/landfills within the catchment) and provide specific flow rates/wastewater characteristics over the sampling period (typically completed by the management team or engineers after the sampling).

Previous experience has shown that obtaining responses to questionnaires and other information requests is challenging for wastewater analysis projects. Wherever possible, efforts were made to simplify the forms and keep them relevant to a specific site. For example, both questionnaires were customised to individual WWTPs, based on the equipment they were operating and the types and numbers of samples to be collected (this information was collected during the recruitment phase). This also allowed the questionnaires to be pre-printed with any known information, i.e. de-identified code, name etc. Questionnaires were issued in both paper and electronic form with contact details in case of questions. The merits of an online form were discussed and, although not logistically feasible for this study, is recommended for future studies in order to ensure consistency of answers and make interrogation of the data easier.

The sampling questionnaire was relatively straightforward, which was reflected in high response rates. The questions focused on timing of sampling and any unusual events that occurred in the catchment or at the WWTP over the sampling period. To prompt responses, examples were included in a separate column on the questionnaire to outline the type of information required (e.g. the effluent auto-sampler was not functioning 9 am to 1 pm on the 5th and only a partial sample was collected; a large music event was held in the catchment on the evening of 7th involving approximately 10,000 people). Most questionnaires were completed in paper form when the operator collected the samples and returned with the samples.

The WWTP questionnaire was based on the version originally developed by Ort and co-workers and used by Thomas et al. (2012) for comparing illicit drug use in 19 European cities through wastewater analysis. Experience with the questionnaire in Europe indicated that the questionnaire was considered long and some questions were challenging to answer. For our study, we therefore made the following adaptations:

- Prioritised the information requirements relative to our research aims and thus reduced the number of questions by approximately one third;
- Identified two priority questions on the first page for which information was requested, irrespective of responses for the rest of the questionnaire, e.g. flow rates and catchment map (to allow census boundaries to be matched);
- Designed the questionnaire in a tabular format so that example answers could be included to prompt the type of information requested or indicate preferred units;
- The questionnaire was reviewed by technical experts to assess the availability of requested information at Australian WWTPs and consistency of terminology with Australian standards;
- The questionnaire was trialled at a local WWTP site prior to use and recommended changes were adopted.

As a result, response rates were ultimately high and were 100% for the priority questions. Assessment of the completeness and quality of the responses is a necessary task; the approach to quality checking the priority data is discussed in Section 3.4.1 with flow rates as an example. Many WWTPs had to be followed up to complete the questionnaire but this was often because the questionnaire did not reach the appropriate person for completion.

3.4.1. Quality checking questionnaire data

Missing questionnaires were followed up systematically with the relevant WWTP personnel over the several months of sampling. Once all questionnaires were received, they were compiled into a database for easier access and reporting.

The most time-critical information from the WWTP questionnaire was the flow data because daily values are required to convert concentrations of analytes in the samples to total mass of chemical excreted by the population. The central reliance on the flow data for reported results necessitated a quality check to ensure the values reported by the WWTPs made sense. This was done by determining the flow versus population ratio and comparing the ratio across all sites. In an earlier study conducted in Australia it was determined that the water flow to a WWTP per person per day is in the order of 250 ± 10 L (O'Brien et al., 2014). Values outside this range were investigated. For example, a very low ratio was reported at a WWTP where wastewater was being lost via a stormwater overflow channel. In this situation the estimated mass load of chemical excreted by the population may have been underestimated because wastewater is being removed from the sewer system.

3.5. Sample management

The protocol for managing > 4000 samples (3600 × 500 mL bottles and 600 × 375 mL jars) included recording sample information in customised databases, comprehensive sample tracking using unique bar codes and acquisition of sufficient frozen storage space. The most labour intensive phases of the project were the immediate pre- and post-sampling stages, i.e. dispatch of sampling kits and return of samples. During these stages, the key objective was to ensure that correctly pre-labelled sampling containers were sent to a site, all samples collected by the site were received back and samples were recorded accurately in the database systems (see Table 4). Once received at our lab, samples were spiked with isotopically labelled standards to allow for any long term degradation occurring during storage to be measured and stored in the Australian Environmental Specimen Bank (AESB) at −20 °C (Table 4). For the purpose of this project, an additional 29 m^3 walk-in freezer was purchased for the AESB to allow systematic archiving of the returned samples.

