Application of long-term collected data for conservation: Spatio-temporal patterns of mortality in Japanese serow

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ABSTRACT.   Monitoring the mortality of wildlife provides basic demographic information to support management plan preparation. The utility of mortality records for conservation measures was investigated in the Japanese serow, focusing on temporal trends and spatial distribution. Using the mortality records of Japanese serow from 2006 to 2018 in Gifu prefecture, cause-specific mortality was categorized into five groups (disease, accident, vehicle collision, parapoxvirus infection, and unknown), and the sex ratios were examined. A state space model was used to analyze the time series for the monthly mortalities, and kernel estimation was used for the spatial distribution of the parapoxvirus infection. Land cover type around the detection point was also reported. Disease, accident, and vehicle collision mortality were similar, and 30% of mortality was of anthropogenic origin. The number of mortality records for males was higher, and the larger home range of males could account for this. The state space model showed moderate increases in monthly mortalities over time and a seasonal variation with the highest level in spring and lowest in winter. Land cover analysis demonstrated a temporal increase in the proportion of human settlement areas, suggesting the change of the Japanese serow habitat. The proximity of Japanese serow to human settlements contributed to increase in mortality records. The point pattern analysis indicated spatial clustering for parapoxvirus infection in the area where an epidemic had occurred in the past. Several measures are recommended; however, mortality records can help develop improved conservation plan.

KEY WORDS: conservation, Japanese serow, mortality, spatial point pattern, time series analysis

The Japanese serow (*Capricornis crispus*) is a solitary ungulate that inhabits forests, from the lower mountain slopes to the subalpine zone, and is endemic to Japan [34]. Adults of both sexes have intrasexually exclusive territories. They are designated as a special natural monument in Japan and are protected in conservation areas established in high altitude mountainous regions. In Gifu prefecture, located in the central region of Honshu, there are three conservation areas for Japanese serow. However, the density of Japanese serow in these areas has been declining recently, as same as almost all conservation areas in Japan from 2000 [47]. Meanwhile, culling has been conducted to protect coniferous plantations from herbivory by Japanese serow outside the conservation areas. Given these contexts, it is crucial to evaluate population status without making a distinction between conservation areas and other habitats. However, limited data are available outside of the conservation areas.

Cause-specific mortality is one of the important demographic parameters in estimating wildlife population dynamics and provides guidelines for management [44]. The mortality of wildlife is ascribed to various causes. For example, vehicle collision is one of the anthropogenic causes of mortality in wildlife and negatively impacts wildlife populations and human safety [2]. Infectious diseases (especially zoonoses) are a huge concern not only for the wildlife population but also for agriculture and human health [8]. Therefore, it is imperative to elucidate mortality caused by disease and non-disease events to assess the population's sustainability and achieve the objective of 'One Health' [14]. In Japanese serow, mortality has various causes, such as pneumonia, enteritis, and traffic accidents [32], and parapoxvirus infection is a well-known cutaneous disease.

An understanding of the spatio-temporal patterns of wildlife mortality is essential to conserve the wildlife population and ensure human health and safety [23, 41]. Unevenly distributed carcasses or sick animals in specified areas imply that a specific disease may be endemic, and are important indicators for the control of infectious diseases [10]. Temporal increases in mortality may indicate the spread of lethal disease [10] or population growth [31], and seasonality reflects species-specific ecology [7].

Surveillance data, especially when collected over a long period, can indicate major causes of mortality. It is also useful in analyzing trends, such as the focalized distribution of a specific disease or seasonality [1]. The Agency of Cultural Affairs requires people to

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report if they find a Japanese serow carcass, such that there is an accumulation of mortality records of Japanese serow inside and outside the conservation area. However, these data have not been sufficiently utilized for management throughout the habitat, even though the necessity of increased conservation efforts has been emphasized recently [48].

In the current study, we verified the utility of mortality records that were administratively collected for conservation. In particular, cause-specific mortality of Japanese serow was described as a basic demographic parameter. In addition, temporal trends and seasonal variations in mortality and the factors that influence them, as well as the spatial distribution of parapoxvirus infection cases were investigated.

