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Spatial and temporal patterns of Ross River virus in South East Queensland, Australia: identification of hot spots at the rural-urban interface

Amanda K. Murphy (amanda.murphy@qimrberghofer.edu.au)
QIMR Berghofer Medical Research Institute https://orcid.org/0000-0002-8993-033X

Julie A. Clennon
Emory University

Gonzalo Vazquez-Prokopec
Emory University

Cassie C. Jansen
Queensland Health

Francesca D. Frentiu
Queensland University of Technology

Louise M. Hafner
Queensland University of Technology

Wenbiao Hu
Queensland University of Technology

Gregor J. Devine
QIMR Berghofer Medical Research Institute

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Abstract

Background: Ross River virus (RRV) is responsible for the most common vector-borne disease of humans reported in Australia. The virus circulates in enzootic cycles between multiple species of mosquitoes, wildlife reservoir hosts and humans. Despite regular outbreaks, ongoing morbidity and substantial economic costs, the underlying determinants of epidemics remain unclear. Public health concern about RRV has recently increased due to rising incidence rates in Australian urban centres, along with increased circulation in Pacific Island countries. Australia experienced its largest recorded outbreak of 9,544 cases in 2015, with the majority reported from South East Queensland (SEQ). We aimed to provide a detailed analysis of disease trends and explore potential links between disease patterns and transmission pathways of RRV.

Methods: We assessed the spatial and temporal distribution of notified RRV cases, and associated epidemiological features in SEQ, from 2001-2016. This included fine-scale analysis of disease patterns across the suburbs of the capital city of Brisbane, and those of 8 adjacent Local Government Areas, and host spot analyses to identify locations with significantly high incidence.

Results: The mean annual incidence rate for the region was 41/100,000 with a consistent seasonal peak in cases between February and May. The highest RRV incidence was in adults aged from 30-64 years (mean incidence rate: 59/100,000), and females had higher incidence rates than males (mean incidence rates: 44/100,000 and 34/100,000, respectively). Spatial patterns of disease were heterogeneous between years, and there was a wide distribution of disease across both urban and rural areas of SEQ. Overall, the highest incidence rates were reported from predominantly rural suburbs to the north of Brisbane City, with significant hot spots located in peri-urban suburbs where residential, agricultural and conserved natural land use types intersect.

Conclusions: Although RRV is endemic across all of SEQ, transmission is most concentrated in areas where urban and peri-urban environments intersect. The drivers of RRV transmission across rural-urban landscapes should be prioritised for further investigation, including identification of specific vectors and hosts that mediate human spillover.

Background

Ross River virus (RRV) is a zoonotic alphavirus commonly circulating in Australia and the Western Pacific, and is responsible for the most widespread and frequently reported mosquito-borne disease in Australia (1, 2). An average of 5,409 RRV notifications were reported across Australia each year between 2006 and 2015 – an increase of 31% compared to the previous decade (1). In humans, symptoms of infection are similar to those of other alphaviruses, such as Barmah Forest, chikungunya and Sindbis, and may include fever, rash, fatigue and polyarthritic muscle and joint pains (3). While not fatal, RRV disease is associated with substantial morbidity and public health impact (4, 5), with symptoms including persistent pain and lethargy for weeks to months following infection (6–8). Because there are

no specific treatments for RRV, symptoms are managed through use of analgesic and anti-inflammatory drugs. It is estimated that notified cases represent only a small proportion of the infected population, as up to 80% of infections may be asymptomatic (9). Although a vaccine has been developed, challenges in determining commercial viability have hindered its progress to market (5, 10).

Ross River virus cases are reported across all Australian states, although Queensland typically reports around half of all annual cases (average of 48%, range 24–65% between 2001–2016). Historically considered a rural disease, cases have increasingly been observed in metropolitan areas of Perth, Brisbane, Sydney and Melbourne since the 1990s (11–16). The largest recorded Australian RRV epidemic occurred from late 2014, and continued through 2015, culminating in a record annual total of 9,544 cases in 2015 (1, 17). Sixty-five percent of these cases (6,193) were from Queensland, with 4,388 reported from the capital, Brisbane – a five-fold increase compared with the previous 4 years (18). Subsequent large outbreaks occurred in the states of New South Wales, Victoria and Western Australia in 2017, with the national total reaching 6,928 cases (1). These coincided with reports of unexpectedly high seroprevalence rates in Australia's neighbouring Pacific Island countries (2, 19). Although sporadic outbreaks in the Pacific Islands had been documented, these recent studies revealed that RRV circulates more regularly outside of Australia than previously thought (20).

RRV prevention relies solely upon mosquito control and avoidance of bites. Development of targeted strategies for RRV management is complicated by uncertainty about the specific vector and reservoir host species that are responsible for mediating epidemics (11, 21). RRV is maintained in enzootic cycles between multiple species of mosquitoes, animal reservoir hosts and humans, but transmission pathways are poorly understood (22–24). The virus infects several vertebrate host and mosquito vector species across different habitats and climate regions of Australia (3, 11). This includes at least 40 different mosquito species, associated with both freshwater and saltwater habitats (11). In coastal areas, the estuarine species Aedes (Ae.) vigilax (in northern Australia) and Ae. camptohynchus (in southern Australia) are considered likely vectors, while major inland vector species include freshwater-breeding Culex (Cx.) annulirostris, Ae. procax and the urban-associated Ae. notoscriptus (3). Many RRV vector species also have diverse host feeding behaviours which likely vary with environmental setting (25).

Vertebrate host species that maintain RRV circulation also vary with einvironmental setting. In rural areas, marsupial mammals are suspected to be a major, but not sole, reservoir (20, 21). Hosts such as humans, livestock, rodents and birds could also play a role in virus maintenance and amplification, particularly in urban areas where marsupials are less common (20). However, the specific determinants of transmission, including the most important mosquito vectors and reservoir hosts in different habitat types and geographic locations, remain unknown (22–24). Factors such as climate, vegetation cover, and human behaviour likely play a role in transmission, but their relative roles are not very well defined (26–30). This diversity in the transmission cycle complicates epidemiological investigation, prediction and control (15, 22). Hence, to be most informative, RRV studies require regional (rather than national-scale) approaches, adapted to specific ecological settings (31).

The South East Queensland (SEQ) region, including the capital city of Brisbane, experiences regular outbreaks and high morbidity caused by RRV. Despite this, few spatial and temporal analyses of RRV trends have been conducted over the past 2 decades, and none that assess the entire region. RRV is known to have been transmitted across both urban and peri-urban areas of Brisbane since at least the 1990's, although the specific determinants of outbreaks remain uncertain (28, 29). Studies of two large outbreaks in Brisbane indicated a wide distribution of cases across the city (14, 17), although increased risk has also been associated with living in proximity to freshwater bushland and wetland environments (29, 32). We explored the contemporary distribution and epidemiological characteristics of RRV in SEQ between 2001 and 2016. Fine-scale spatial and temporal trends in distribution of RRV were assessed in urban and rural areas, with the aim to provide a detailed analysis of disease trends, and explore potential links between disease patterns and transmission pathways of RRV.

Methods

Study location

Queensland is Australia's third most populated state, with 4.9 million inhabitants. We explored patterns of notified RRV cases in nine Local Government Areas (LGAs) of South East Queensland (SEQ): City of Brisbane, City of Ipswich, Moreton Bay Region, Redland City, Logan City, City of Gold Coast, Scenic Rim Region, Sunshine Coast Region and Shire of Noosa (Fig. 1). These 9 LGAs encompass an area of approximately 22,000 km^2 and together comprise 80% of the state's population (3.2 million), including 1.1 million in the capital city of Brisbane. The region has a sub-tropical climate, and diverse natural ecosystems including freshwater and estuarine wetlands, Mangrove shrubland and saltmarsh, Eucalypt and Melaleuca woodlands, and rainforest (33).

RRV notification data

Daily RRV notification data were obtained from the Queensland Department of Health's Notifiable Conditions Surveillance System (NoCS) for the 16-year period between 1st January 2001 and 31st December 2016. Case data included: disease onset date (an estimate, based on reported onset of illness at the time of presentation to a medical clinic), age, gender, and geographical location of case residence. The residential addresses of cases were aggregated to two different geographical unit classifications used by State and Territory Local Government Departments, and described in the Australian Standard Geographical Classification (34). These were the LGA and the State Suburb Code (SSC). The broadest scale unit was the LGA, which is equivalent to a large municipal area, with a population of up to 1,100,000 people (average of 40,000); while SSC represented the finest geographical unit size, equivalent to a suburb or neighbourhood, comprising populations up to 50,000 (average 1,500). The study area included a total of 774 SSCs across 9 LGAs, of which 17 were unpopulated and excluded from the analyses. Population data for LGAs and SSCs were extracted from census data from the Australian Bureau of Statistics (ABS) for census years between 2001 and 2016 (35). Annual population figures, and

population by gender and age-group, were matched to the notification data for each LGA and SSC for the calculation of incidence rates.