3.6. Sample archiving and access

An archive of frozen wastewater samples collected over time allows for retrospective wastewater analyses. Typically, only a fraction of each sample is needed for laboratory tests and the rest can be catalogued and stored for re-analysis in the event that a new drug emerges onto the market or a chemical exposure becomes of interest in the future. The value of such sample archives lies in their assured quality (e.g. no cross contamination, minimal degradation) and accessibility (samples managed through an up to date database with retrievable metadata). Thus archiving protocols (see Table 5) are of vital importance. Given the wide range of research questions that can be investigated using the census samples, strict access protocols became warranted to maximise the research potential from each (limited volume) sample (Table 5).

3.7. Census information

Once census data are available, the WWTP catchment population can be estimated by overlaying geo-referenced high resolution catchment maps (provided by the WWTPs through the WWTP questionnaire, SI Section S-3) with publically available geo-referenced census population data (from the ABS).

In brief, wastewater catchment boundary maps can be provided as PDF or geospatial files (such as shape files) and geo-referenced into freely available GIS software such as QGIS, i.e. the physical map is associated with spatial locations in the GIS software. When census GIS files are available, they can then be imported into the software and overlain with the WWTP catchment map. The census data (population count, demographics etc.) are reported for individual mesh blocks (polygons) (Fig. 4) which is the highest resolution available and each mesh block comprises between 30 and 60 dwellings with 99.9% of these containing < 500 people. There are approximately 358,000 mesh blocks which cover Australia without gaps or overlaps. The GIS software calculates the area of each mesh block. The area ratio of mesh blocks before and after overlapping the census and WWTP catchment GIS files can be used to determine the population (and associated

Table 4
Study logistics and QA/QC approach for sample management.

Process	Detail
Sample and archiving databases	Details for every participating WWTP's sampling activities were held in two databases: 1. **Sampling database**: de-identified code, contact personnel and details, available equipment (autosamplers, freezers), type of sampling to be undertaken, dates of sampling, numbers of sampling containers needed, instructions to be sent and whether site-specific instructions were required, courier slip numbers (to chase lost shipments), questionnaires (how sent, electronic or paper copy, and to whom) 2. **Archiving database**: all sampling containers sent to WWTPs were pre-allocated a unique code in the archiving database with site information and archiving location (in the freezers) recorded in the system.
Sample tracking	Pack lists were produced from the sampling database to help ensure the correct sampling kit, including pre-labelled containers detailing WWTP and sampling day and site-specific instructions and questionnaires, was packed for each site. Each sample kit was double checked before sealing Waterproof bar code stickers with a unique archive number were attached to every container sent out Returned samples were scanned (using bar codes) and the return information uploaded to the archiving database Missing samples could be identified using the archiving database (and followed up with the courier using the courier slip details recorded in the sampling database)
Chain of custody	A formal chain of custody process was not deemed necessary as all sampling bottles were pre-labelled and bar coded as part of the archiving system and the (pre-filled) return courier slips were kept as evidence of the sample return
Dispatch of sampling kits	Dispatch of sampling kits to WWTPs was aimed for two weeks prior to the sampling week, although this was not achieved for some sites due to complications with the courier company Dispatch was confirmed by email with each WWTP so that non-arrival could be notified allowing sufficient time to track down lost kits or re-send. Of the 638 boxes sent out to participating WWTPs, 1% were lost and had to be re-sent
Return of sampling kits	The freezer storage space was setup prior to sampling with labelled boxes to allow archiving to take place quickly once samples started arriving The return courier slip was retained and the condition of each sample carefully recorded Archiving space was consolidated once all samples were returned to allow easy access to samples (see also Section 3.6)
Compromised samples	The few samples that were returned in a compromised condition (e.g. empty or broken container, insufficient sample collected (so concentration of preservative would be too high)) were discarded and the record updated in the archiving database
Assessing degradation	Each returned influent and effluent bottle was spiked (onto the frozen sample) with an equal mix of two deuterated compounds (carbamazepine-d8 which is highly stable and paracetamol-d4 which is rapidly degraded (O'Brien et al., 2017)). When samples are subsequently analysed, the relative masses of the two standards indicate the potential for degradation to have occurred during storage

ancillary data) within the WWTP catchment, based on proportional area. An example catchment and terminology is explained in Fig. 4.

