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# Detecting signals of chronic shedding to explain pathogen persistence: *Leptospira interrogans* in California sea lions

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## Summary

**1.** Identifying mechanisms driving pathogen persistence is a vital component of wildlife disease ecology and control. Asymptomatic, chronically infected individuals are an oft-cited potential reservoir of infection, but demonstrations of the importance of chronic shedding to pathogen persistence at the population-level remain scarce.

**2.** Studying chronic shedding using commonly collected disease data is hampered by numerous challenges, including short-term surveillance that focuses on single epidemics and acutely ill individuals, the subtle dynamical influence of chronic shedding relative to more obvious epidemic drivers, and poor ability to differentiate between the effects of population prevalence of chronic shedding vs. intensity and duration of chronic shedding in individuals.

**3.** We use chronic shedding of *Leptospira interrogans* serovar Pomona in California sea lions (*Zalophus californianus*) as a case study to illustrate how these challenges can be addressed. Using leptospirosis-induced strands as a measure of disease incidence, we fit models with and without chronic shedding, and with different seasonal drivers, to determine the time-scale over which chronic shedding is detectable and the interactions between chronic shedding and seasonal drivers needed to explain persistence and outbreak patterns.

**4.** Chronic shedding can enable persistence of *L. interrogans* within the sea lion population. However, the importance of chronic shedding was only apparent when surveillance data included at least two outbreaks and the intervening inter-epidemic trough during which fade-out of transmission was most likely. Seasonal transmission, as opposed to seasonal recruitment of susceptibles, was the dominant driver of seasonality in this system, and both seasonal factors had limited impact on long-term pathogen persistence.

**5.** We show that the temporal extent of surveillance data can have a dramatic impact on inferences about population processes, where the failure to identify both short- and long-term ecological drivers can have cascading impacts on understanding higher order ecological phenomena, such as pathogen persistence.

**Key-words:** asymptomatic infection, birth pulse, critical community size, epidemic drivers, maintenance host, marine mammal stranding, partially observed Markov process, pathogen reservoir, seasonal transmission, subclinical shedding

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## Introduction

With the continued burden of infectious diseases around the world, understanding the mechanisms underlying pathogen persistence is essential to inform disease control and wildlife conservation efforts (Buhnerkempe *et al*. 2015; Tompkins *et al*. 2015; Plowright *et al*. 2016). Classically, the potential for pathogen persistence within a host population has been defined by the critical community size, which is the number of susceptible individuals necessary to maintain unbroken chains of transmission within a population more often than not (Bartlett 1960). However, more recent studies have shown that the relationship between host population size and pathogen persistence may be modified by other processes, such as spatial structure [e.g. phocine distemper virus (Swinton *et al*. 1998)] and indirect transmission via vectors [e.g. plague (Buhnerkempe *et al*. 2011b)] or environmental reservoirs [e.g. avian influenza (Breban *et al*. 2009; Rohani *et al*. 2009)]. Similarly, chronic shedding of the pathogen by a subset of infected hosts may allow for pathogen persistence (Monack, Mueller & Falkow 2004), but parsing the role of chronic shedding remains a challenge in disease ecology (Buhnerkempe *et al*. 2015; Plowright *et al*. 2016).

Although some pathogens such as hepatitis B virus (Edmunds *et al*. 1993) nearly always result in long-term infections, there is greater uncertainty regarding the role of chronic shedding in persistence of pathogens that can cause either acute, clinical illness or chronic, asymptomatic infection [e.g. feline coronavirus in cats (Foley *et al*. 1997), *Leptospira* spp. in domestic animals (Adler & de la Peña Moctezuma 2010) and *Salmonella* Typhi in humans (Gonzalez-Escobedo, Marshall & Gunn 2011)]. Such pathogens can cause conspicuous short-term outbreaks that are dominated by acute infections and are often followed by refractory periods where observable infection is rare and disease fadeout is more likely; these refractory periods are also known as inter-epidemic troughs (Lloyd-Smith *et al*. 2005; Grassly & Fraser 2006). In these scenarios, chronic shedders could act as an endogenous bridging mechanism between outbreaks [i.e. infection persists without being reintroduced (Buhnerkempe *et al*. 2011b)]. However, detecting and assessing the role of chronic shedding using population surveillance data are hampered by several challenges – time-scale of surveillance, low prevalence and/or intensity of chronic shedding, case underreporting and interacting signals of epidemic drivers (Plowright *et al*. 2016).

First, surveillance data are often confined to single outbreak events, which deviate from the mechanistic time-scale inherent in signatures of persistence drivers (Rohani *et al*. 2009). Thus, the duration of surveillance is likely to have profound effects on any inference made (Buhnerkempe *et al*. 2015; Lessler *et al*. 2015), especially about chronic shedding. Additionally, chronic shedders may have reduced infection loads leading to lower pathogen-shedding intensities (Leonard *et al*. 1992; Pathak *et al*. 2010), or, for many host–pathogen systems, most hosts experiencing acute illness recover and eliminate the pathogen rather than becoming chronic shedders (Monack, Mueller & Falkow 2004; Pathak *et al*. 2010). Either common, low-intensity chronic shedders or rare, high-intensity chronic shedders may have subtle but significant impacts on disease dynamics and management, but distinguishing between these scenarios is problematic. These inference problems are exacerbated by the universal problem of incomplete case reporting. Asymptomatic cases, whether acute or chronic, will be underreported in conventional data streams that rely on detection using obvious signs of infection (Lessler *et al*. 2015). Without observing these individuals directly, signatures of chronic shedding may be detected via their influence on patterns of overtly ill individuals.

Short-term epidemic drivers can further complicate inference about the presence of chronic shedders and their role in persistence. Specifically, seasonal transmission and contact patterns have been recognized as important drivers of seasonal outbreaks (Altizer *et al*. 2006; Grassly & Fraser 2006). Similarly, seasonal reproduction can drive outbreaks and affect pathogen persistence through pulsed recruitment of new susceptible individuals (Peel *et al*. 2014). The focus on short-term outbreak mechanisms, however, largely ignores the mechanisms that operate during inter-epidemic troughs to enable pathogen persistence. Interactions between short-term drivers, such as seasonal transmission and susceptible recruitment, and long-term drivers, such as chronic carriage, will be key to understanding persistence (Lloyd-Smith *et al*. 2005; Breban *et al*. 2009; Rohani *et al*. 2009; Brown *et al*. 2013). Discerning the signal of chronic shedders operating in inter-epidemic troughs from epidemic drivers operating through multiple epidemic cycles remains a challenge in wildlife systems [(Lloyd-Smith *et al*. 2005; Buhnerkempe *et al*. 2015), although research has progressed on the related problem of environmental reservoirs (Breban *et al*. 2009; Rohani *et al*. 2009; Brown *et al*. 2013)].