Rural and urban classifications

We classified each SSC as rural or urban according to the Australian Statistical Geography Standard (ASGS), which defines urban areas of Australia (36). Urban or rural designation is based on population density and urban infrastructure criteria for each Section of State (SOS), obtained from census data. These were matched to each SSC using Geographical Information System (GIS) software ArcMap 10.6 (ESRI, Redlands, CA, USA). The ASGS groups urban areas into the sub-categories: 'Major Urban'; a combination of Urban Centres with a total population of 100,000 or more, and 'Other Urban'; a combination of Urban Centres with a population between 1,000 and 99,999. Rural areas also have two sub-categories: 'Bounded Locality'; a population centre of between 200 and 999 residents, and 'Rural Balance'; which forms the Remainder of the State/Territory. The ABS considers both categories 'Major Urban' and 'Other Urban' as urban, while 'Bounded Locality' and 'Rural Balance' are rural. In our dataset, 292 SSCs were classified as rural and 465 as urban.

Land use data

Land use maps were obtained from the Queensland Government Land Use Mapping Program (QLUMP), available from the Queensland Spatial Catalogue (37). The QLUMP maps and assesses land use patterns and changes across the state, according to the Australian Land Use and Management (ALUM) Classification (Australian Department of Agriculture, version 8, October 2016) (38). The ALUM Classification is a detailed national standard that classifies land use types in order of increasing levels of modification of the natural landscape. The 6 classes are: 1. Conservation and Natural Environments: Land is used primarily for conservation purposes, based on the maintenance of essentially natural ecosystems already present; 2. Production from Relatively Natural Environments: Land is used mainly for primary production based on limited change to the native vegetation; 3. Production from Dryland Agriculture and Plantations: Land is used mainly for primary production, based on dryland farming systems; 4. Production from Irrigated Agriculture and Plantations: Land is used mainly for primary production, based on irrigated farming; 5. Intensive uses: Land is subject to substantial modification, generally in association with closer residential settlement, commercial or industrial uses; and 6. Water. Land use maps were imported into ArcMap 10.6 (ESRI, Redlands, CA, USA) for visualisation.

Data analysis

RRV notification epidemic features were explored over time, with monthly, annual and mean incidence rates calculated for LGAs, SSCs and the overall SEQ region. Spatial and temporal patterns of RRV notification rates were compared by age and sex, and by rural versus urban classification. Differences were tested for statistical significance using the Kruskal-Wallis or Mann-Whitney non-parametric tests, implemented in Statistical Package for the Social Sciences (SPSS) Statistics software (IBM New York USA; version 23), with a significance level of 0.05.

Spatial analyses included calculation of smoothed incidence rates and hot spot analysis, both performed using GeoDa software (Luc Anselin, version 1.12.1.161, September 2018). Spatial smoothing of rates were performed using spatial Empirical Bayes (EB) smoothing technique, which corrects for outliers in raw (crude) rates to increase precision, especially where raw rates are unstable or have a high variance. The spatial EB technique recalculates the incidence rate for each geographical unit (SSC), applying a weighted average to each that is between its raw rate and that of its immediate neighbouring geographical units (queen continguity scheme) (39). This has the effect of reducing the rates of extremely high-rate SSCs, while also increasing the rate of low-rate SSCs. Weights applied to the calculation for each SSC are proportional to the population at risk, so that smaller populations will have their rates adjusted considerably, whereas rates for larger populations will change less. In our dataset, high variances were observed due to the presence of small populations with low case numbers in some areas.

Hot spot analyses employed the Getis and Ord for G* local spatial autocorrelation statistic (40) for detecting hot and cold spot SSCs. Hot spot analysis was conducted using both raw and smoothed annual and mean incidence rates for SSCs, using queen contiguity neighbourhood criteria (adjusting rates by the average of immediate neighbouring SSCs in any direction). We identified and reported hot and cold spots that were significant in the raw analysis, and those that were shared between raw and smoothed analyses. Because hot/cold spots identified using mean rates were heavily influenced by the large magnitude outbreaks in 2014 and 2015, we also identified hot and cold spots that were persistently detected across at least two individual years. For this, we counted how many years an individual SSC was identified as a hot/cold spot, and defined those present in at least 2/16 years as persistent. Hot spots were then mapped against urban/rural classification and land use types of SEQ. Maps of population distribution, incidence rates and hot spots were created using ArcMap 10.6 (ESRI, Redlands, CA, USA).

Results

Demographic trends

For the period 2001–2016, a total of 18,115 RRV notifications were analysed across the SEQ region. During this period, the mean annual RRV notification rate across all of SEQ was 41 cases/100,000 population. Mean annual rates were higher in females versus males, at 44/100,000 population compared to 34/100,000, respectively, though this difference was not significant (Mann-Whitney $U = 84$, $p = 0.102$). For both genders, the highest incidence rates occurred in the 40–44 and 45–49 age categories (69 and 66/100,000, respectively) and the lowest in the two age categories < 10 years (1 and 3/100,000, respectively) (Fig. 2). The overall trend showed a gradual increase in incidence from birth up to age 29 years (mean incidence of 17/100,000 across these age groups), peaking between ages 30–64 years (mean 59/100,000), and dropping again ≥ 65 years (mean 27/100,000). This trend was consistent across all years of this study. Statistical comparisons within and between these three broad age groups indicated that rates did not differ significantly between adults from 30–64 years (Kruskal-Wallis $H = 8$, $p =$

0.239), but that this group's rates were significantly higher than those aged ≤ 29 years (Mann-Whitney U = 1175, $p < 0.0001$), and ≥ 65 years (U = 1589, $p < 0.0001$).

Temporal trends

RRV disease occurs in SEQ throughout all months of the year; however, a distinct seasonal pattern in the timing of annual notification peaks was shared amongst all LGAs. The peak annual notification period was typically between February and May, and this was consistent across years with higher and lower notifications (Fig. 3). These months also show the highest variability in case numbers (with a monthly average of 186 cases across SEQ, SD 246). Conversely, notifications during the winter and spring months between June-November are generally low and relatively stable across the region (monthly average of 46 cases, SD 35). The relative magnitude of RRV outbreaks across the region were variable between years, with larger and smaller outbreaks occurring in intermittent years (the monthly temporal trend is shown in Additional file 1). Long-term trends indicated that outbreaks generally occurred synchronously across the region, rather than initially occurring in one LGA and then spreading to another. This was also the case during largest recorded outbreak of 2015, which began earlier than usual (in late 2014) and peaked in February-March 2015, with timing consistent across all SEQ LGAs.

Spatial trends

Spatiotemporal patterns of RRV disease were variable between the 9 LGAs, with the highest case numbers in the most populated LGA, Brisbane City (5,352 total cases), and the lowest in the sparsely populated Scenic Rim Region (393 total cases) (Table 1). Annual case numbers for each LGA can be found in Additional file 2. Mean annual incidence rates across the 16-year period, and ranged from 30/100,000 in Gold Coast City to 130/100,000 in Noosa Shire (Table 1), with rates in Noosa Shire being significantly higher than all other LGAs (Mann-Whitney U = 269, $p < 0.0001$). Rates were also routinely high in the Sunshine Coast Region and sporadically high in the Scenic Rim Region. These 3 areas (Noosa Shire, Scenic Rim Region and Sunshine Coast Region) had the highest incidence rates overall, and had more rural characteristics (lower population density and proportion of urban SSCs) compared with the 4 largest cities: Brisbane, Gold Coast, Ipswich and Logan (Table 1). Conversely, these higher-density cities together had significantly lower rates than all other LGAs (Mann-Whitney U = 1098, $p < 0.0001$), but were not significantly different from each other (Kruskal-Wallis H = 3, $p = 0.361$).

Table 1
Summary characteristics of each Local Government Area (LGA) of South East Queensland during the study period, 2001–2016.

LGA name*	Average population (2001–2016)	Population density (per km^2)	Total cases (2001–2016)	Proportion of urban SSCs	Mean annual incidence rate (per 100,000)
Brisbane City	1,023,663	762	5,352	94.2%	33
Gold Coast City	475,169	356	2,252	74.1%	30
Ipswich City	157,963	146	1,044	58.0%	41
Logan City	271,082	283	1,456	81.7%	34
Moreton Bay Region	357,532	175	3,097	58.5%	54
Noosa Shire	41,008	47	850	29.2%	130
Redland City	134,328	250	991	63.6%	46
Scenic Rim Region	35,443	8	393	2.8%	69
Sunshine Coast Region	256,982	114	2,680	50.4%	65
Total	2,753,170	188	18,115	61.0%	41

* See Fig. 1 for location of each LGA within the study area.