MATERIALS AND METHODS

Study area

The survey was conducted in Gifu prefecture, located in the central region of Honshu, Japan. The mean annual precipitation and temperature are 1,827.5 mm and 15.8°C in the southern region, respectively, and 1,699.5 mm and 11.0°C in the northern region, respectively [15]. Forested land (8,620 km^2) occupies almost 80% of the whole area (10,620 km^2), and 45% of the forest is covered with conifer plantations [16]. Other forested land is dominated by broad-leaved deciduous forests in the middle to the northern region and broad-leaved evergreen forest in the southern area [13]. The Japanese serow is widely distributed due to extensive areas of suitable habitat, namely, rich broad-leaved deciduous forests [34]. The culling of Japanese serow first began in Gifu prefecture in 1979 to prevent damage to young conifer plants, and other prefectures followed. The local government of Gifu prefecture has considerable experience in the management of the Japanese serow, and has well organized mortality records of this animal; therefore, Gifu was considered a suitable study area.

Data

A total of 1,202 mortality records of Japanese serow was collected across Gifu prefecture from March 2006 to December 2018 in compliance with the Law for Protection of Cultural Property. In principle, when a local community member detected a carcass of Japanese serow, the discoverer was obliged to report to the local educational board. The local educational board staff then went to the detection point and recorded the following information; date of detection, geographical point of detection, cause of mortality, age, sex, body length, withers height, and horn length. The cause of mortality was determined by veterinarian or non-expert staff through gross observation and the situation when the carcass was detected. For example, parapoxvirus forms papular or nodular lesions on the lips, eyelids, oral mucosa, muzzle, or skin of the udder, and feet [37]; therefore, the diagnosis of parapoxvirus infection was generally based on macroscopic characteristics of lesions. All mortality data were reported to Gifu prefectural government, and the geographical information regarding the point of detection and the attributes of the carcasses were converted to GIS data format. All geographical data were analyzed using QGIS 3.4.15 [39] and projected to the UTM zone 53N coordinate system (EPSG: 6690).

Classification of cause of mortality

Because the raw data on the causes of mortality were documented in free format, they were converted to a classification for statistical analysis. This was achieved by arranging the cause of mortality in the raw data in a procession of morbidity events, in accordance with the rules and guidelines adopted by the World Health Assembly and applied in medical science [50]. In principle, the underlying cause of mortality was determined as the first event in the sequence of morbid events. For example, if the raw data said, "died by hemorrhagic shock due to a train collision", the underlying cause of mortality was noted as "train collision," which was the primary event. In cases where a single underlying cause of mortality could not be determined, recording multiple causes was allowed.

Underlying causes were then categorized into five groups; vehicle collision (Vehicle collision), accidents except for vehicle collision (Accident), parapoxvirus infection (Parapox), diseases except for parapoxvirus infection (Disease), and unknown (Unknown). The incidence of parapoxvirus infection could have a serious impact on the population of Japanese serow [30] and was therefore categorized independently.

Statistical analysis

The χ^2 test was used to test deviation from 1:1 sex ratio (male or female; unknown sex data was not included in the statistical test) in each of the five groups. A P value <0.05 was considered statistically significant.

The temporal trend and seasonal variation in the number of mortality records (March 2006 to December 2018; 154 months) were analyzed for all-cause mortality, Disease, Accident, and Vehicle collision (Parapox did not contain enough data for analysis) by time series analysis using a state space model represented by the following formulae:

$$y_t \sim Poisson\left(\exp(\theta_t)\right) \quad \dots (1),$$

$$\theta_t = \mu_t + \gamma_t \quad \dots (2),$$

$$\mu_{t+1} = \mu_t + v_t + \xi_t \text{ with } \xi_t \sim N(0,\sigma_\xi^2) \quad \dots (3),$$

$$v_{t+1} = v_t + \zeta_t \text{ with } \zeta_t \sim N(0,\sigma_\zeta^2) \quad \dots (4),$$

$$\sum_{j=0}^{11} \gamma_{t+1-j} = \omega_t \text{ with } \omega_t \sim N\left(0,\sigma_\omega^2\right) \quad \dots (5),$$
t=1, 2, …., 154.

where formula (1) represents the observation model, y_t is the observed value (the number of mortality records per one month), and formula (2) represents the state model, θ_t stands for the unobservable state in time t which was composed by trend (μ_t) and seasonal (γ_t) component (represented by formula (3), (4), and (5)). The model considering the trend and seasonality is called the basic structural time series model, which should be verified the first time the model is constructed [33] and plays a prominent part in structuring time series analysis [12]. ξ_t, ζ_t, and ω_t are disturbances and mutually independent. σ_ξ^2, σ_ζ^2, and σ_ω^2 are variances. j stands for the jth 'month' in a year [12]. The trend component of the model considers both the level (μ_t) and the slope term (v_t), which are varying over time. The model with this trend component structure is called a local linear trend model and is a popular choice for modeling trends [6]. Importance sampling, which was the simulation method used for the adjustment of sampling-distribution bias generated by the model approximations, was conducted for 1,000 times.