As a case study in addressing the above challenges, we use leptospirosis in California sea lions (*Zalophus californianus*). Leptospirosis, caused by spirochetes of the genus *Leptospira* that are transmitted primarily via urinary shedding (either directly or indirectly through environmental contamination), is a disease of global concern and affects most mammals including humans (Levett 2001; Bharti *et al*. 2003). In 1970, *Leptospira interrogans* serovar Pomona was identified as the cause of a large stranding event in California sea lions where high numbers of sick or dead sea lions were observed on the coast of California and Oregon (McIlhattan *et al*. 1971; Vedros *et al*. 1971). Surveillance in stranded sea lions has revealed yearly leptospirosis outbreaks occurring between July and December since at least 1984 (Fig. 1; Gulland *et al*. 1996; Lloyd-Smith *et al*. 2007). These outbreaks follow shortly after the recruitment of susceptible yearlings to the coastal population, which occurs following weaning in the spring, and coincide with altered mixing patterns linked to seasonal migrations in late summer and fall (Fig. S1, Supporting

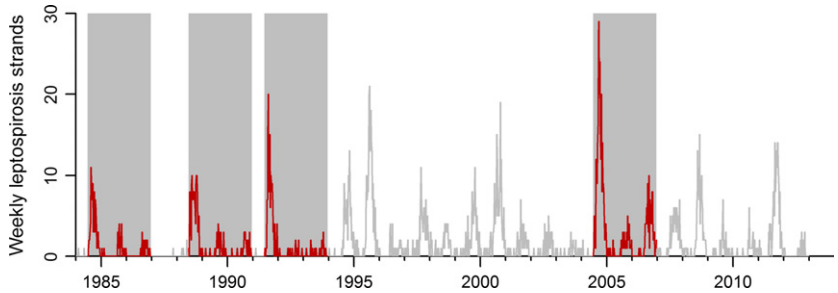


**Fig. 1.** Weekly strands of California sea lions due to leptospirosis. Highlighted regions indicate the four stranding eras over which models were fit (grey boxes; 1984–1987, 1988–1991, 1991–1994 and 2004–2007). Note that the eras from 1995–1997, 2000–2003 and 2009–2011, while qualitatively similar to the four eras specified above, are not considered because they are preceded by relatively large outbreaks so the criterion of initial population susceptibility is not satisfied. [Colour figure can be viewed at wileyonlinelibrary.com]

Information; Gulland *et al.* 1996; Zuerner *et al.* 2009). Genetic analyses of circulating *Leptospira* strains have revealed that strains isolated from California sea lions are distinct from other known strains of *L. interrogans* serovar Pomona, indicating that repeated spillovers from other hosts are unlikely to have driven such patterns and that *Leptospira* has persisted enzootically in this population for at least 30 years (Zuerner & Alt 2009). The mechanisms underlying this enzootic persistence are unclear, but chronic shedding has been hypothesized as important (Gulland *et al.* 1996; Lloyd-Smith *et al.* 2007). Shedding by asymptomatic individuals has been demonstrated, and this shedding can last for 12 weeks and potentially longer (Dierauf *et al.* 1985; Prager *et al.* 2013, 2015). With asymptomatic shedders, a long history of disease surveillance, and seasonality in both transmission and susceptible recruitment, the sea lion/leptospirosis system provides a unique opportunity to assess chronic shedding as a mechanism of pathogen persistence in wildlife, and to highlight challenges researchers may face conducting similar assessments in other systems.

Here, we assess the predicted role of chronic shedding in the transmission dynamics of *Leptospira* infection in California sea lions. We show that chronic shedding can enable *Leptospira* persistence within this host population, but the ability to detect signatures of chronic shedding is complicated by the time-scale of surveillance data and trade-offs among duration, prevalence and intensity of chronic shedding. Finally, we show how seasonal susceptible recruitment and seasonal transmission combine with chronic shedding to create observed seasonal patterns in incidence. To do this, we use knowledge of sea lion demography and *Leptospira* biology to build mechanistic models of pathogen spread. We fit these models to leptospirosis surveillance data from stranded sea lions using recent advances in model fitting for partially observed incidence time series (Ionides *et al.* 2015) and use simulations to study persistence.

## Materials and methods

### STRANDING DATA

We used stranding data collected by The Marine Mammal Center (TMMC, Sausalito, CA, USA) from 1984 to 2012 (collection methods described elsewhere; Gulland *et al.* 1996; Lloyd-Smith *et al.* 2007). The TMMC stranding range includes the central and northern California coast (Fig. S2). This range is determined by jurisdictional boundaries (counties in California) rather than by animal distribution, and it covers the central portion of the migratory range of a genetically distinct population of California sea lions, the Pacific Temperate population, that extends from the US–Mexico border into Canada and Alaska (Fig. S2; Schramm *et al.* 2009). The Pacific Temperate population of California sea lions give birth and breed on the California Channel Islands in June and July. Adult females then nurse a pup for 10–12 months and remain associated with the rookery islands throughout the year (Fig. S1). Although some juveniles (i.e. yearlings to 4 years old) remain around rookery islands, most males and non-breeding females this age migrate north to the central California coast in fall and overwinter there. Subadult and adult males migrate north from the rookeries following the breeding season and overwinter in coastal waters from central California to British Columbia, returning south in the spring. Thus, juveniles and non-nursing adult females are the age classes primarily found in the TMMC stranding range outside the breeding season, with subadult and adult males passing through the range in the autumn and spring on their north and south migrations.

Infection with *L. interrogans* serovar Pomona in this population exhibits clear, yearly outbreaks in the fall during northward migration, typically peaking from July to December. Evidence of leptospirosis is rarely observed outside this outbreak season, including in animals returning from north of the TMMC stranding range in the spring (Figs S1 and S2; Gulland *et al.* 1996; Greig, Gulland & Kreuder 2005; Lloyd-Smith *et al.* 2007) or in pups and females inhabiting the rookery islands year-round south of the stranding range (Figs S1 and S2; R.L. DeLong, unpublished data); available data thus indicate that seasonality in leptospirosis-induced strandings reflects the true seasonality of infection in this system. In addition, leptospirosis outbreaks have exhibited clear 3–5 year cycles that are reflected in data from stranded sea lions (Gulland *et al.* 1996; Greig, Gulland & Kreuder 2005; Lloyd-Smith *et al.* 2007). Changes in climatic and oceanographic conditions, such as El Niño events, impact sea lion demography (Weise, Costa & Kudela 2006; Melin *et al.* 2010, 2012) and may also impact the survival of leptospires (Johnson & Harris 1967). The impacts of changing environmental conditions on both sea lions and *Leptospira* are expected to alter periodicity in the system (Gulland *et al.* 1996). Deviations from the regular cyclic pattern are particularly noteworthy during the late 1990s (around the historically strong El Niño event of 1997–1998) when relatively large outbreaks were observed annually for

several years (Fig. 1). Because we are focused on intrinsic disease drivers, large environmental perturbations may obscure signals of these drivers and are beyond the scope of this study. Hence, we focused on four 2·5-year eras in the stranding record that are typical of the regular cyclic pattern – an isolated large outbreak (i.e. not preceded by another large outbreak) followed by two smaller outbreaks (1984–1987, 1988–1991, 1991–1994 and 2004–2007; Fig. 1). Here, the large first outbreak depletes the susceptible pool, increasing the likelihood of post-epidemic fadeout and maximizing the potential to detect signatures of chronic shedders as a persistence mechanism. Weekly data for all stranding eras began on 17 June of the first year specified to fully capture the initial outbreak for each stranding era. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.j15ns (Buhnerkempe *et al*. 2017).