At the SSC level, spatial trends were also highly varied. The spatial trend in high-incidence SSCs changed from one year to the next (recent annual patterns are shown in Additional file 3), but overall the SSCs with the highest raw and smoothed incidence rates were primarily located in Noosa Shire and the Sunshine Coast Region (Fig. 4, Additional file 4). However, the highest rate of all SSCs was for Amberley in Ipswich City LGA (raw mean rate 676/100,000 and smoothed mean rate of 562/100,000). The distribution of incidence rates for rural and urban SSCs in each LGA are shown in Fig. 5. The trend across LGAs showed that SSCs with the highest incidence rates tended to have low-mid range population densities. Mean annual RRV rates were 70 cases/100,000 (smoothed rate 85/100,000) in rural SSCs, and 44/100,000 (smoothed rate 43/100,000) in urban SSCs. Both raw and smoothed incidence rates were significantly higher in rural SSCs compared to urban SSCs (Mann-Whitney $U = 58{,}388$, $p = 0.001$ for raw; and $U = 24{,}359$, $p < 0.0001$ for smoothed).

Hot spot analyses

Using raw mean annual incidence rates, a total of 127/757 (17%) SSCs were identified as hot spots and 352/757 (47%) as cold spots in any individual year. These increased to 178 and 458 SSCs, respectively, using smoothed rates. A comparison of raw versus smooth analyses for annual hot and cold spots across individual years was shown in Additional file 5. In either raw or smoothed analyses, 86 hot spots and 272 cold spots were persistent (present in ≥ 2 years). There were 14 SSCs that were both hot and cold spots in ≥ 2 years that were excluded, leaving 72 persistently hot and 258 persistently cold spots. Of these, 45 hot and 154 cold spots were shared between both analyses (shown in Additional file 6). Persistent hot spots were similar to those identified using mean annual rates (n = 56 mean hot spots), while mean cold spots differed (n = 47 mean cold spots). Although hot spots were geographically dispersed across all LGAs, the SSCs with the most persistent hot spots were in the Sunshine Coast Region and Noosa Shire LGAs. The same hot spots were rarely detected in consecutive years, although some were detected in multiple years, up to 7/16 years (Additional file 7). Conversely, cold spots tended to persist more in the same SSCs across several years, particularly in the Scenic Rim Region where there were very low populations and cases (Additional file 6).

A visualisation of hot spots relative to rural and urban areas of SEQ is shown in Fig. 6a. Hot spots tended to be most focused around the edges of where major urban and rural areas intersect. Of the 72 persistent hot spots detected in either raw or smoothed analyses, 35 were located in urban and 37 in rural SSCs (for the 45 hot spots shared between both analyses, 19 were urban and 26 rural). There also appeared to be diverse land use types within or adjacent to hot spot SSCs (Fig. 6b). All hot spots contained some degree of urban infrastructure, and many were located in close proximity to either dryland agriculture and plantations or to major water bodies. While some more inland hot spots were surrounded largely by conservation and natural environments, these environments appeared to be most often identified as cold spots (Fig. 6, Additional file 6). Very few hot spots were located centrally within major urban areas, rather than on the edge. Only one persistent hot spot SSC was identified within Brisbane City LGA (Chelmer, identified in 2/16 years) using raw rates only. The largest cities including Brisbane, Gold Coast and Logan LGAs were more commonly dominated by cold spots rather than hot spots (Additional files 5 and 6).

Discussion

This study sought to describe RRV spatial and temporal incidence patterns in SEQ, and to identify epidemiological trends that help elucidate the drivers of virus spillover. We found that incidence rates were highest for females, for age groups between 30–64 years, and for residents of rural suburbs (SSCs), especially those north of Brisbane. Suburbs around the edges of major urban areas were persistent annual hot spots for RRV disease. This suggests that suburbs in rural and peri-urban areas possess characteristics that promote circulation of RRV, possibly related to specific habitat or land use types present. The specifc contributors to human infection in different environments are uncertain, and this requires further investigation. In particular, identification of RRV vectors and hosts in areas where natural and urbanised environments meet.

The demographic trends we observed were comparable with those of previous Australian studies, with the highest rates in females, and in age groups between 30 and 64 years (3, 8, 9, 11, 41). Although male to female prevalence ratios have varied slightly in previous studies, no overall gender-related risk has been apparent (3, 8). Clinical studies report that children show fewer symptoms than adults, presumably due to age-related differences in immune responses, while symptoms tend to persist longer in adults (8, 11, 41, 42). However, seroprevalence studies have shown RRV antibody seroconversion to increase with age (43–45) suggesting that the true incidence of infection may be higher in younger age groups than indicated by notified cases. In all likelihood, exposure to RRV is probably equivalent across all ages and genders, with higher reports in adults due to differing disease manifestations with age. Disease rates will also vary with geographical region, as the tropical northern regions of Australia have higher rates than the temperate south (1). Because few comprehensive seroprevalence studies have been conducted in SEQ, the true age-related burden is unknown.

There was a strikingly consistent seasonal trend in outbreaks across SEQ, peaking annually between the months of February and May. This coincides with periods of relatively high temperature and rainfall from late austral summer to early autumn, when SEQ's average daily temperatures are 20–24°C (46). Although climate alone does not predict outbreak occurrence, it influences vector and wildlife host species' abundance (47–50). Favourable temperature conditions, rainfall, high tides and low-level flooding have all been associated with elevated RRV risk in previous studies (26–28, 51–53). Temperature also impacts viral replication, with the ideal temperature for RRV transmission at 26.4 degrees celsius (transmission range of 17 to 31.5°C) (54). However, variations in weather patterns and vector-host ecology mean that climate-based predictions are only valid locally or, at best, regionally, rather than nationally (55, 56). Hence, although suitable weather conditions are a requisite precursor to outbreaks, outbreak occurrence ultimately depends on availability of, and interactions between, sufficient competent vectors and susceptible hosts (57, 58).

The variable annual spatial trend observed suggests that conditions supporting transmission occur sporadically in particular SSCs, and may change from one year to the next. This might be due to local climatic and environmental variations which influence vector and host abundance in both freshwater and saltwater habitats (23, 27). Freshwater vectors Cx. annulirostris, Ae. notoscriptus, Ae. procax, and Ae. vittiger as well as saltwater vectors Ae. vigilax, Cx. sitiens and Verallina funerea were associated with large outbreaks in Brisbane and the Sunshine Coast Region during the 1990s (14, 59). The most recent outbreaks of 2014–2015 were linked to increased abundance of the freshwater vectors Cx. annulirostris and Ae. procax in Brisbane following high rainfall (17). Many of the implicated vectors share similar or overlapping habitat types and have broad host-feeding behaviours (25). Hence, the relative contribution of different vectors is to human outbreaks is challenging to disentangle. It is possible that multiple vectors in different habitats contribute to varying degrees, at different times (60, 61).

Similarly, a number of different hosts that maintain RRV circulation across SEQ could contribute to epidemics. Although few studies have investigated the role of specific wildlife hosts in human RRV outbreaks, opportunistic serosurveys of wildlife together with a handful of experimental infection studies

have generated some hypotheses (21). Potential hosts theorised to contribute to RRV transmission include birds, small mammals and marsupials (including rodents, possums, flying foxes) in urban areas; and larger mammals and marsupial macropods (such as horses and cattle, kangaroos and wallabies) in peri-urban and rural areas (15, 20, 21, 57). However, current evidence identifying important RRV hosts is limited, and broader investigations of the transmission potential of wildlife are much needed (57). In the absence of these, it can be assumed from RRV's wide geographic and habitat range that there is flexibility in both vectors and hosts. In SEQ, the seasonal composition of vectors and hosts in peri-urban habitats, especially those in proximity to hot spot suburbs, should be a particular focus for future RRV transmission studies.

We identified both high incidence rates and the most persistent hot spots overall in Noosa Shire and Sunshine Coast Region, in which there are low-medium human population densities and diverse land use types present. The specific drivers of high rates of RRV in these LGAs are uncertain, but could relate to the proximity of peri-urban human populations to rural vector and wildlife habitats. Interactions between humans, vectors and wildlife in or near particular land use types in peri-urban areas could create a 'perfect storm' of factors supporting RRV transmission. Human-modified and fragmented landscapes are known to influence the risk of vector borne diseases, either positively or negatively, through altering ecological relationships between wildlife, vectors and humans. (62–64). Land use changes such as deforestation and agricultural development have been linked to increased risk of West Nile virus and malaria infection (65, 66), and have been linked to arboviral disease risk in Australia (67, 68). While our findings do not confirm a link between land use and RRV risk, they do suggest this could be worthy of investigation. Given RRV's expansion into urban and outer metropolitan areas of Australia in recent years, it is conceivable that urban expansion and alteration of wildlife habitats may have implications for RRV risk.

The absence of hot spots, and concentration of cold spots, in the Scenic Rim LGA suggests that it lacks sufficient human, wildlife host and vector populations to maintain persistent outbreaks. This is despite the Scenic Rim having a large proportion of natural conservation and irrigated agricultural areas, which could theoretically support mosquito and wildlife habitats. This might be explained by the low human population in this LGA, which results in sporadically high but inconsistent incidence rates, and unstable spatial patterns of disease (both hot and cold spots in the same suburbs in different years). This pattern could potentially change if human populations in the Scenic Rim were to increase. Again, analyses of different land use types, their association with specific vector and host habitats, and RRV risk would assist understanding of how and where these factors inter-relate.