Point density for Parapox was nonparametrically estimated as intensity by isotropic Gaussian kernel estimation. A bandwidth was optimized by the likelihood cross-validation method [3]. To elucidate the spatial characteristics of Parapox, the analysis area was generated as the concatenation of the 1,000 m radius buffer zone from each point in all-cause mortality. Intensity values were reported as counts per square kilometer. For detecting deviations from spatial homogeneity, the L-function was used to test the range of spatial structures. L-function ($L(r)$) is a transformation of Ripley's K-function ($K(r)$), which makes a visual assessment of deviations much easier ($L(r) = \sqrt{K(r)/\pi}$) [3]. In computing the K-function at scale r, hypothetical circles of radius r were placed around each point location, and the average number of points within those circles was calculated [27]. The null hypothesis of complete spatial randomness (CSR) was graphically verified using global envelope, which was determined by finding the maximum deviation from the theoretical L-function for CSR ($L_{theo}(r)$) through simulations. If the graph of the estimated L-function for the data ($\hat{L}_{obs}(r)$) transgresses upper or lower limit of global envelopes at any scaled distance r along the horizontal axis, it is statistically significant with a P-value of $1/(m+1)$ where m is the number of simulated patterns [3]. We set $m=19$, namely significance level 0.05 was given. When the null hypothesis of CSR was rejected, point pattern was classified as clustered ($\hat{L}_{obs}(r) > L_{theo}(r)$) or regular ($\hat{L}_{obs}(r) < L_{theo}(r)$).

All analyses were conducted using R 3.6.1 [40] with the KFAS package [18] for analyzing the state space model, and spatstat package [3] for analyzing spatial distribution.

Land cover type analysis

The proportion of human settlement area to all land cover types was calculated in the buffer area, centered at the points where carcasses were detected [11]. Firstly, each detection point was buffered with a radius of 300, 400, and 500 m. These buffer radii were selected on the basis of the size of the Japanese serow's home range [36, 38, 45, 51]. Each buffer was spatially laid on a 1/50,000 vegetation map [4]. The vegetation data were clipped using the buffers, and the area of each land cover type was calculated. The land cover types were classified into two categories, namely 'land specifically related to human activity (residential area, industrial area, developed land, cropland, paddy field, pasture, and golf course)' and 'other (the specific type of vegetation such as 'coniferous plantation', '*Quercus serrata* forest' for example, or 'open water', or 'cut-over area')', followed by the calculation of the proportion of the former category in each year.

RESULTS

Cause of mortality and sex-specific characteristics

All causes of mortality documented in free format were successfully categorized into five groups (Table 1): Disease (28.5%), Accident (28.8%), Vehicle collision (26.2%), Parapox (4.2%), and Unknown (12.8%). In 10 cases, underlying cause of mortality could not be categorized as one cause, and relevant two causes were assigned. In Accident, drowning was predominant, followed by fall. A total of 23 cases of accidental entanglement with artificial fencing or net were included in the subgroup of 'captured between objects.' In Disease, details were unknown in more than 60% of cases, 40% were due to senility, starvation, infectious disease, and disorders in specific organs or in particular situations. In Vehicle collision, the proportion of the unknown groups reached almost 90%, and were documented as only 'vehicle collision' in the raw data. However, it was assumed that the true percentage of automobile collisions must be higher than the result obtained in our study (19/315, see Table 1) because most of their detection points were roadside. Fifty-one cases of Parapox were recorded over the study period.

The sex ratio was significantly different from one, and the proportion of male (57.4%) was higher than female (42.6%, $P<0.001$). This was true in the case of Disease ($P=0.019$), Accident ($P<0.001$), and Vehicle collision ($P=0.0041$) (Table 2). No statistically significant differences in Parapox and Unknown were detected.

Temporal trend and seasonal variation

The level of the monthly mortalities showed a moderate increase through the study period (Fig. 1a) except for an irregular peak in February 2015 in all-cause mortality. The highest value of the level, 12.3 in March 2018 and February 2015 was almost four times the lowest value of 3.2 in November 2006. In Disease, Accident, and Vehicle collision, the overall trend of the temporal increase of the level was also observed (Fig. 1b, 1c, and 1d). Furthermore, Disease showed irregular peak in February 2015.