## MODEL

To study chronic shedding in this system, we used a discrete-time, stochastic model with weekly time steps that was based on a continuous-time process model and simulated using the Euler–Maruyama method implemented in the POMP package (King *et al*. 2015; King, Nguyen & Ionides 2016) in R statistical software version 3.1 (R Development Core Team 2014). Here, a susceptible ($S$) – exposed ($E$) – acutely infected ($I$) – recovered ($R$) model was modified to incorporate progression from acute to chronic shedding (C; eqns 1–8). To avoid confounding effects between chronic infection and over-dispersed acute infectious periods, we divided the acutely infected period into four stages ($I_1$–$I_4$; eqns 3–6) so the acute infectious period was gamma-distributed (Conlan *et al*. 2010).

$$\frac{dS}{dt} = b(t)N - \beta(t)S\frac{[(I_1 + I_2 + I_3 + I_4) + \varepsilon C]}{N} - \mu S \qquad \text{eqn 1}$$

$$\frac{dE}{dt} = \beta(t)S\frac{[(I_1 + I_2 + I_3 + I_4) + \varepsilon C]}{N} - E(\mu + v) \qquad \text{eqn 2}$$

$$\frac{dI_1}{dt} = vE - I_1\left(\frac{\gamma}{4} + \mu + \alpha\right) \qquad \text{eqn 3}$$

$$\frac{dI_2}{dt} = \frac{\gamma}{4}I_1 - I_2\left(\frac{\gamma}{4} + \mu + \alpha\right) \qquad \text{eqn 4}$$

$$\frac{dI_3}{dt} = \frac{\gamma}{4}I_2 - I_3\left(\frac{\gamma}{4} + \mu + \alpha\right) \qquad \text{eqn 5}$$

$$\frac{dI_4}{dt} = \frac{\gamma}{4}I_3 - I_4\left(\frac{\gamma}{4} + \mu + \alpha\right) \qquad \text{eqn 6}$$

$$\frac{dC}{dt} = \rho\frac{\gamma}{4}I_4 - C(\mu + \delta) \qquad \text{eqn 7}$$

$$\frac{dR}{dt} = (1 - \rho)\frac{\gamma}{4}I_4 + \delta C - \mu R \qquad \text{eqn 8}$$

State variables and parameters are described in Table 1. We assumed transmission is frequency-dependent (based on the total population $N$) similar to phocine distemper in harbour seals (Swinton *et al*. 1999). Chronic shedders were a fraction, $0<\varepsilon<1$, as infectious as acutely infected individuals, to account for weak or intermittent shedding. Transmission intensity, $\beta(t)$, from both acute and chronic shedders was seasonal, to reflect changes in sea lion mixing and possible environmental influences, and was modelled using a three-step function (Table 1; Fig. S1). In this formulation, $\beta_1$ represents transmission during the reproductive season when many individuals are concentrated around rookery islands [Fig. S1; 17 June–4 August (Riedman 1990)]. $\beta_2$ represents transmission during the post-breeding period when sea lions (other than pups and nursing mothers) migrate north along the coast [Fig. S1; 5 August–17 November (Riedman 1990)], coinciding with the period when sea lions strand at highest rates due to leptospirosis (Fig. S1). $\beta_3$ represents transmission during the inter-epidemic trough when very few sea lions strand in the TMMC stranding range due to leptospirosis [Fig. S1; 18 November–16 June (Gulland *et al*. 1996)].

After infection, individuals transitioned from exposure to acute infection at rate $v$. We assumed this incubation period lasted 1 week on average (Levett 2001). During acute infection, individuals experienced disease-induced mortality at rate $\alpha$ and recovered at total rate $\gamma$. We assumed acute infection lasted 2 weeks on average [i.e. the mean total residence time in $I_1$, $I_2$, $I_3$ and $I_4$ described by a $\Gamma(4, 4\gamma)$ distribution], which was consistent with the duration of clinical disease seen in stranded sea lions (Dierauf *et al*. 1985; Prager *et al*. 2015). Alternative assumptions of 1- or 3-week acute infectious periods did not change qualitative model results (Figs S3 and S4). After the acute infection phase, a proportion of individuals, $\rho$, became chronically infected, while the rest recovered. Chronic shedders recovered and ceased shedding at rate $\delta$. We assumed that chronic shedders suffered no disease-induced mortality, in line with current understanding of leptospirosis in other maintenance hosts (Ellis 2015) and the observation of asymptomatic shedders amongst both wild-caught and stranded sea lions (Prager *et al*. 2013, 2015). We assumed that immunity against the same serovar was lifelong (Adler & de la Peña Moctezuma 2010; Evangelista & Coburn 2010).

We assumed that sea lions experienced natural mortality at a constant rate, $\mu$, and were capable of recruiting new susceptibles at rate $b_1$. Because pups on the rookery islands are (almost) never observed to be infected and yearlings frequently strand due to leptospirosis (Greig, Gulland & Kreuder 2005), we assumed that recruitment into the susceptible class occurred during the period from March to May when pups wean and leave the rookery islands, with susceptible recruitment set to zero for the rest of the year (Table 1; Fig. S1; Melin *et al*. 2000). Demographic parameters were estimated using survival and reproduction data from a long-term mark–resight study using branded California sea lions [(Melin *et al*. 2012); Table 1]. We estimated the natural mortality rate, $\mu$, from the complement of yearly survival probability, averaged over age- and sex-based differences using the stable age-distribution for the population, and converted to a rate assuming exponential waiting times (Table 1). Again using the stable age-distribution, we calculated the average per capita birth rate from age-specific yearly natality estimates; because we assume susceptible recruitment occurs following weaning, we corrected for pup mortality before weaning to determine the annual susceptible recruitment rate. This annual rate was rescaled to account for the duration of the susceptible recruitment window to arrive at the per capita susceptible recruitment rate during the weaning period, $b_1$ (Table 1).

**Table 1.** Description of models, parameters and their values. For models SR, CR and CS, only parameters that differ from those in CSR are shown. For parameters that were fit to the data, the range of random start values is shown in brackets

| Parameter | Description | Value*,† |
|---|---|---|
| *CSR – chronic shedding, seasonal transmission, and seasonal susceptible recruitment* | | |
| $\mu$ | Natural mortality rate | 0·0029 |
| $b_1$ | Per capita susceptible recruitment rate from 1 March to 20 May | 0·019 |
| $v$ | Rate of progression from exposure to acute infection | 1 |
| $\gamma$ | Rate of recovery from acute infection | 0·5 |
| $S(0)$ | Initial fraction of the population that was susceptible | 0·8 |
| $E(0)$ | Initial fraction of the population that was exposed | 0·0025 |
| $I_1(0)$, $I_2(0)$, $I_3(0)$, $I_4(0)$ | Initial fraction of the population that was in each of the four acute infection stages | 0·001 |
| $C(0)$ | Initial fraction of the population that was chronically infected | 0·005 |
| $R(0)$ | Initial fraction of the population that was recovered and immune to infection | 0·1885 |
| $N(1984)$ | Total population size in 1984 | 70 240 |
| $N(1988)$ | Total population size in 1988 | 98 681 |
| $N(1991)$ | Total population size in 1991 | 150 338 |
| $N(2004)$ | Total population size in 2004 | 220 349 |
| $\beta_1$ | Transmission coefficient from 17 June to 4 August | [0, 50] |
| $\beta_2$ | Transmission coefficient from 5 August to 17 November | [0, 50] |
| $\beta_3$ | Transmission coefficient from 18 November to 16 June | [0, 50] |
| $\alpha$ | Disease-induced mortality rate | [0, 1] |
| $\rho$ | Proportion of acutely infected individuals that become chronically infected | [0, 1] |
| $\varepsilon$ | Proportional shedding intensity of chronic individuals compared to acutely infected individuals | [0, 1] |
| $\delta$ | Rate at which chronically infected individuals recover | [0, 2] |
| $p_{obs}$ | Probability of observing an individual dying of leptospirosis as a strand | [0, 1] |
| *SR – seasonal transmission and seasonal susceptible recruitment* | | |
| $E(0)$ | Initial fraction of the population that was exposed | 0·0035 |
| $I_1(0)$, $I_2(0)$, $I_3(0)$, $I_4(0)$ | Initial fraction of the population that was in each of the four acute infection stages | 0·002 |
| $C(0)$ | Initial fraction of the population that was chronically infected | 0 |
| $\rho$ | Proportion of acutely infected individuals that become chronically infected | 0 |
| *CR – chronic shedding and seasonal susceptible recruitment* | | |
| $\beta_0$ | Transmission coefficient for the entire year | [0, 50] |
| *CS – chronic shedding and seasonal transmission* | | |
| $b_0$ | Per capita susceptible recruitment rate for the entire year | 0·0040 |

*Rate parameters have units 1/weeks.
†Values of fit parameters are given in Tables S1–S4.