This study is the first to describe long-term epidemiological trends of RRV across SEQ. We report RRV disease patterns at both broad (LGA) and fine (SSC) scales, and identify characteristics associated with higher RRV risk that can inform future investigations. However, the study was limited by its reliance on routinely collected public health data, and the associated challenges with passive disease reporting. In Queensland, notification processes for RRV do not include individual case interview, nor information on the timing and location of RRV exposure, which is often unknown. We used the case's reported onset date

of symptoms and residential address as a proxy for this. While we expect that many residents will be bitten and infected in their home suburb, this will not be true for all, and there is no way to correct for this. Nevertheless, given RRV's high incidence and wide geographical range across SEQ, using the case residence seems a reasonable proxy for location of infection. Socioecological factors not accounted for in our study may also have influenced the demographic trends we observed. For instance, healthcare-seeking practices likely differ between genders and age-groups, and exposure to mosquitoes through occupational or leisure activities could also differ between demographic groups. The impact of socioeconomic factors on infection risk has also varies by geographic region (22, 23, 28). However, within our study region, previous studies indicate that socioeconomic variation is unlikely to have had a significant impact on our results (28, 32).

Conclusions

Overall, our findings contribute to understanding of RRV disease patterns and public health risk in SEQ. To further progress understanding of RRV transmission risk and improve future disease prediction and prevention, greater understanding of seasonal variation in distribution and abundance of potential vectors and hosts is essential. For SEQ, this is especially important in urban fringe areas, and areas undergoing urban expansion. Peri-urban suburbs at the rural-urban interface, especially where different land use types intersect, could be most capable of supporting adequate densities of vectors, hosts and humans to allow persistent transmission. We recommend that hot spots identified in these 'edge' locations be targeted for further investigation of RRV transmission pathways. Clarifying the relative importance of specific contributors to RRV epidemics is a priority for developing targeted disease prevention strategies – and may have flow-on benefits for prevention of other endemic and imported mosquito-borne infections in Australia.

Declarations

Ethics approval and consent to participate

Access and use of the human notification data for SEQ was approved by the Human Research Ethics Committees of QIMR Berghofer Medical Research Institute (QIMRB) and Queensland University of Technology (QUT) (Reference number P2238). Approval to access and use case data was obtained from the Queensland Department of Health as data custodian.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AM, WH, and GD conceived the project. AM carried out the analyses and drafted the manuscript, with input from JC, GVP, CJ, WH and GD. FF and LH aided interpretation. All authors reviewed and revised the final manuscript and agreed to its submission.

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References

1. National Notifiable Diseases Surveillance System: Commonwealth of Australia; Australian Government. [Available from: http://www9.health.gov.au/cda/source/cda-index.cfm.
2. Lau C, Aubry M, Musso D, Teissier A, Paulous S, Despres P, et al. New evidence for endemic circulation of Ross River virus in the Pacific Islands and the potential for emergence. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2017;57:73-6.
3. Harley D, Sleigh A, Ritchie S. Ross River virus transmission, infection, and disease: a cross-disciplinary review. Clin Microbiol Rev. 2001;14(4):909-32, table of contents.
4. Ratnayake JTB. PhD Thesis: The Valuation of Social and Economic Costs of Mosquito-Transmitted Ross River Virus: Griffith University; 2006.
5. Woodruff R, Bambrick H. Climate change impacts on the burden of Ross River virus disease. National Library of Australia.: Commonwealth of Australia; Garnaut Climate Change Review. June 2008.
6. Condon RJ, Rouse IL. Acute symptoms and sequelae of Ross River virus infection in South-Western Australia: a follow-up study. Clinical and diagnostic virology. 1995;3(3):273-84.

7. Suhrbier A, La Linn M. Clinical and pathologic aspects of arthritis due to Ross River virus and other alphaviruses. Curr Opin Rheumatol. 2004;16(4):374-9.
8. Selden SM, Cameron AS. Changing epidemiology of Ross River virus disease in South Australia. Med J Aust. 1996;165(6):313-7.
9. Aaskov JG, Ross P, Davies CE, Innis MD, Guard RW, Stallman ND, et al. Epidemic polyarthritis in northeastern Australia, 1978-1979. Med J Aust. 1981;2(1):17-9.
10. Tomerini DM, Dale PE, Sipe N. Does mosquito control have an effect on mosquito-borne disease? The case of Ross River virus disease and mosquito management in Queensland, Australia. J Am Mosq Control Assoc. 2011;27(1):39-44.
11. Russell RC. Ross River virus: ecology and distribution. Annual review of entomology. 2002;47:1-31.
12. Lindsay M, Oliveira N, Jasinska E, Johansen C, Harrington S, Wright AE, et al. An outbreak of Ross River virus disease in Southwestern Australia. Emerging infectious diseases. 1996;2(2):117-20.
13. Brokenshire T, Symonds D, Reynolds R, Doggett S, Geary M, Russell R. A cluster of locally-acquired Ross River virus infection in outer western Sydney. N S W Public Health Bull. 2000;11(7):132-4.
14. Ritchie SA, Fanning ID, Phillips DA, Standfast HA, McGinn D, Kay BH. Ross River virus in mosquitoes (Diptera:Culicidae) during the 1994 epidemic around Brisbane, Australia. J Med Entomol. 1997;34(2):156-9.
15. Claflin SB, Webb CE. Ross River Virus: Many Vectors and Unusual Hosts Make for an Unpredictable Pathogen. PLoS pathogens. 2015;11(9):e1005070.
16. Tong S, Bi P, Hayes J, Donald K, Mackenzie J. Geographic variation of notified Ross River virus infections in Queensland, Australia, 1985-1996. The American journal of tropical medicine and hygiene. 2001;65(3):171-6.
17. Jansen CC, Shivas MA, May FJ, Pyke AT, Onn MB, Lodo K, et al. Epidemiologic, Entomologic, and Virologic Factors of the 2014–15 Ross River Virus Outbreak, Queensland, Australia. Emerging infectious diseases. 2019;25(12).
18. Notifiable Conditions Reports: Queensland Government; Queensland Department of Health. [Available from: https://www.health.qld.gov.au/clinical-practice/guidelines-procedures/diseases-infection/surveillance/reports/notifiable/annual/default.asp.
19. Aubry M, Finke J, Teissier A, Roche C, Broult J, Paulous S, et al. Silent Circulation of Ross River Virus in French Polynesia. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2015;37:19-24.
20. Flies EJ, Lau CL, Carver S, Weinstein P. Another Emerging Mosquito-Borne Disease? Endemic Ross River Virus Transmission in the Absence of Marsupial Reservoirs. BioScience. 2018;68(4):288-93.
21. Stephenson EB, Peel AJ, Reid SA, Jansen CC, McCallum H. The non-human reservoirs of Ross River virus: a systematic review of the evidence. Parasites & vectors. 2018;11(1):188.
22. Tong S, Dale P, Nicholls N, Mackenzie JS, Wolff R, McMichael AJ. Climate variability, social and environmental factors, and ross river virus transmission: research development and future research

needs. Environ Health Perspect. 2008;116(12):1591-7.