Catchment populations can also be described by the available demographic and socioeconomic metrics collected in the census. Preliminary analyses of the 2016 ABS data indicate there is significant age stratification across the catchments in this study (data not presented here), which may be driving patterns in, for example, pharmaceutical use. This approach also allows for improved comparisons between other location-based data sources, such as policing or health data, and can contextualise and supply further understanding to the study's outputs.

The delay in the release of census data is one constraint to this methodology as census data may only become available up to a year after the census date every 5 years, limiting the timeliness of these comparisons. The static nature of the census also precludes studies on short-term temporal changes in demographics (< 5 years) to be investigated. Yet, population count estimates released quarterly post-census provide some additional temporal population estimates. In addition, the catchment populations could be modelled between two or more census periods to estimate population at any given time point, or to forecast future population based on annual or monthly population growth rates. Such estimates could be applied to ongoing longitudinal wastewater analysis studies to provide population estimates at the resolution of the study.

3.8. Other catchment information

In addition to the demographic data, other georeferenced metadata for a specific wastewater catchment may be required to properly interpret wastewater analysis data. For example, a pharmaceutical manufacturer located within a small population catchment may discharge significant amounts of pharmaceuticals which would otherwise be interpreted as disproportionally high per capita pharmaceutical consumption. Even with questions in the questionnaire about types of industry, trade waste is usually recorded on a volume basis or in terms of biological or chemical oxygen demand. In this example, unless the pharmaceutical manufacturer discharges significant amounts of trade waste it might not get reported on a questionnaire. This emphasizes the

Table 5
QA/QC approach for sample archiving and access.

Process	Detail
Sample processing	Prior to analysis, samples need to be defrosted. Defrosting increases the risk of degradation of poorly stable chemicals and this was minimised by establishing multiple aliquots when a given sample was first used. In our specific case, the acidified samples from the influent were first defrosted and a number of aliquots were subsampled including 2 × 10 mL (one filtered) and 4 × 2 mL (two filtered)
Sample archiving	Samples and aliquots were stored in the archiving facility at −20 °C with alarm notifications of extended temperature increases (outside of routine defrosting cycles) One 10 mL aliquot from each sample was archived at −80 °C to maintain the integrity of the sample biochemistry (as much as possible) Details of all samples were recorded in the archiving database, with the future intention of including remaining sample volume and links to analytical results and publications Access to samples was initially unrestricted, but strict access protocols were soon put in place
Sample access	Protocols to access samples were established in conjunction with the archiving facility's overall processes Procedures were put in place to prioritise and approve usage of a sample, and particularly to control access to the samples collected over the census night Where possible, analytical methods were combined to increase the number of quantified analytes achieved per sample The ultimate aim is to analyse each sample using non-target approaches (on high resolution mass spectrometers) and to archive the mass spectra for future interrogation (creating an Environmental Data Bank companion archive to the Environmental Specimen Bank)