The full model that contains chronic shedding ('C'), seasonal transmission ('S') and seasonal susceptible recruitment ('R') is hereafter referred to as 'CSR' (Table 1). To determine the importance of chronic shedding, we formulated an alternative model without chronic shedding ('SR,' Table 1) by eliminating transitions into the chronic class (i.e. setting $\rho = 0$, Table 1).

## INITIAL CONDITIONS

For all models, the initial population size, $N(0)$, was the estimated number of sea lions in the Pacific Temperate population at the beginning of the stranding era considered (Table 1; Lowry & Maravilla-Chavez 2005). Because the stranding eras were chosen to begin with a large outbreak preceded by several relatively quiet years, we assumed most of the population was initially susceptible (i.e. $S(0) = 0{\cdot}8$; Table 1). For model SR, we used the same initial conditions, with initial chronic shedders divided evenly between the exposed and acutely infected stages (Table 1). Our assumed value of $S(0)$ is consistent with preliminary attempts to fit initial conditions, which showed that the highest-likelihood models had $S(0) > 0{\cdot}7$ (results not shown), and with the proportion of sea lions that were seronegative for anti-*Leptospira* antibodies in July of 2004 (0·77 among those stranding for reasons other than leptospirosis), the only stranding era for which we have randomly sampled serology data (Lloyd-Smith *et al.* 2007). Results were qualitatively similar for lower initial proportions of susceptible individuals ($S(0) = 0{\cdot}5$; results not shown).

## PARAMETER ESTIMATION

We used maximum likelihood to estimate disease parameters that were not fixed based on independent data (Table 1). To calculate model likelihoods, the stranding time series was linked to model outputs through an observation process depending on disease severity. Individuals stranding due to clinical leptospirosis are typically severely ill with 60–70% mortality in rehabilitation despite receiving supportive clinical care (Gulland *et al.* 1996). We assumed that without clinical care, these severely ill animals would die in the wild. Thus, we linked disease-induced stranding to the modelled flux of sea lions dying due to leptospirosis. We included a parameter for the proportion, $p_{obs}$, of individuals dying due to leptospirosis that are observed as a strand, and used a partially observed Markov process model to account for this observation process.

The model was fit to weekly counts of leptospirosis strands using a multiple iterated filtering algorithm to find the maximum likelihood across parameter space, including 50 random start values for the free parameters (Table 1; Ionides *et al.* 2015), implemented using the POMP package (King *et al.* 2015; King, Nguyen & Ionides 2016) in R statistical software version 3.1

(R Development Core Team 2014). For full details of settings (e.g. cooling schedules, number of particles, random walk standard deviation) used in the filtering algorithm, see Appendix S1.

To provide the most complete analysis of the role of chronic shedding in this system, we began by fitting the CSR and SR models to the full 2·5 years of surveillance data from the four stranding eras.

### CHALLENGES IN DETECTING SIGNATURES OF CHRONIC CARRIAGE

#### *Surveillance duration*

We expect that inference on the importance of chronic shedding for pathogen persistence will depend critically on the duration of disease surveillance. We thus fit CSR and SR to all four stranding eras over three surveillance durations: 2·5, 1·5 and 0·5 years, which capture three, two and one outbreaks respectively. Within each stranding era/surveillance duration combination, we compared models using the Akaike information criterion (AIC), which rewards model fit while penalizing model complexity (Burnham & Anderson 2003). We compared the probability of *Leptospira* persistence predicted by each best-fit model. Persistence was assessed using the proportion of 100 stochastic simulations where the disease was maintained after 30 years (the minimum observed persistence time in this population).

#### *Prevalence, intensity and duration of chronic shedding*

It is also desirable to characterize the prevalence, intensity and duration of chronic shedding, but distinguishing among these properties is challenging. To explore this issue, we jointly varied the probability of becoming chronically infected, $\rho$, the rate at which chronic shedders recover, $\delta$, and the relative transmission intensity of chronic shedders, $\varepsilon$, over the possible ranges of values for these parameters, while leaving all other parameters at their maximum likelihood estimates, using model CSR fit to the full 2·5 years of data from 1984 to 1987. We calculated the likelihood of these parameter sets to determine the likelihood surface for the parameters related to chronic infection.

#### *Presence of seasonal epidemic drivers*

Interactions between chronic shedding and seasonal epidemic drivers, such as seasonal transmission and seasonal susceptible recruitment, may be important for persistence across inter-epidemic troughs, but the primary drivers of seasonality in our system are poorly understood. To probe these relationships, we formulated two additional models – 'CR', which removed seasonal transmission by assuming constant transmission throughout the year, and 'CS', which removed seasonal susceptible recruitment by assuming that recruitment occurs at a constant level throughout the year while requiring annual susceptible recruitment to be equivalent to the seasonal model (Table 1).

## Results

Predictions from the full model with chronic shedders, seasonal transmission and seasonal susceptible recruitment (CSR) closely mirrored the pattern of leptospirosis strandings observed in California sea lions (Figs 2a and 5). The force of infection during outbreak seasons was dominated by transmission from acute infections (Fig. 2a, b). During inter-epidemic troughs, transmission from acute infections decreased markedly (Fig. 2b), and chronic shedders became much more important (Fig. 2c). Even though total transmission from chronic shedders was low throughout the year (Fig. 2b), the presence of sustained transmission during inter-epidemic troughs has potential to impact long-term dynamics by creating a stable reservoir of chronic shedders. Although not predictive of actual strandings, simulations (using parameters fit to the 1984–1987 era) showed that this mechanism could enable persistent pathogen circulation over a 30-year time span (Fig. 2d). Furthermore, in all stranding eras (1984–1987, 1988–1991, 1991–1994 and 2004–2007), CSR was clearly preferred over the model without chronic shedding (SR); the preferred model, CSR, predicted persistence in all simulations when the models were fit to data spanning multiple outbreak cycles (i.e. 2·5 years of data; Fig. 3; Tables S1–S4).

### SURVEILLANCE DURATION

Inference on the importance of chronic shedding depended strongly on the surveillance duration used (Fig. 3) – either 2·5 years (multiple outbreaks and inter-epidemic troughs), 1·5 years (two outbreaks with a single inter-epidemic trough) or 0·5 years (one outbreak and no inter-epidemic trough). The CSR model was preferred by AIC with 2·5 and 1·5 years of surveillance data across all stranding eras (Fig. 3b, Tables S1–S4). However, with only 0·5 years of surveillance, model SR was preferred across all stranding eras (Fig. 3b, Tables S1–S4). The effect of surveillance duration on predicted persistence was even more striking (Fig. 3c, Tables S1–S4). With 2·5 or 1·5 years of data, simulations of CSR with parameters fit to any stranding era generated persistence of *Leptospira* in greater than 98% of simulations (Fig. 3c, Tables S1–S4). However, when parameterized to 0·5 years of surveillance data (i.e. no inter-epidemic trough), simulations of this same model failed to generate persistence when fit to two of the four stranding eras (1988–1991 and 1991–1994, Tables S2 and S3). In these instances, the inferred parameter values governing chronic shedding rendered this mechanism negligible (Tables S2–S3), and the persistence behaviour of CSR more closely resembled that of SR, which failed to generate meaningful persistence at any surveillance duration (Fig. 3c, Tables S1–S4).