23. Kelly-Hope LA, Purdie DM, Kay BH. Ross River virus disease in Australia, 1886-1998, with analysis of risk factors associated with outbreaks. J Med Entomol. 2004;41(2):133-50.
24. Jardine A, Neville PJ, Lindsay MD. Proximity to mosquito breeding habitat and Ross River virus risk in the Peel region of Western Australia. Vector borne and zoonotic diseases. 2015;15(2):141-6.
25. Stephenson EB, Murphy AK, Jansen CC, Peel AJ, McCallum H. Interpreting mosquito feeding patterns in Australia through an ecological lens: an analysis of blood meal studies. Parasites & vectors. 2019;12(1):156.
26. Hu W, Nicholls N, Lindsay M, Dale P, McMichael AJ, Mackenzie JS, et al. Development of a predictive model for ross river virus disease in Brisbane, Australia. The American journal of tropical medicine and hygiene. 2004;71(2):129-37.
27. Tong S, Hu W, Nicholls N, Dale P, MacKenzie JS, Patz J, et al. Climatic, high tide and vector variables and the transmission of Ross River virus. Intern Med J. 2005;35(11):677-80.
28. Hu W, Clements A, Williams G, Tong S, Mengersen K. Bayesian spatiotemporal analysis of socio-ecologic drivers of Ross River virus transmission in Queensland, Australia. The American journal of tropical medicine and hygiene. 2010;83(3):722-8.
29. Muhar A, Dale PE, Thalib L, Arito E. The spatial distribution of ross river virus infections in Brisbane: Significance of residential location and relationships with vegetation types. Environ Health Prev Med. 2000;4(4):184-9.
30. Ng V, Dear K, Harley D, McMichael A. Analysis and prediction of Ross River virus transmission in New South Wales, Australia. Vector borne and zoonotic diseases. 2014;14(6):422-38.
31. Flies EJ, Weinstein P, Anderson SJ, Koolhof I, Foufopoulos J, Williams CR. Ross River Virus and the Necessity of Multiscale, Eco-epidemiological Analyses. J Infect Dis. 2018;217(5):807-15.
32. Hu W, Tong S, Mengersen K, Oldenburg B. Exploratory spatial analysis of social and environmental factors associated with the incidence of Ross River virus in Brisbane, Australia. The American journal of tropical medicine and hygiene. 2007;76(5):814-9.
33. Regional ecosystem descriptions Queensland Government. [updated April 16, 2019. Available from: https://apps.des.qld.gov.au/regional-ecosystems/list/?bioregion=12.
34. Australian Standard Geographical Classification (AGSC) Canberra, AustraliaAustralian Bureau of Statistics; October 5, 2011. [Available from: http://www.abs.gov.au/AUSSTATS/abs@.nsf/Previousproducts/1216.0Contents11999?opendocument&tabname=Summary&prodno=1216.0&issue=1999&num=&view=.
35. Australian census data by geography: Commonwealth of Australia; Australian Bureau of Statistics. [Available from: https://www.abs.gov.au/websitedbs/D3310114.nsf/Home/Census?OpenDocument&ref=topBar.
36. Australian Statistical Geography Standard (ASGS): Volume 3 - Non ABS Structures. Australian Bureau of Statistics.

37. Queensland Spatial Catalogue - QSpatial: Queensland Government; Queensland State Department of Natural Resources, Mines and Energy. [updated June 4, 2019. Available from: http://qldspatial.information.qld.gov.au/catalogue/custom/index.page.
38. Australian Land Use and Management Classification Version 8. Canberra, AustraliaDepartment of Agriculture and Water Resources, Australian Bureau of Agricultural and Resource Economics and Sciences.
39. Anselin L, Lozano-Gracia N, Koschinky J. Rate Transformations and Smoothing. Technical Report., Spatial Analysis Laboratory DoG; 2006.
40. Ord JK, Getis A. Local Spatial Autocorrelation Statistics: Distributional Issues and Application. Geographical Analysis. 1995;27(4).
41. Hawkes RA, Boughton CR, Naim HM, Stallman ND. A major outbreak of epidemic polyarthritis in New South Wales during the summer of 1983/1984. Med J Aust. 1985;143(8):330-3.
42. Mackenzie JS, Smith DW. Mosquito-borne viruses and epidemic polyarthritis. Med J Aust. 1996;164(2):90-3.
43. Boughton CR, Hawkes RA, Naim HM, Wild J, Chapman B. Arbovirus infections in humans in New South Wales. Seroepidemiology of the alphavirus group of togaviruses. Med J Aust. 1984;141(11):700-4.
44. Hawkes RA, Pamplin J, Boughton CR, Naim HM. Arbovirus infections of humans in high-risk areas of south-eastern Australia: a continuing study. Med J Aust. 1993;159(3):159-62.
45. Doherty RL. Surveys of haemagglutination-inhibiting antibody to arboviruses in Aborigines and other population groups in Northern and Eastern Australia, 1966-1971. Trans R Soc Trop Med Hyg. 1973;67(2):197-205.
46. Climate Data Online: Commonwealth of Australia; Australian Bureau of Meteorology. [Available from: http://www.bom.gov.au/jsp/ncc/climate_averages/temperature/index.jsp.
47. Williams CR, Fricker SR, Kokkinn MJ. Environmental and entomological factors determining Ross River virus activity in the River Murray Valley of South Australia. Aust N Z J Public Health. 2009;33(3):284-8.
48. Jacups SP, Whelan PI, Markey PG, Cleland SJ, Williamson GJ, Currie BJ. Predictive indicators for Ross River virus infection in the Darwin area of tropical northern Australia, using long-term mosquito trapping data. Tropical medicine & international health : TM & IH. 2008;13(7):943-52.
49. Ritchie EG, Bolitho EE. Australia's savanna herbivores: bioclimatic distributions and an assessment of the potential impact of regional climate change. Physiol Biochem Zool. 2008;81(6):880-90.
50. Martin GA, Yanez-Arenas C, Roberts BJ, Chen C, Plowright RK, Webb RJ, et al. Climatic suitability influences species specific abundance patterns of Australian flying foxes and risk of Hendra virus spillover. One Health. 2016;2:115-21.
51. Tall JA, Gatton ML. Flooding and Arboviral Disease: Predicting Ross River Virus Disease Outbreaks Across Inland Regions of South-Eastern Australia. J Med Entomol. 2019.

52. Woodruff RE, Guest CS, Garner MG, Becker N, Lindesay J, Carvan T, et al. Predicting Ross River virus epidemics from regional weather data. Epidemiology. 2002;13(4):384-93.
53. Tong S, Bi P, Donald K, McMichael AJ. Climate variability and Ross River virus transmission. J Epidemiol Community Health. 2002;56(8):617-21.
54. Shocket MS, Ryan SJ, Mordecai EA. Temperature explains broad patterns of Ross River virus transmission. Elife. 2018;7.
55. Jacups SP, Whelan PI, Currie BJ. Ross River virus and Barmah Forest virus infections: a review of history, ecology, and predictive models, with implications for tropical northern Australia. Vector borne and zoonotic diseases. 2008;8(2):283-97.
56. Kelly-Hope LA, Purdie DM, Kay BH. El Nino Southern Oscillation and Ross River virus outbreaks in Australia. Vector borne and zoonotic diseases. 2004;4(3):210-3.
57. Carver S, Bestall A, Jardine A, Ostfeld RS. Influence of hosts on the ecology of arboviral transmission: potential mechanisms influencing dengue, Murray Valley encephalitis, and Ross River virus in Australia. Vector borne and zoonotic diseases. 2009;9(1):51-64.
58. Koolhof IS, Carver S. Epidemic host community contribution to mosquito-borne disease transmission: Ross River virus. Epidemiology and infection. 2017;145(4):656-66.
59. Ryan PA, Do KA, Kay BH. Spatial and temporal analysis of Ross River virus disease patterns at Maroochy Shire, Australia: association between human morbidity and mosquito (Diptera: Culicidae) abundance. J Med Entomol. 1999;36(4):515-21.
60. Hu W, Mengersen K, Dale P, Tong S. Difference in mosquito species (Diptera: Culicidae) and the transmission of Ross River virus between coastline and inland areas in Brisbane, Australia. Environmental entomology. 2010;39(1):88-97.
61. Hu W, Tong S, Mengersen K, Oldenburg B, Dale P. Mosquito species (Diptera: Culicidae) and the transmission of Ross River virus in Brisbane, Australia. J Med Entomol. 2006;43(2):375-81.
62. Steiger DB, Ritchie SA, Laurance SG. Land Use Influences Mosquito Communities and Disease Risk on Remote Tropical Islands: A Case Study Using a Novel Sampling Technique. The American journal of tropical medicine and hygiene. 2016;94(2):314-21.
63. Kilpatrick AM, Randolph SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet. 2012;380(9857):1946-55.
64. Gottdenker NL, Streicker DG, Faust CL, Carroll CR. Anthropogenic land use change and infectious diseases: a review of the evidence. Ecohealth. 2014;11(4):619-32.
65. Gomez A, Kilpatrick AM, Kramer LD, Dupuis AP, 2nd, Maffei JG, Goetz SJ, et al. Land use and west nile virus seroprevalence in wild mammals. Emerging infectious diseases. 2008;14(6):962-5.
66. Fornace KM, Abidin TR, Alexander N, Brock P, Grigg MJ, Murphy A, et al. Association between Landscape Factors and Spatial Patterns of Plasmodium knowlesi Infections in Sabah, Malaysia. Emerging infectious diseases. 2016;22(2):201-8.

67. Meyer Steiger DB, Ritchie SA, Laurance SG. Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics. Parasites & vectors. 2016;9(1):387.
68. Walsh MG. Ecological and life history traits are associated with Ross River virus infection among sylvatic mammals in Australia. BMC Ecol. 2019;19(1):2.

Additional Files

Additional file 1 (.TIF Image) Fig S1. Monthly trend of RRV notifications in South East Queensland, 2001-2016.

Monthly case notifications are shown for each of the 9 Local Government Areas (LGAs) in the study area. A major flooding event that occurred in the region in early 2011 likely reduced case numbers of that year by inundating vector breeding sites with fast-flowing water. The largest ever recorded peak in monthly cases occurred for all LGAs during February and March 2015.

Additional file 2 (.docx Table) Table S2. Summary of annual case counts for each Local Government Area (LGA).

Additional file 3. (.TIF Image) Figure S3. Annual RRV incidence in South East Queensland: 2013-2016.