There was explicit seasonality for all-cause mortality with the highest value in April and the lowest in December (Fig. 2a). In cause-specific mortality, the seasonal variations were similar between Accident (Fig. 2c) and Vehicle collision (Fig. 2d), with high in spring (April to May) and low in winter (November to February). Compared to Accident and Vehicle collision, the peak shifted to March and the number of mortalities did not show salient decrease in January to February in Disease (Fig. 2b).

Table 1. Cause-specific mortality of Japanese serow in five categorized groups

Disease (343) [a]	Accident (346) [b]	Vehicle collision (315)	Parapox (51)	Unknown (154)
Details unknown (209)	Drowning (135)	Details unknown (281)	Parapoxvirus infection (51)	No listed (153)
Senility (42)	Fall (94)	Train (14)		Euthanasia (1)
Circulatory diseases (33)	Trauma (41)	Automobile (19)		
Starvation (29)	Captured between objects (26)	Bicycle (1)		
Respiratory diseases (9)	Details unknown (15)			
Infectious disease (6)	Predation / fight (14)			
Perinatal disease (4)	Asphyxia (10)			
Scabies (3)	Meteorological factor (9)			
Dermatosis (2)	Electrocution (2)			
Gait disturbance (3)	Poaching (1)			
Capture myopathy (1)				
Cataracts (1)				
Digestive diseases (1)				
Peritonitis (1)				
Urinary system diseases (1)				

The number in parenthesis represents the number of cases. Data of mortality record were collected in Gifu prefecture form March 2006 to December 2018. Disease: diseases except for parapoxvirus infection, Accident: accidents except for vehicle collision, Vehicle collision: vehicle collision, Parapox: parapoxvirus infection, Unknown: unknown. [a] In two cases, two underlying causes categorized to Disease were contained, and we counted only one as five group categorization. [b] In one case, two underlying causes categorized to Accident were contained, and we counted only one as five group categorization.

Table 2. Comparison of cause-specific mortality in Japanese serow within sex

		Sex			Sex ratio	P-value [a]
		Male	Female	Unknown		
Cause of mortality	Disease	181	139	23	0.57	0.019
	Accident	196	133	17	0.60	<0.001
	Vehicle collision	177	127	11	0.58	0.0041
	Parapox	29	19	3	0.60	0.15
	Unknown	41	42	71	0.49	0.91
Total		619	459	124	0.57	<0.001

Data of mortality record were collected in Gifu prefecture form March 2006 to December 2018. Disease: diseases except for parapoxvirus infection, Accident: accidents except for vehicle collision, Vehicle collision: vehicle collision, Parapox: parapoxvirus infection, Unknown: unknown. [a] $P<0.05$ was considered statistically significant.

The percentage of the human settlements demonstrated an overall increase during the study period, independent of buffer size, although values fluctuated slightly between the years (Fig. 3). This value was lowest in 2006, and highest in 2018 and 2015, increased by 2 to 2.5 times.

*Spatial distribution of parapoxvirus infection cases*
The null hypothesis of CSR was rejected at the 0.05 significance level (Fig. 4a). Point patterns were diagnosed as clustered when *r* was over approximately 10 km. The intensity showed a moderate increase in the middle-west area and was notably high in the south-east area of Gifu prefecture (Fig. 4b).

DISCUSSION

Cause of mortality as baseline data
Baseline data on the causes of Japanese serow mortality were determined, although some exact causes in each category are still unknown. Because Disease, Vehicle collision, and Accident contributed almost the same levels to overall mortality, it could be considered that these are the three leading causes of mortality in Japanese serow in Gifu prefecture. However, the limitation in using mortality records is that the records reflect only the mortality inside the human activity areas, and the true percentages of causes of mortality in Japanese serow might be different. Even after considering this limitation, anthropogenic causes (vehicle collisions, accidental entanglement with fencing, electrocution, and poaching) must have a significant effect on the population because they constituted almost 30% of all-cause mortality on record in Gifu prefecture.