### PREVALENCE, INTENSITY AND DURATION OF CHRONIC SHEDDING

The estimated probability of becoming a chronic shedder ($\rho$), and the estimated intensity ($\varepsilon$) and duration of chronic shedding ($\delta$) were variable across model fits (Tables S1–S4), pointing to challenges with identifiability

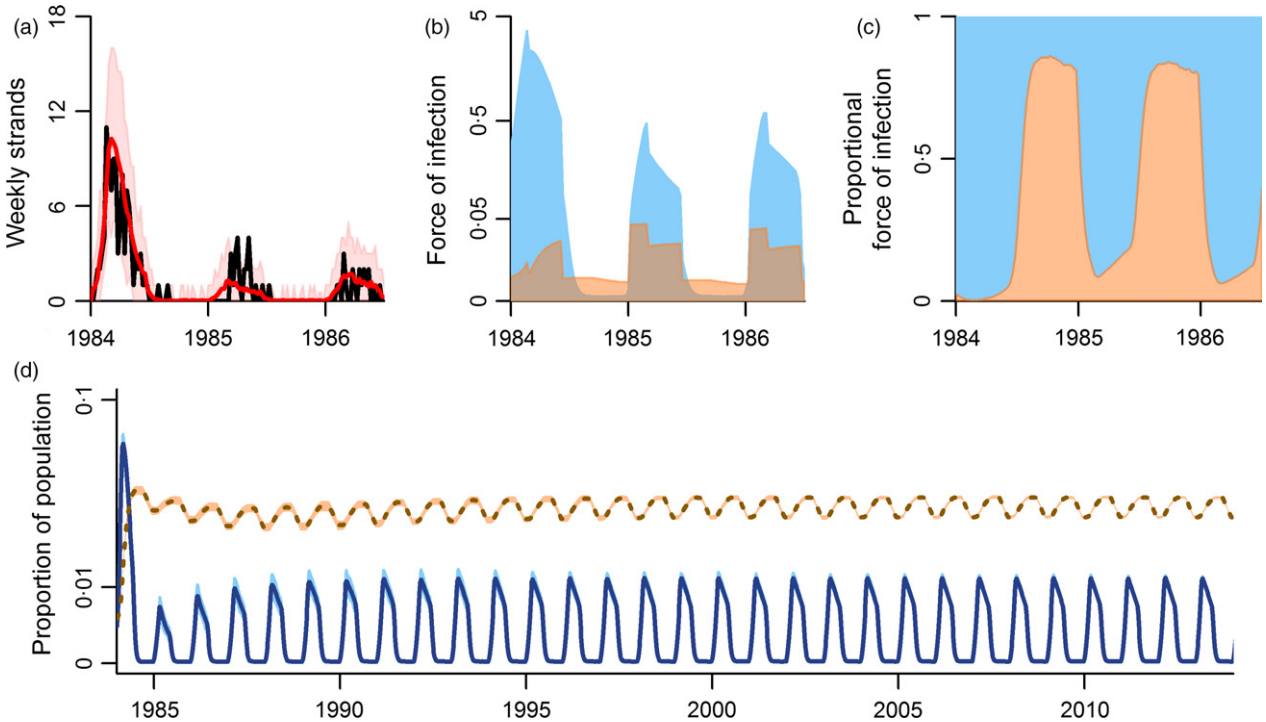


**Fig. 2.** Chronic shedding, seasonal transmission, and seasonal susceptible recruitment (CSR) fit to 2·5 years of stranding data starting in 1984. (a) Model fit showing the average number (solid red line) and 95% confidence interval (pink region) of weekly strandings predicted by CSR fit to data from the 1984–1987 stranding era (black line). (b) Force of infection from acutely infected (blue) and chronically infected (orange) individuals on a log scale. (c) Proportional force of infection from acutely (blue) and chronically infected (orange) individuals. (d) Predicted proportion of the population that is acutely infected (blue line) and chronically infected (orange line) over a 30-year period. Shaded regions represent the 95% confidence intervals from 100 simulations. Simulations began on 17 June, and axis ticks marking the years occur on this date.

in these quantities. The likelihood space for these parameters revealed several patterns (Fig. 4). First, if chronically infected individuals are assumed to be common (Fig. 4a), chronic shedding intensity must be very low (0·3–1% of acute shedding intensity) with a mean chronic shedding duration longer than 30 weeks to achieve high model likelihoods. If chronic shedding is less common (Fig. 4b), chronic shedding intensity has to increase to compensate (10–30% of acute shedding intensity), and the mean duration of chronic infections has to span a year or more for high model likelihoods. If chronic infections are extremely rare (Fig. 4c), chronic shedding intensity still has to be 10–30% of acute shedding intensity but now mean durations must be considerably longer (2 years or more) to achieve high model likelihoods.

### PRESENCE OF SEASONAL EPIDEMIC DRIVERS

Identifying important seasonal drivers was critical to fully understanding the role of chronic shedding in the system (e.g. does chronic shedding link periods of elevated susceptible supply or does it just bridge over seasons of low transmission?). In all four stranding eras, models with seasonal transmission were preferred by AIC, whether or not they had seasonal susceptible recruitment (Fig. 5, Tables S1–S4). The model lacking seasonal transmission (CR) was generally not able to capture the second and third seasonal stranding peaks (Fig. 5a,b,d), and fit reasonably only in 1992–1993 when these peaks were much less pronounced (Fig. 5c). This inability to fit the data arose from the less pronounced cycles in acute infections in the model including only seasonal susceptible recruitment (CR; Figs S5b–S8b). In contrast, the model lacking seasonal susceptible recruitment (CS) was either preferred outright or indistinguishable from CSR using data from 1984–1987, 1988–1991 and 2004–2007 (Fig. 5a,b,d) and generated large, persistent, seasonal fluctuations in acute infections (Figs S5c–S8c).

In addition, although the probability of long-term persistence was largely unchanged with the removal of either seasonal driver (Tables S1–S4), preferred models (either CSR, CS or both) generated a large, persistent reservoir of chronically infected individuals in the long term (Figs S5–S8). This reservoir contrasted with the declining influence of chronic shedding in non-preferred models (CR) over a 30-year period (Figs S5–S8).