Annual incidence patterns are shown for State Suburb Codes (SSCs) within each of the 9 Local Government Areas in the years before, during and after the largest recorded epidemic in 2015.

Additional file 4 (.docx Table) Table S4. Locations with the highest overall rates across all years, 2001-2016.

Additional file 5 (.TIF Image) Figure S5. Annual hot and cold spots for RRV incidence in South East Queensland: 2013-2016.

Significant high- and low-incidence (hot and cold) spots identified through two different analysis techniques are overlaid: local G* analysis of raw (crude) annual incidence rates for State Suburb Codes (SSCs), and smoothed annual rates for SSCs (Empirical Bayes Spatial smoothing technique). Disagreement occurred where an SSC was hot in one analysis and cold in the other, or vice versa.

Additional file 6 (.TIF Image) Figure S6. Persistent and mean RRV hot spots in South East Queensland: 2001-2016.

Significant high- and low-incidence (hot and cold) spots shared between raw and smoothed incidence rate analyses are shown by State Suburb Code (SSC): **a)** 45 persistent hot and 154 persistent cold spots (present in ≥ 2 years) present in both raw and smoothed analyses; and **b)** 56 mean hot and 47 mean cold spots present in both raw and smoothed analyses. In a) SSC colours are graduated according to the number of years identified as a hot/cold spot, including 14 additional SSCs that were both hot and cold in ≥ 2/16 years.

Additional file 7 (.docx Table) Table S7. Summary of 45 persistent hot spots identified in both raw and smoothed incidence analyses from 2001-2016.

Figures

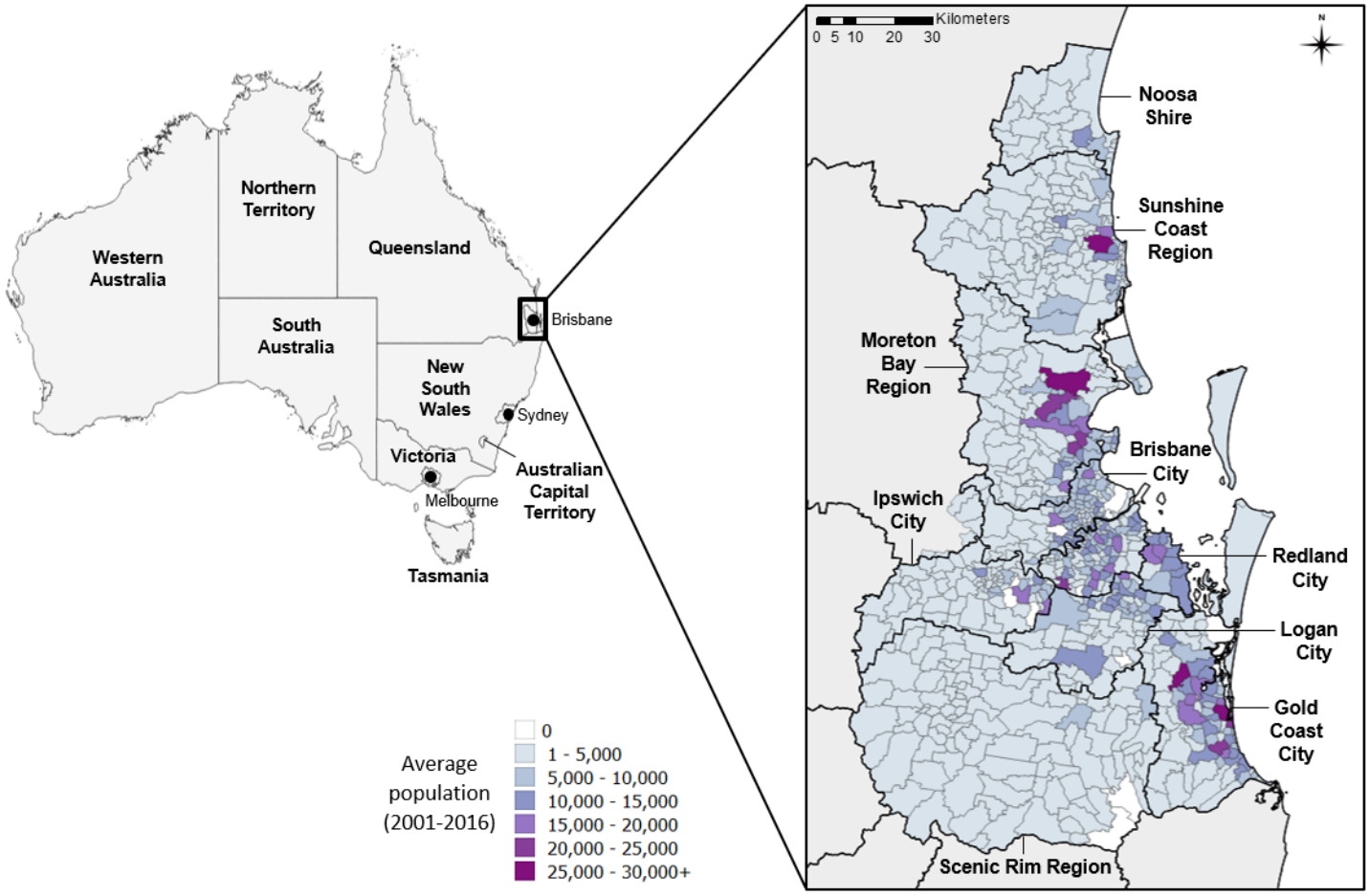

Figure 1

Map of Australia and South East Queensland. On left, the 8 Australian states are shown including three largest Australian cities Sydney, Melbourne and Brisbane, marked by black dots. Inset, South East Queensland, and the 9 local government areas (LGAs) included in the study, each sub-divided into smaller State Suburb Code (SSC) units. The population distribution for SSCs is indicated by graduated shading.

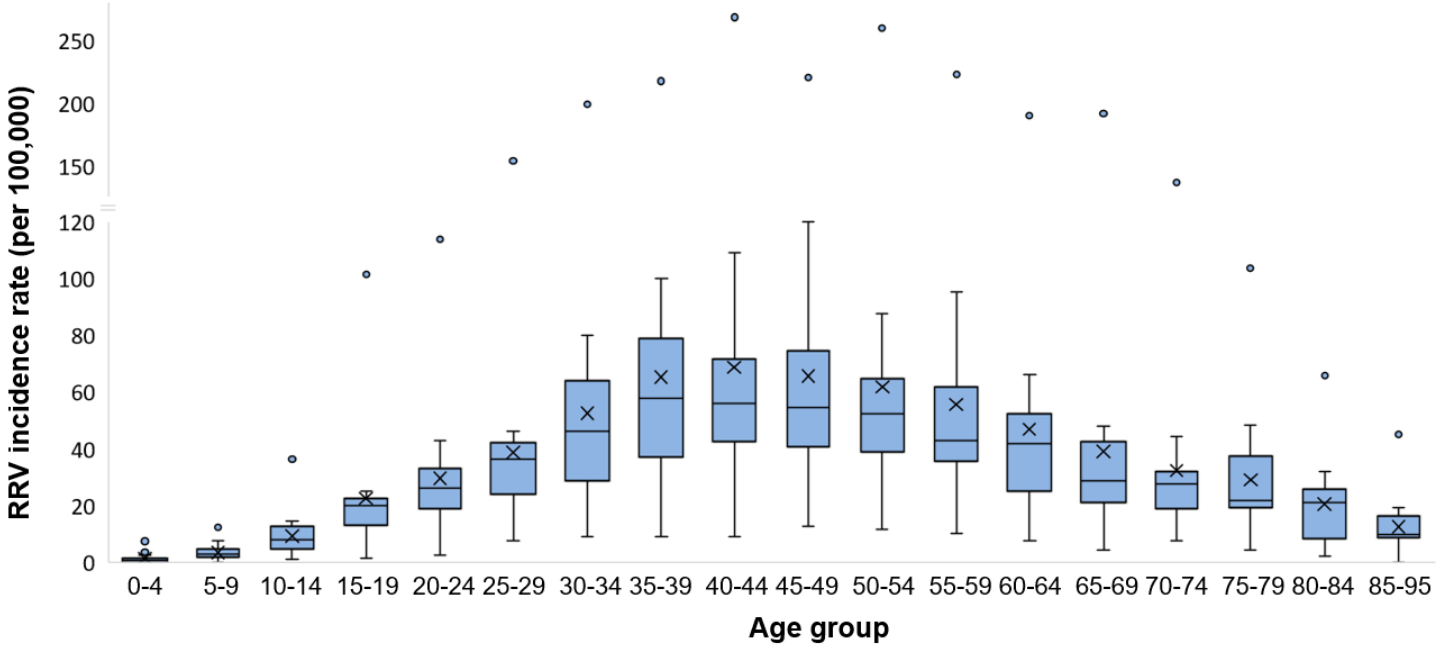

Figure 2

Mean annual RRV incidence in South East Queensland by age-group, 2001-2016. Age-adjusted mean annual incidence rates are grouped into 18 age groups of SEQ, between 0 and 95 years. Mean rates per group are indicated by X, and the median by the horizontal line across each box. Whiskers indicate the estimated minimum (lower whisker) and maximum (upper whisker) incidence values per age group (equal to the 1st and 3rd quartile -/+ 1.5 x the inter-quartile range, respectively). Outlying points indicate extreme values reported during large outbreak years, e.g. during the outbreaks of 2014 and 2015.