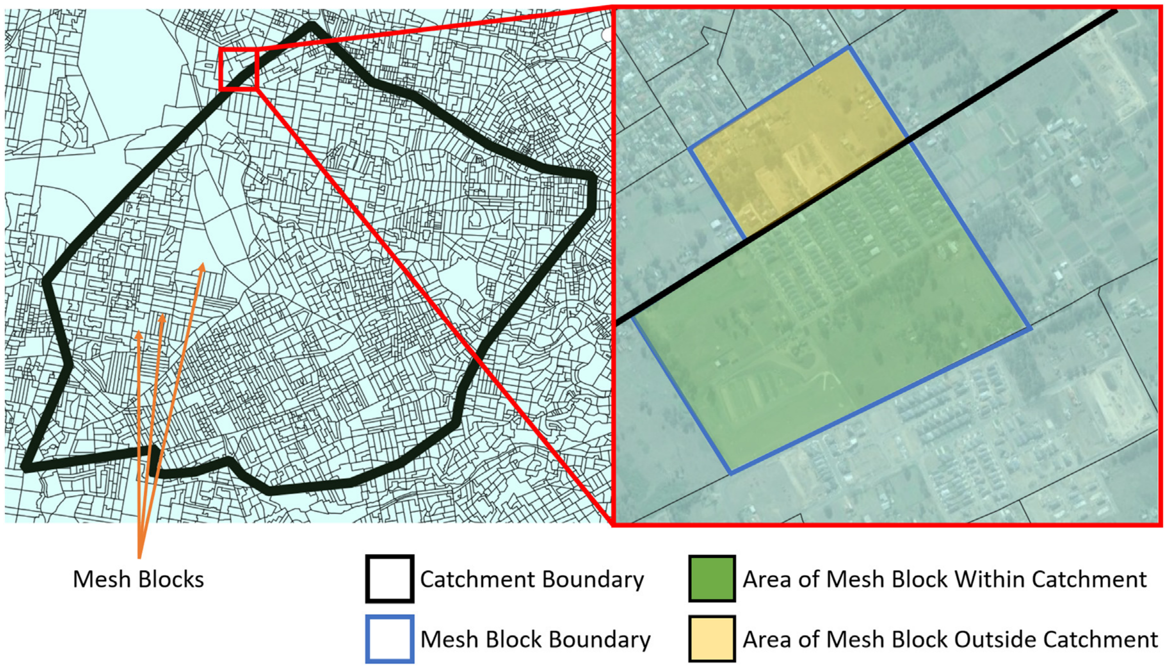

Fig. 4. Example catchment boundary map overlain onto the census mesh block map and inset: mesh blocks at the fringes of the catchment that are intersected by the catchment boundary with sections of the mesh block inside (green) and outside (yellow) of the catchment boundary. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

importance of obtaining accurate catchment maps which can be overlayed with other georeferenced data.

4. Summary of key recommendations for future studies

One measure of success for this study was the number of participating WWTPs recruited which exceeded expectations. As the first study of this kind, a number of recommendations to consider for future studies were identified (Table 6).

5. Outlook and future applications

The samples and data generated by this national study represent a unique, high-resolution baseline data set that can be utilised to address a wide range of research questions. A major utility of this approach lies in the combination of wide geographical and population coverage and the feasibility of regular data collections, demonstrated by successful anniversary collections in two subsequent years, to facilitate longitudinal studies.

Longer term population-level data are critical to monitoring public health. Alcohol and tobacco use are good examples of the public health utility of wastewater analysis given the burden of disease attributable to the use of both drugs and the challenges in monitoring population trends in alcohol and tobacco use via surveys (e.g. under-reporting, declining survey response rates and the expense of conducting surveys which means that they can only be done annually at most and more often (as in Australia) three yearly). Wastewater analysis data on these drugs has the potential for much more frequent sampling and much better geographic data on prevalence (Lai et al., 2018). Our ultimate aim is to create companion Environmental Specimen and Environmental Data Banks as an invaluable record of population and environmental health. These data banks can be interrogated retrospectively as new and relevant target analytes and parameters emerge and questions about historical trends arise. This approach has potential to become a widely used policy tool for public and environmental health and to be applied in diverse fields, such as criminology and toxicology.

Wastewater analysis studies are becoming more and more widely accepted as a reliable approach to determine population-level trends in chemical consumption and exposure (Tscharke et al., 2018). Going forward, continued validation of the approach is required. This may be achieved through triangulation of the data outputs, i.e. validating wastewater studies outputs against other sources of data, for example, prescriptions or sales of pharmaceuticals. Appropriate collection, handling and storage of samples is also of the utmost importance in future wastewater studies to ensure integrity and reliability of the study outputs. Organising studies on a national scale has far reaching application and benefits but is logistically challenging. The current study contributes to addressing these challenges by clearly demonstrating the study aspects that worked effectively and how the study design and operation can be improved for future collections.

The utility of the wastewater analysis approach was clearly demonstrated by the census project and it is hard to put a limit on the future application of such studies. If humans excrete a chemical that is relatively stable in the sewer system and it can be measured in samples, then this approach can be used. As analytical instruments become more sensitive, an increasing number of analytes will be able to be retrospectively quantified in archived samples.

The census data provide accurate population and ancillary data to calculate and interpret trends and understand inter-regional differences in chemical consumption and exposure. Accessing additional social and regulatory data sources will enable researchers to study links between drug (licit or illicit) and alcohol use with crime rates, hospitalisation, drug busts and suicide rates. By combining wastewater data with census and health data, questions of exposure and effect can also be investigated, as demonstrated in a recent proof of concept study. Here, wastewater data combined with census population counts and data from WWTP questionnaires showed that: (i) there is a relationship between the per capita loads of histamine burden and antihistamine biomarkers, (ii) per capita loads of the histamine burden marker were within the range reported by metabolomics studies, and (iii) hydraulic retention time of sewer systems did not appear to explain variations in biomarker loads (Choi et al., 2018).