## Discussion

Chronic shedding by asymptomatic individuals remains an oft-cited but relatively understudied mechanism driving pathogen persistence in wildlife disease systems. Although chronic shedding is readily observed in longitudinal studies of individuals, identifying it in cross-sectional disease

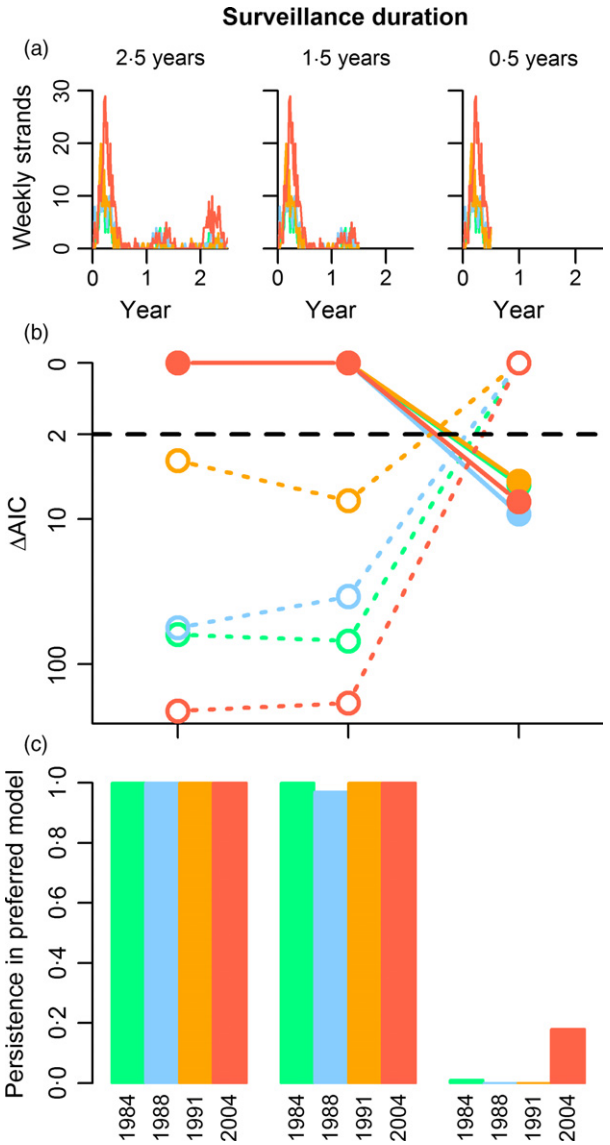


**Fig. 3.** Comparison of models with and without chronic shedding over multiple surveillance durations. (a) Stranding data for all four stranding eras at surveillance durations of 2·5, 1·5 and 0·5 years. (b) Differences in Akaike information criterion (AIC) scores (ΔAIC – beginning with the best models at 0 at the top) between chronic shedding, seasonal transmission, and seasonal susceptible recruitment (CSR) (filled circles on solid lines) and seasonal transmission and seasonal susceptible recruitment (SR) (open circles on dashed lines) at each surveillance duration in each stranding era. A ΔAIC score of less than 2 (denoted by the dashed black line) is generally considered to be a preferred model. (c) Probability of persistence for the model preferred between CSR and SR (as determined by ΔAIC in panel b). In all panels, colours denote the stranding era: 1984–1987 (green), 1988–1991 (blue), 1991–1994 (orange) and 2004–2007 (red).

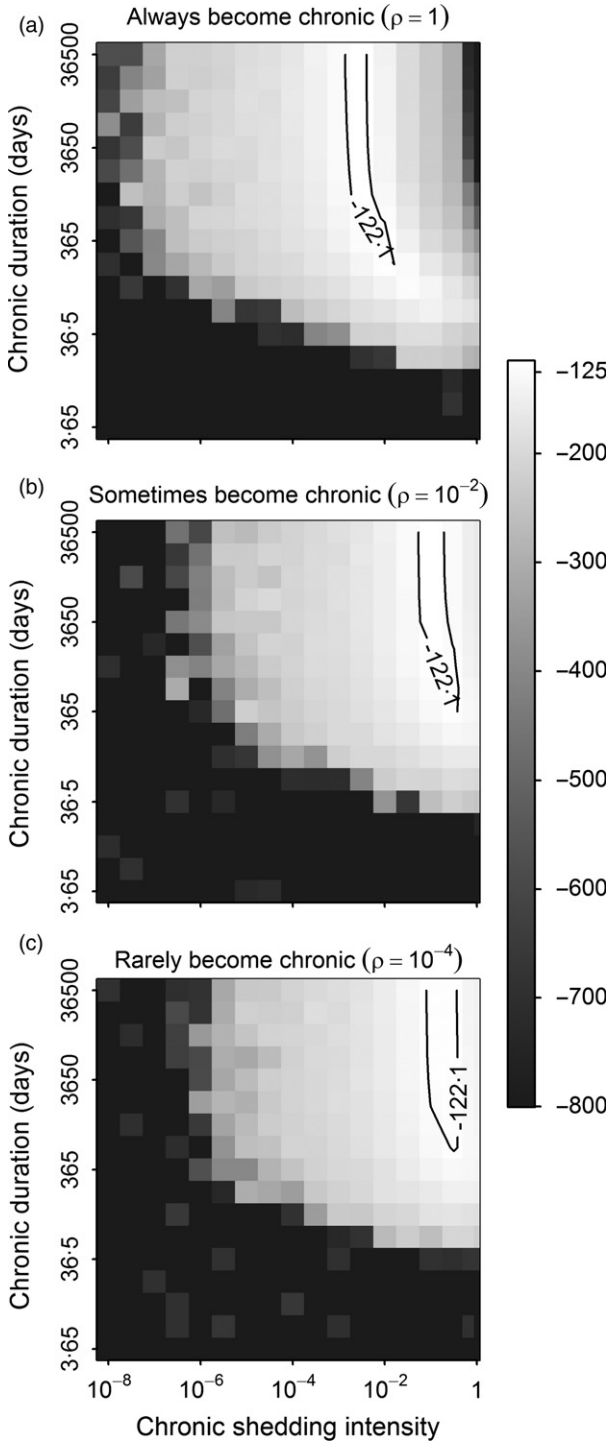


**Fig. 4.** Likelihood space for chronic shedding parameters. Using the estimated parameter values for the CSR model fit to the 1984–1987 stranding era with 2·5 years of surveillance data, the average duration of chronic infection ($1/\delta$) and the transmission efficiency of chronic shedding relative to acute shedding ($\varepsilon$) were varied, and the log-likelihood of the parameters was recalculated. This was done for three values of the probability of becoming a chronic shedder ($\rho$), (a) 1, (b) 0·01 and (c) 0·0001. Log-likelihood ranges from high (white) to low (dark grey). The contour line gives the 99% confidence region for a likelihood ratio test when all parameters except for the three chronic shedding parameters are fixed. We note that this is not a true profile confidence region due to computational constraints and is meant to be only illustrative.

surveillance data is difficult. We have shown that the role of chronic shedding can be characterized if: (i) observation occurs over at least two outbreak seasons including the intervening inter-epidemic trough; (ii) specific inference about the prevalence, intensity or duration of chronic shedding is not required; and (iii) the analysis