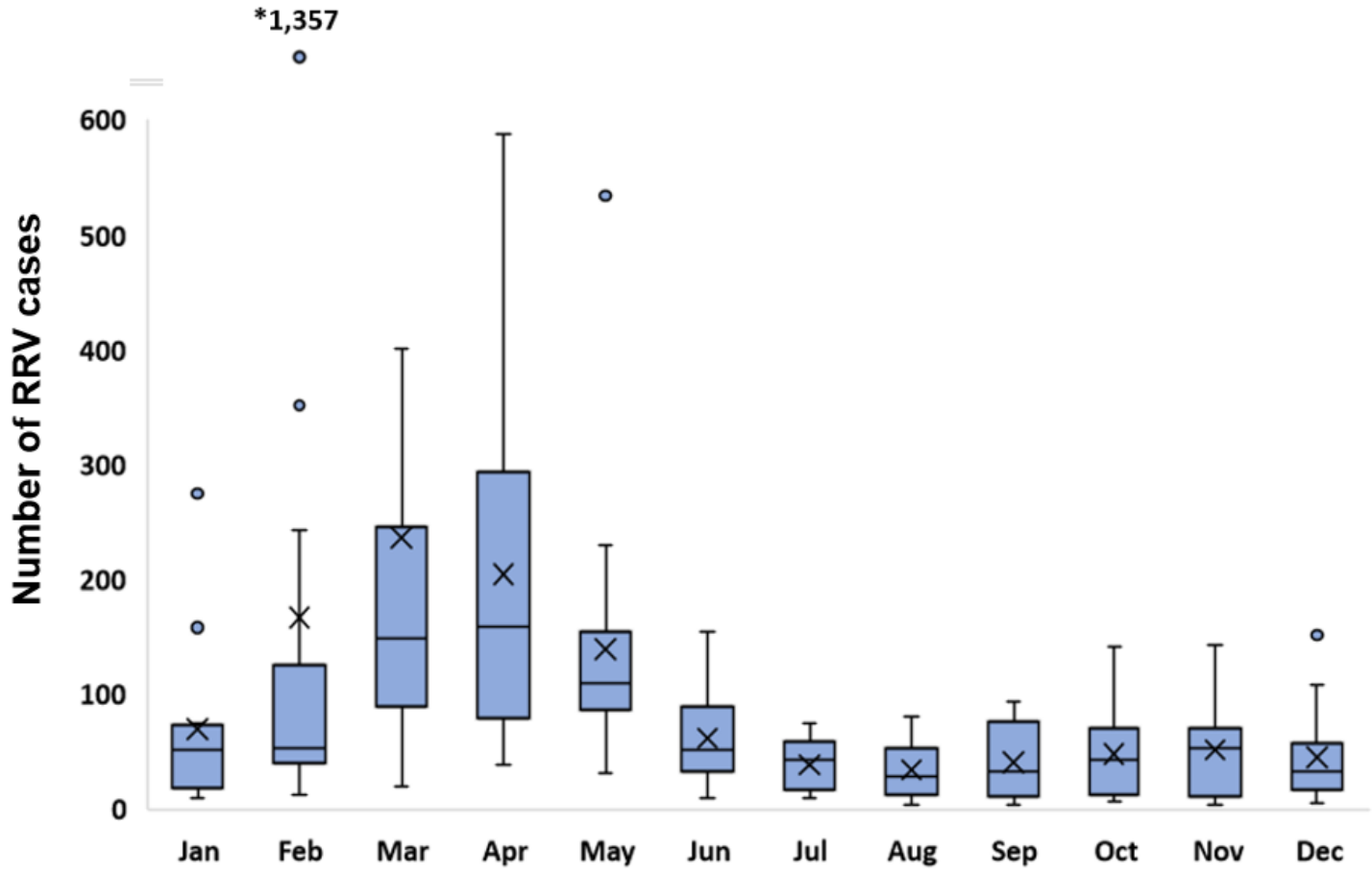

Figure 3

Mean monthly trend of RRV case notifications in South East Queensland, 2001-2016. Mean monthly case numbers across the 16-year period are shown, with extreme case report values from large outbreak years indicated as outliers. Mean rates per group are indicated by X, and median by the horizontal line crossing each box. Whiskers indicate the estimated maximum and minimum case numbers per month across the 16 years (equal to the 1st and 3rd quartile -/+ 1.5 x the inter-quartile range, respectively). *Total cases reported in February 2015 (1,357) were the highest ever reported in a single month.

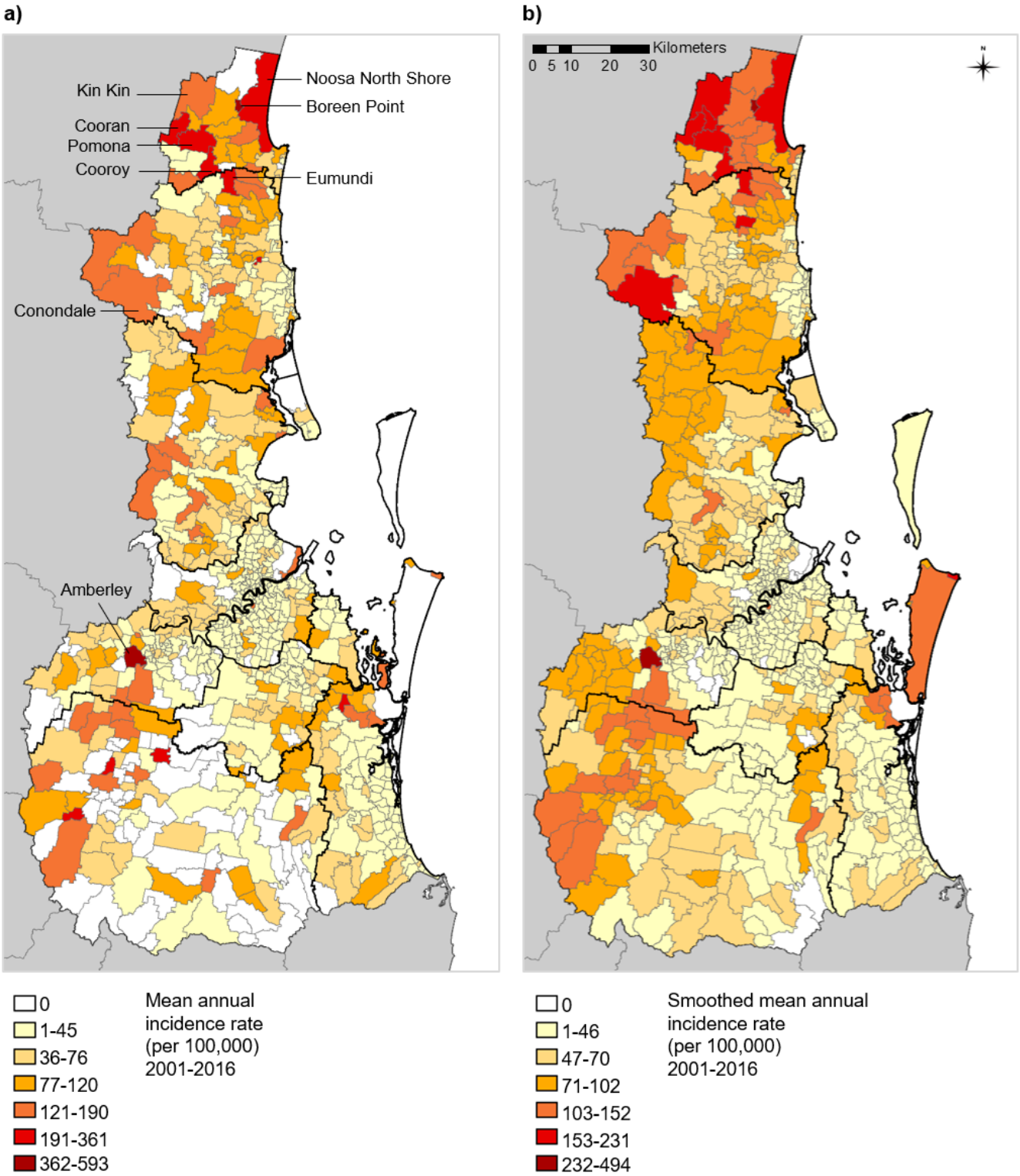

Figure 4

Mean annual RRV incidence of State Suburb Codes (SSCs) in South East Queensland, 2001-2016. a) Mean annual incidence rate, and b) smoothed mean annual incidence rate for SSCs of South East Queensland over the 16-year study period. Seventeen unpopulated SSCs are indicated by the white (zero case) locations in b).