Collection of paired influent and effluent samples, as included in the

Table 6
Recommendations to consider for future studies.

Task/phase	Recommendation
WWTP recruitment	Early and targeted collaboration with state or regional governments may lead to a faster approach to recruit a higher number of WWTPs compared to individual direct approaches
	A different approach for privately run WWTPs may be more successful – e.g. top down approach with clear communication lines to operational personnel
	Establish clear cut-offs for WWTP participation to avoid last minute recruitments
	When the study team was not directly involved, communication channels sometimes broke down. Whilst opportunistic approaches, such as advertisements via existing networks, were generally successful, in future the wording of third party advertisements and other communiqués need to be more closely controlled to ensure WWTPs understand what is required before agreeing to participate
Sampling	The rationale for some of the critical QA/QC requirements could be better explained to WWTP personnel to help optimise sample handling (e.g. explain why samples should remain frozen and the significance of collecting close to the requested total volume of the sample in each bottle, i.e. a 400 mL 'fill to' mark was included on the label but smaller volumes were collected in some instances which would increase the concentration of the added preservative)
	Instructions could be delivered in the form of a short video that covers both the how and why of sampling
	WWTPs tended to default to their own procedures for sampling if the study's sampling procedure conflicted with their own, which may result in risks to sample integrity (for the study's purposes). A good understanding of each WWTP process for sampling would allow any conflicts to be identified, discussed up front and acceptable compromises struck. Greater flexibility with sampling dates may have allowed operators to coordinate our sampling and their sampling at different times to allow "best practice" to be implemented for our purposes
	Mobile pump units to collect samples were considered but not developed for this study. Whilst only time proportional sampling, rather than preferred flow proportional sampling could be achieved with such a pump, a low cost mobile solution would be of benefit to national studies where high spatial coverage is required. Further development of passive sampling of wastewater should also be considered where purchase of mobile units is cost prohibitive or impractical (e.g. no accessible power source near the wastewater channel)
Transport	It is better to send sampling kits well ahead of sample collection. Most sites have space to store the boxes and early dispatch takes pressure off the study team, the WWTP personnel and the courier company, and allows time to manage lost sampling kits
	The logistics of dispatching > 600 sampling kits was underestimated. It would be highly advantageous to have the chosen courier company understand the project aims and work with the study team to find solutions to labelling and other practicalities
	The use of temperature 'buttons' i.e. miniature temperature data loggers, placed inside the returned packing box would indicate if and for how long samples were not frozen. This would be particularly advantageous at sites with long transport periods such as remote sites
Questionnaires	Online questionnaires would ensure consistency of responses and ease of data interrogation
	Whilst the WWTP questionnaire was reduced in size as much as possible, ongoing evaluation of the need for each information request should be considered. Significant time was invested in getting questionnaires completed and returned and the length and complexity of the questionnaire are likely deterrents
	During initial discussions with the WWTP during the recruitment phase, identify the person/people who have the knowledge to complete the WWTP questionnaire and send the copy directly to them with a follow up call
Sample management	Whilst bar coding is the recommended way to manage sample returns, systematic checking of the quality of printed labels and matching the correct labels to sampling containers is crucial
Sample access	Controlled access to samples to monitor finite volumes, with an agreed approach to prioritise or rank requests to analyse samples should be established from the outset

study concept reported here, further opens up the current and future applications of national studies of this type. A study that includes a high number of WWTPs that utilise different treatment processes allows for greater understanding of changes in treatment efficiencies (through analysis of influent versus effluent samples) over time and whether these efficiencies are achieved on a national scale.

Samples from the present study may also inform our understanding of sources and fate of chemicals via their behaviour in the WWTP system. For example, recent observations of increased levels of perfluorinated compounds (chemicals of concern historically used in fire-fighting foams) in effluent compared to paired influent samples suggested new formation pathways not considered previously (Gallen et al., 2018). Overall, the breadth of current and anticipated future applications highlight the value of wastewater analysis studies and the concomitant need for effective WWTP recruitment and sound sample collection and handling practices to deliver high quality, high resolution, reliable data.

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Appendix A. Supplementary data

Supplementary information to this article including "S-1 Polyethylene (PE) passive sampler development and deployment" and "S-2 Sampling questionnaire used for the study" can be found online at https://doi.org/10.1016/j.envint.2018.12.003.

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