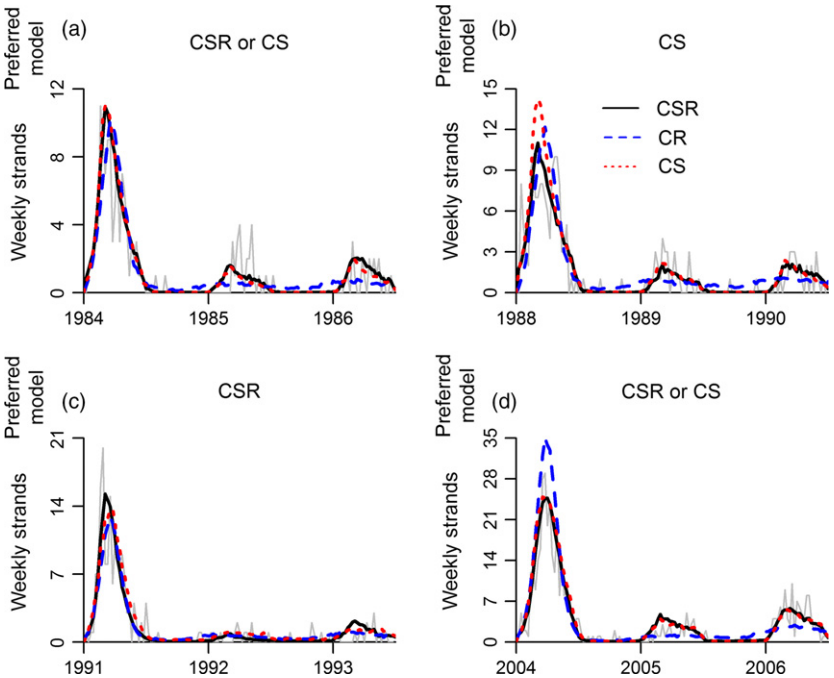


**Fig. 5.** Comparison of models with chronic shedding that either had seasonal transmission, seasonal susceptible recruitment or both. (a–d) give results for models fit to 1984–1987, 1988–1991, 1991–1994 and 2004–2007 stranding eras respectively. Models CSR (solid black line), CS (dotted red line) and CR (dashed blue line) were compared. Because of the importance of chronic infection over longer surveillance durations (Fig. 3), all models were fit with 2·5 years of stranding data. Preferred models for each dataset, as determined by Akaike information criterion (AIC) (Tables S1–S4), are shown above each panel. Simulations began on 17 June, and axis ticks marking the years occur on this date.

accounts for interactions with short-term epidemic drivers. For leptospirosis in California sea lions, we have demonstrated that chronic shedding has the potential both to describe the observed patterns in strands and to drive long-term persistence of *Leptospira* in this population (Figs 2 and 3).

The apparent importance of chronic shedding to *Leptospira* persistence depended on the time-scale of observation. When fitting data from only a single outbreak, simpler models without chronic shedding were preferred (Fig. 3). However, after observing just a single inter-epidemic trough, models with chronic shedding were strongly preferred, and these models predicted markedly higher probability of pathogen persistence (Fig. 3). The recognition that inference about population dynamics depends on the time-scale of observation has gained traction in conservation biology and ecology more generally (Hastings 2010; Buhnerkempe *et al.* 2011a) but is not yet widely appreciated in disease ecology. We postulate that this is largely due to a historical focus on the basic reproduction number, $R_0$ (the expected number of secondary infections caused by a single infectious individual in a wholly susceptible population), which is typically estimated during initial invasion of a population or at a long-term steady state. $R_0$ alone is ill-suited to address the non-equilibrium dynamics occurring in inter-epidemic troughs that operate on intermediate time-scales and ultimately govern pathogen persistence (Grassly & Fraser 2006). As we have demonstrated, observations and models must reflect the time-scales relevant to the ecological drivers being studied (Hastings 2010).

Clear identifiability issues between the properties of chronic shedding rendered it difficult to distinguish between signals of common low-intensity shedding and rare high-intensity shedding (Fig. 4). Wild captures of apparently healthy California sea lions during a leptospirosis outbreak revealed that 39% of individuals were asymptomatic shedders, suggesting the possibility of relatively common chronic shedding (Prager *et al.* 2013). However, surveys to detect changes in the proportions of symptomatic and asymptomatic shedders across seasons are needed to understand how fluctuations in the prevalence of chronic shedding impact persistence. Additionally, the best model fits indicated that mean chronic durations of a year or more were needed to promote persistence (Fig. 4). This relatively long-lived infection is consistent with existing data, where sea lions were observed shedding leptospires for at least 12 weeks (Prager *et al.* 2015) or 22 weeks (Dierauf *et al.* 1985); however, the intensity and intermittency of chronic shedding are unknown for sea lions and most other species (Ellis 2015). In this study, the reduced transmission coefficient for chronic shedders can represent either low-intensity, constant shedding or intermittent shedding following acute infection, but studies employing quantitative PCR approaches are needed to differentiate these scenarios in sea lions. As a point of comparison, Norway rats (*Rattus norvegicus*) in Brazil exhibit 84–88% prevalence of chronic shedding of *L. interrogans* serovar Copenhageni, shedding consistently and at relatively high levels, almost exclusively without symptoms (Costa *et al.* 2015). These traits align Norway rats with the classical 'maintenance host' paradigm for *Leptospira*. Our findings suggest that California sea lions, by exhibiting dramatic outbreaks, high morbidity and mortality, and probably lower prevalence of chronic shedding, represent a different class of 'maintenance hosts' that still can enable population persistence of *Leptospira*.

Inference about chronic shedding depended on epidemic drivers such as seasonal transmission and seasonal susceptible recruitment. Models without seasonal susceptible recruitment generally could not be differentiated from the full model, indicating that pulsed susceptible introduction plays a smaller role in outbreak dynamics (Fig. 5; Tables S1–S4). The relatively tight window for susceptible recruitment in our system is expected to increase the amplitude of oscillations in prevalence, and increase disease fadeout as a result (Peel *et al*. 2014). However, the long life span of sea lions (c. 17 years for males and 20 for females) may combine with the potential for extended duration of chronic shedding to mute the impact of the susceptible pulse on the expected oscillatory behaviour (Peel *et al*. 2014). We also assumed susceptible recruitment was associated with springtime weaning when pups join the coastal population. Individual variation in weaning time (Melin *et al*. 2000) or when pups first become at risk of leptospirosis could impact assessment of the role of seasonal susceptible recruitment.

In contrast, models without seasonal transmission were strongly selected against, indicating that seasonal transmission strongly shapes outbreak patterns (Fig. 5; Tables S1–S4). The probability of persistence was similar in the full model and models lacking either of the short-term drivers, further implicating chronic shedding as the principal driver of persistence (Tables S1–S4). Overall, our findings show that seasonal transmission is the predominant driver of seasonality in outbreaks of leptospirosis in California sea lions, while chronic shedding provides the bridge over periods when transmission was generally low (Fig. 2). We modelled seasonal transmission phenomenologically without a specific mechanistic underpinning, but seasonal transmission is likely to be governed by migration patterns, geographic differences in haul-out substrates, and animal density and mixing patterns at haul-outs (Riedman 1990; Zuerner *et al*. 2009), all acting in concert with environmental changes. However, characterizing seasonality in this system is potentially limited by stranding data that only covers a portion of the migratory range of this population (Fig. S2). Despite this potential limitation, we believe that the stranding data reflect true seasonality in the system given that the major age classes affected by leptospirosis [juveniles and subadults (Greig, Gulland & Kreuder 2005; Lloyd-Smith *et al*. 2007)] remain within the TMMC stranding range following the outbreak season. Additionally, there is no evidence of different seasonal signatures outside the TMMC stranding range, including (i) extremely rare signs of current or recent infection amongst older age classes during their southward migration from north of the stranding range and (ii) extremely rare evidence of infection in pups in the US population (R.L. DeLong, unpublished data) that inhabit the rookery islands south of the stranding range year-round. These observations support the assumption that persistence via continuous chains of transmission between acutely infected individuals to the north or south of the TMMC stranding range is unlikely. However, because migration patterns and leptospirosis prevalence differ between age and sex classes (Dierauf *et al*. 1985; Gulland *et al*. 1996; Greig, Gulland & Kreuder 2005; Lloyd-Smith *et al*. 2007), future field and modelling studies that account for sea lion demographic structure could elucidate the mechanisms underlying seasonal transmission.