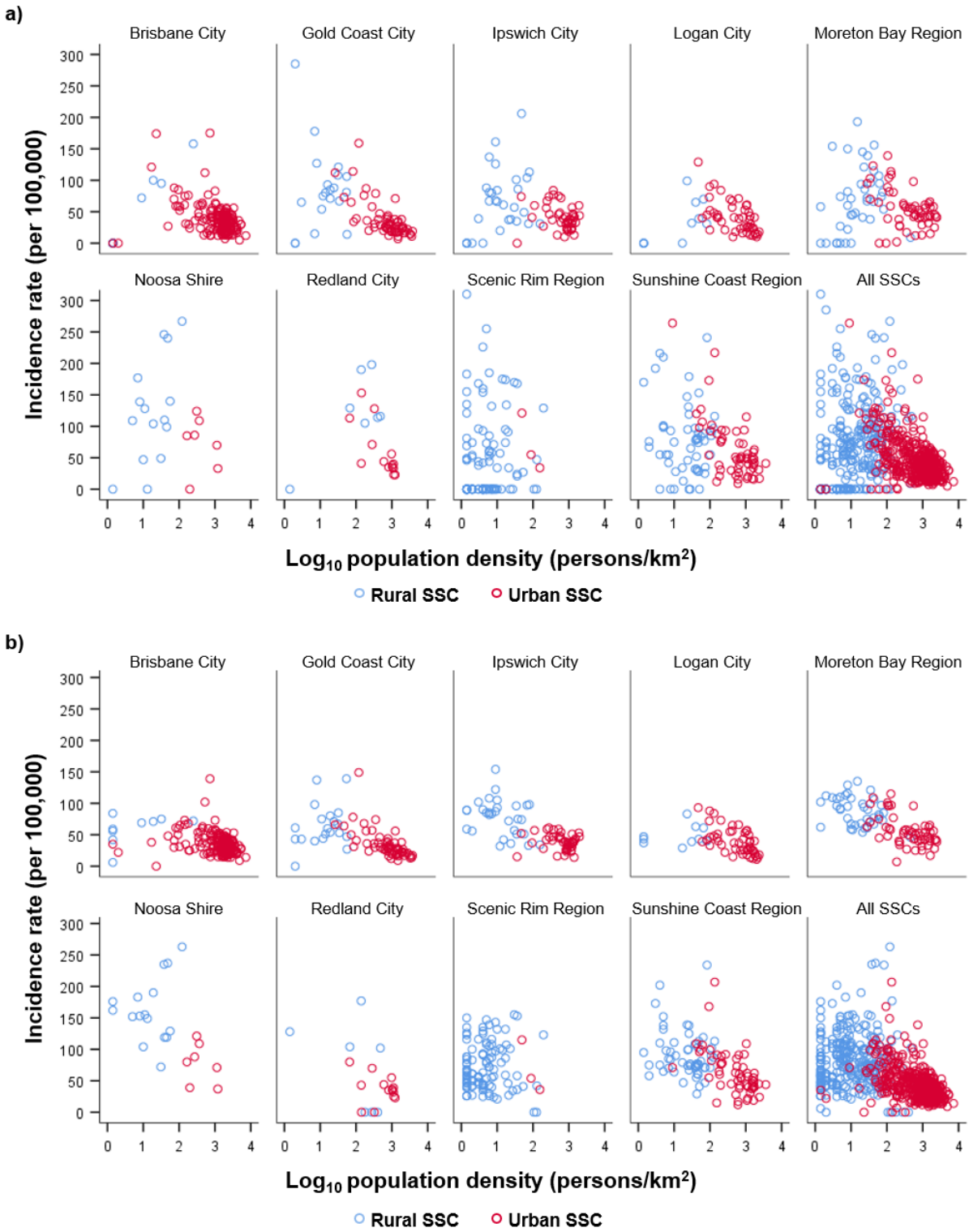

Figure 5

Mean annual RRV incidence of rural and urban State Suburb Codes (SSCs), 2001-2016. a) raw incidence rates, and b) smoothed incidence rates for the 757 SSCs of South East Queensland, shown according to the 9 Local Government Areas (LGAs) they are located within, and all SSCs combined. Rural and urban SSCs are indicated by blue and red circles, respectively. Two outlying SSCs (in Ipswich City and Noosa

Shire) had incidence values beyond the scale shown here, but are listed in Additional file 4. See Fig. 1 for location of each LGA within the study area.

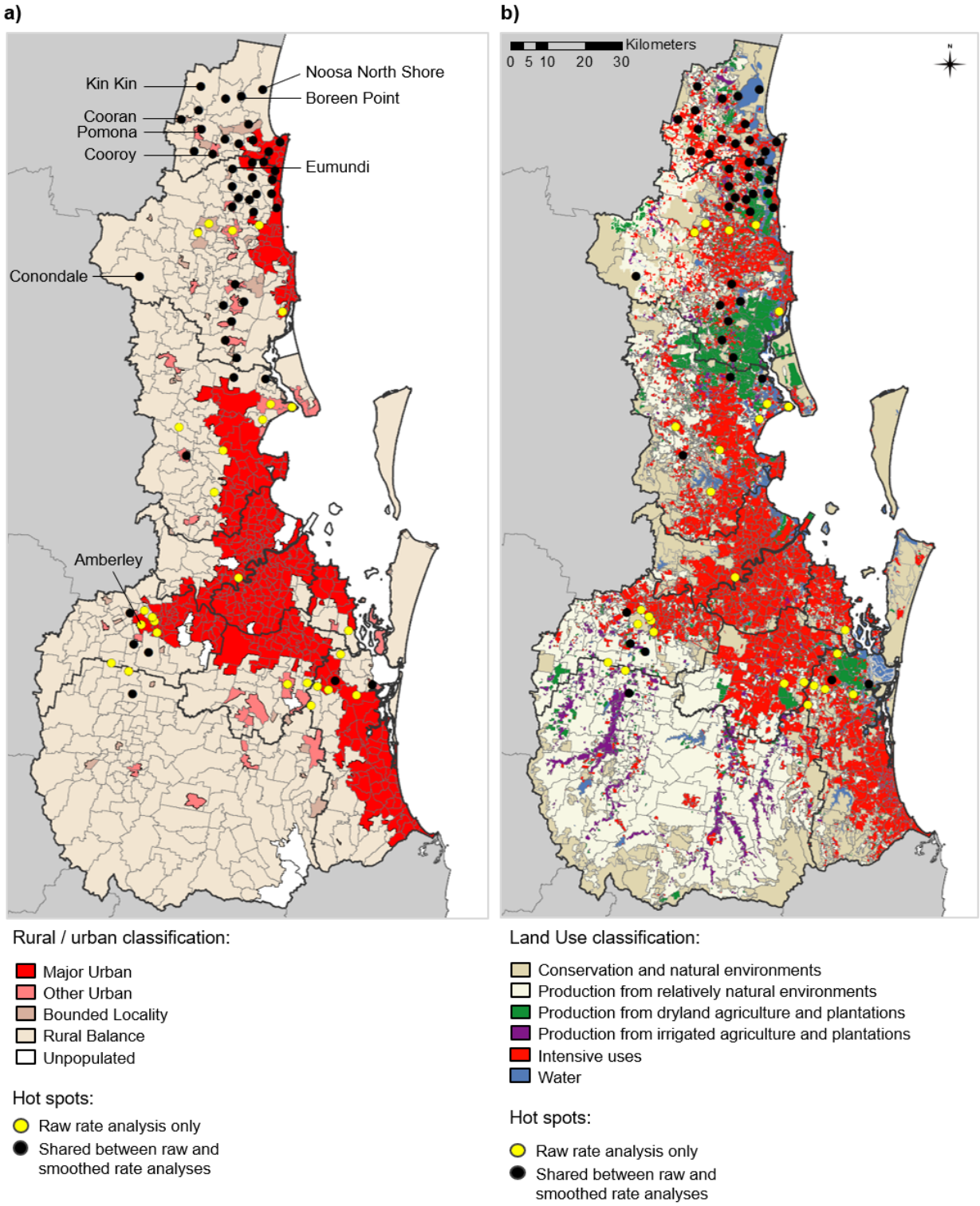

Figure 6

Persistent high incidence hot spots in South East Queensland, 2001-2016. Persistent hot spots identified for State Suburb Codes (SSCs) are shown relative to a) urban and rural areas of South East Queensland; and b) different land use types of South East Queensland. Points indicate the centroid of each hot spot

SSC. Hot spots detected using raw incidence rates only (yellow points; n=27 SSCs) and those detected in both raw and smoothed incidence analyses (black points; n=45 SSCs) are indicated. Note: in b) ‘Intensive uses’ refers to residential areas and urban infrastructure. See methods for further description.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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