Our model also allows for a link between observed infections in the stranding record and total incidence in the population. In our system, the disease mortality rate, $\alpha$, and the probability of observing a dying sea lion as a strand, $p_{obs}$, together determine the probability that a new infection will be observed. Identifiability concerns between disease mortality and the observation probability make it difficult to tease apart the specific determinants of case observation (Fig. S9), but the combination of these two parameters yields a consistent probability of an infection resulting in an observed stranding event (3·3 to 6·3 of every 1000 infections stranding and being observed; Fig. S9). Because of the simplicity of our model, precise estimates of this probability are unlikely to be accurate, but qualitatively, we find that total incidence may be at least two orders of magnitude greater than the observed strandings. We did not explore potential seasonal variation in the probability of observing a strand, which could arise from seasonality in human visits to coastal sites or from sea lion migratory patterns that cause seasonal variation in the proportion of the population found within the TMMC range. However, seroprevalence data suggest that, at least during major outbreak years, *Leptospira* infection is likely widespread in the California sea lion population off the coast of the US, representing a significant reservoir of this zoonotic pathogen in the California coastal ecosystem (Lloyd-Smith *et al*. 2007).

The observed role of chronic shedding in *Leptospira* persistence is strikingly similar to that of environmental transmission in recurrent epidemics of avian influenza (Breban *et al*. 2009; Rohani *et al*. 2009; Brown *et al*. 2013). Environmental transmission also has a relatively small influence during outbreaks but even low levels of environmental contamination can serve to generate secondary outbreaks (Breban *et al*. 2009; Rohani *et al*. 2009; Brown *et al*. 2013). Environmental contamination can also play a role in the transmission of *Leptospira* (Levett 2001; Adler & de la Peña Moctezuma 2010), and *Leptospira* DNA has been detected in urine-contaminated sand around stranded pinnipeds (Cameron *et al*. 2008). We did not account for this mode of transmission in the current model, but the long-term viability of leptospires detected at haul-out sites remains an open question (Cameron *et al*. 2008). In addition, persistence in wildlife disease systems can be achieved by spillover from alternate host reservoirs (Haydon *et al*. 2002). Pathogenic *Leptospira* is known to infect many mammal species (Levett 2001; Adler & de la Peña Moctezuma 2010). This

potential persistence mechanism was not incorporated in our model, because genetic typing of *L. interrogans* serovar Pomona has shown that all strains isolated from California sea lions are distinct from strains previously isolated from terrestrial species (Zuerner & Alt 2009). Our group and others are actively seeking to characterize *Leptospira* strains in the California coastal ecosystem, which will strengthen (or overturn) the evidence for no external reservoir, but existing evidence indicates that spillover from alternative hosts does not play an important role in disease dynamics in this sea lion population. Finally, transmission from spatially distinct subpopulations can support pathogen persistence. Sea lions in the California coastal ecosystem are thought to be a genetically distinct population that migrates through the TMMC stranding range during the year (Schramm *et al.* 2009). Evidence of *Leptospira* infection has been reported in another population of California sea lions further south on the Pacific coast of the Baja peninsula (Schramm *et al.* 2009), which may encounter our study population during its migration (particularly older males, which are likely to migrate northward following the breeding season). Currently available data on *Leptospira* diagnostics in the Baja population show different serologic response profiles (potentially indicating a different infecting strain) and demographic infection patterns (including evidence of infection in pups) that do not resemble patterns seen in our study population (Avalos-Téllez *et al.* 2016). Further research is needed to identify the infecting strain in the Mexican population and to investigate possible connectivity or epidemiological interactions between these populations, to determine whether spatial coupling may contribute to pathogen persistence in this system.

Although our work has shown that chronic shedding can provide a bridge over periods of low transmission, the processes that cause inter-annual variability in outbreak sizes remain unclear. Here, by assuming a large proportion of the population is initially susceptible entering each stranding era, we have chosen implicitly to focus on the build-up of susceptible individuals through reproduction as the primary driver of the observed large outbreaks (Gulland *et al.* 1996). However, changes in transmissibility of *Leptospira* may also underlie variability in outbreak size with climatic and oceanographic conditions, such as El Niño events, potentially impacting sea lion condition or contact patterns (Weise, Costa & Kudela 2006) or survival of leptospires in the environment (Johnson & Harris 1967). Accounting for these mechanisms explicitly in future work will allow for a fuller understanding of transmission dynamics in this system, but such work is unlikely to undermine the fundamental importance of chronic shedding for long-term persistence.

Chronic shedding can be an important mechanism for pathogen persistence, but signals of chronic shedders in disease surveillance data can be cryptic. These signals are most easily detected from long-term surveillance datasets, which enable identification of short-term epidemic drivers alongside characterization of interactions between these short-term drivers and long-term drivers operating in inter-epidemic troughs. Longitudinal data from experimental infection or clinical studies have tremendous value for confirming the existence of chronic infections, and potentially to differentiate common low-intensity from rare high-intensity chronic shedding; this also provides unique opportunities to merge within-host models of disease progression with population models of disease transmission (Gog *et al.* 2015). Continued development of approaches to study chronic shedding, from individual- and population-scale data, will provide insights on the persistence of specific pathogens and on the broader problem of the role of inapparent infections in disease dynamics.

## Authors' contributions

M.G.B., K.C.P., C.C.S. and J.O.L-S. conceived the ideas and designed methodology; K.C.P., D.J.G., and F.M.D.G. collected the stranding data; J.L.L., S.R.M. and R.L.D. provided the background information on demography needed to parameterize the model; M.G.B., K.C.P. and C.C.S analysed the data; M.G.B., K.C.P. and J.O.L-S. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## Data accessibility

Weekly numbers of leptospirosis strands for all four stranding eras can be found on the Dryad Data Repository: https://doi.org/10.5061/dryad.j15ns (Buhnerkempe *et al.* 2017).

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## Supporting Information

Details of electronic Supporting Information are provided below.

**Appendix S1.** Details of POMP model fitting.

**Table S1.** Model selection results and parameter estimates for models fit to the 1984–1987 stranding era.

**Table S2.** Model selection results and parameter estimates for models fit to the 1988–1991 stranding era.

**Table S3.** Model selection results and parameter estimates for models fit to the 1991–1994 stranding era.

**Table S4.** Model selection results and parameter estimates for models fit to the 2004–2007 stranding era.

**Fig. S1.** Timeline for model transmission and reproduction parameters in relation to seasonality in leptospirosis strands and sea lion biology.

**Fig. S2.** Map showing the overlap between the Marine Mammal Center's stranding range and the entire range of the Pacific temperate California sea lion population.

**Fig. S3.** Model comparison for CSR and SR with a mean acute infection duration of 1 week.

**Fig. S4.** Model comparison for CSR and SR with a mean acute infection duration of 3 weeks.

**Fig. S5.** Predicted number of acutely and chronically infected individuals for models CSR, CR and CS fit to the 1984–1987 stranding era.

**Fig. S6.** Predicted number of acutely and chronically infected individuals for models CSR, CR and CS fit to the 1988–1991 stranding era.

**Fig. S7.** Predicted number of acutely and chronically infected individuals for models CSR, CR and CS fit to the 1991–1994 stranding era.

**Fig. S8.** Predicted number of acutely and chronically infected individuals for models CSR, CR and CS fit to the 2004–2007 stranding era.

**Fig. S9.** Likelihood space for parameters related to the probability of an infection resulting in a strand.