The 51 mortalities categorized to Parapox were assumed to be severe cases of infection. Although the parapoxvirus infection itself is not thought to be fatal, the infected Japanese serow can become emaciated and will die because it cannot forage or walk due to the sore mouth or feet, or through secondary contamination with suppurative microorganisms [19, 52] and complications [22].
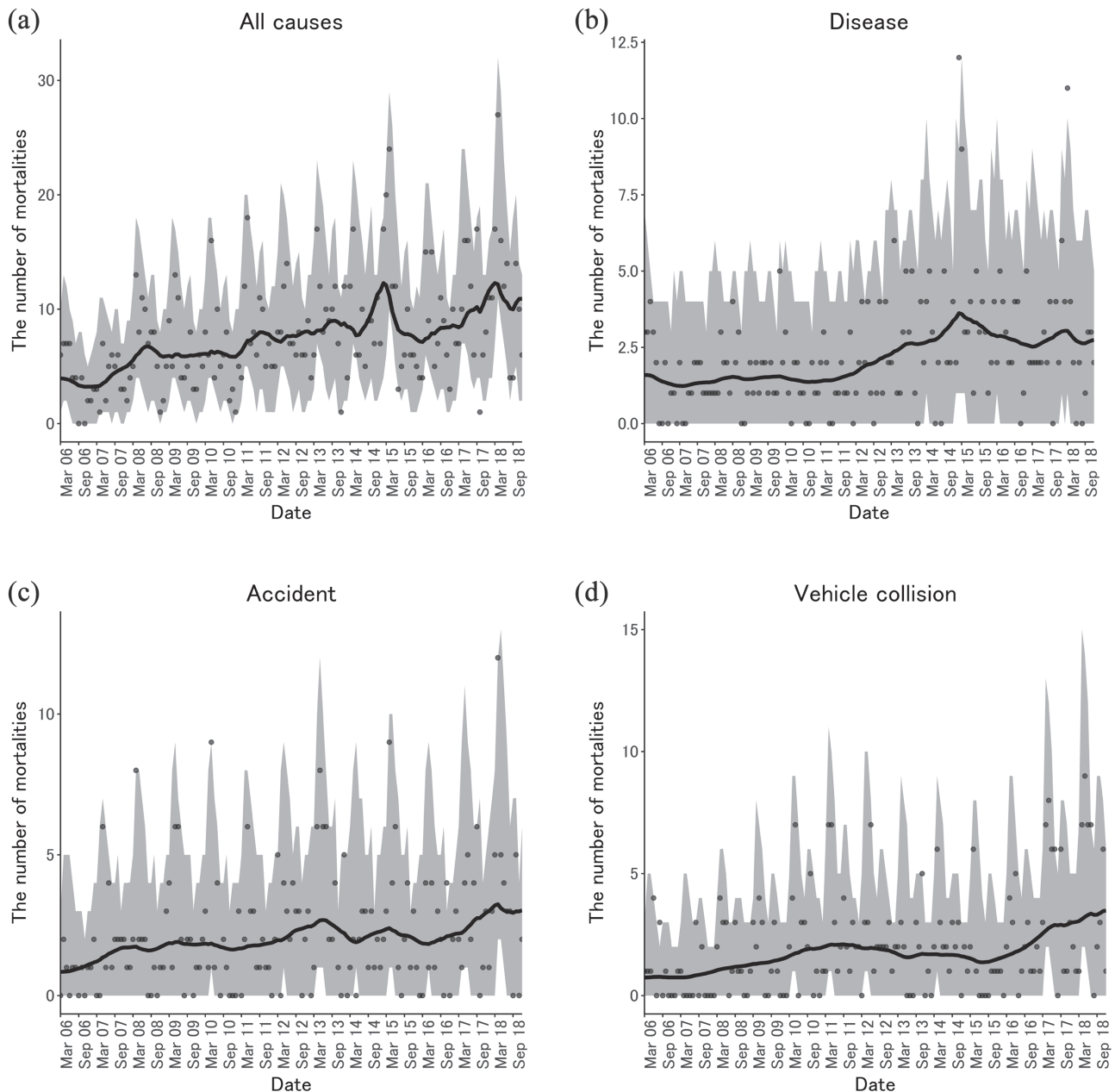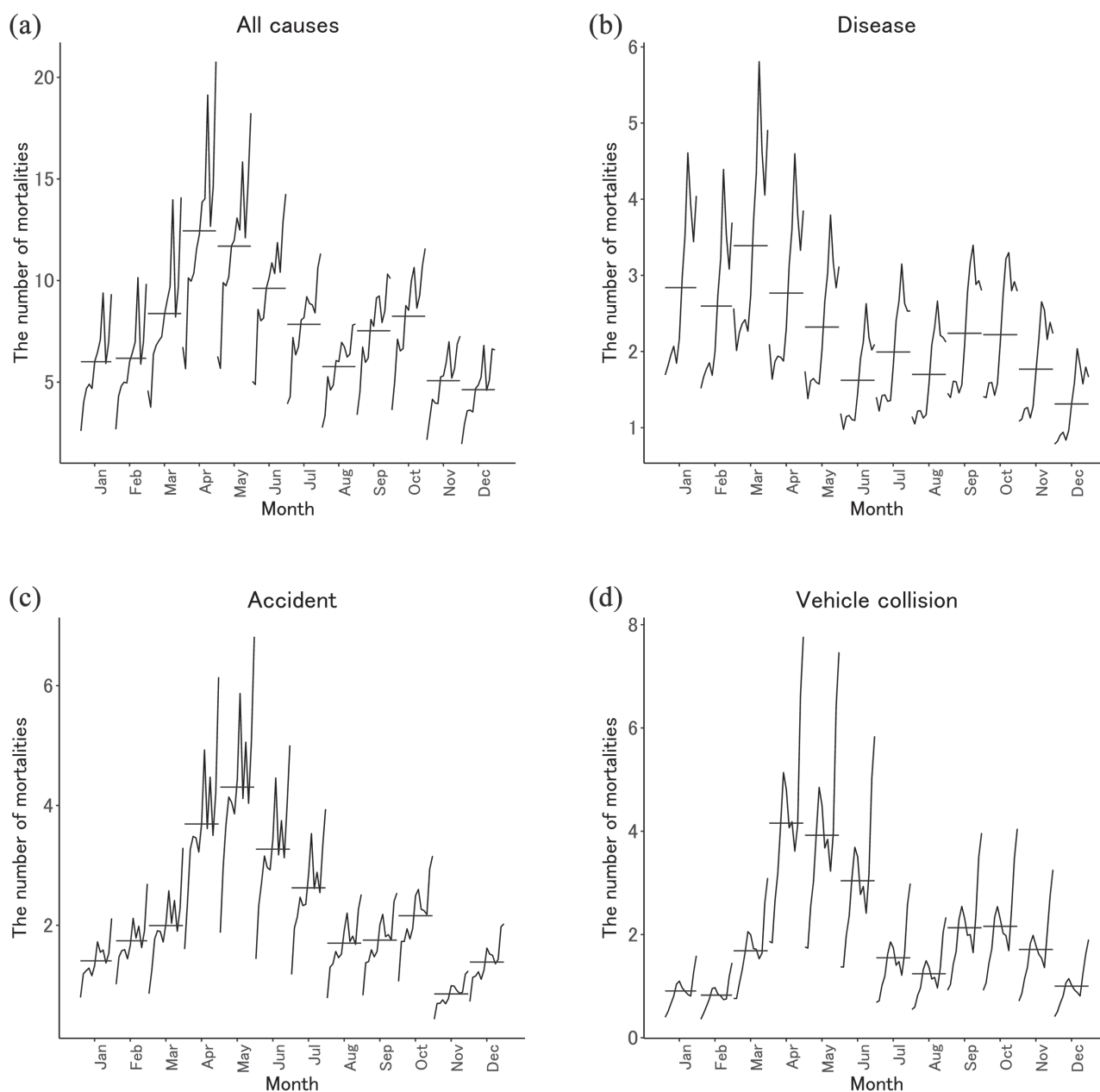
Fig. 1. The trend component estimated by the state space model for the number of mortality records of Japanese serow in Gifu prefecture, from March 2006 to December 2018; (a) All causes, (b) Disease, (c) Accident, and (d) Vehicle collision. Black line represents the level which is the unobservable state. Gray dots are observed values; gray band line denotes predicted interval of observed value. Disease: diseases except for parapoxvirus infection, Accident: accidents except for vehicle collision, Vehicle collision: vehicle collision.

The number of mortality records differed by sex

The more frequent detection of male carcasses could be ascribed to the larger home range of males [25, 35, 36]. As males have a larger home range, they would be more likely to appear near humans, concomitantly increasing the risk of accidents and the probability of their carcasses being discovered. On the other hand, the number of Parapox cases did not differ significantly by sex. Considering the previous study on culled animals that reported higher morbidity rate of parapoxvirus infection in females than in males [28], the mortality due to parapoxvirus infection was presumed to be more frequent in females in nature. Thus, the higher detection rate in male carcasses was offset, resulting in no significant difference in mortality record. In summary, the mortality record in the Japanese serow reflects sex-specific ecology.

Temporal increase and seasonal variation in the number of monthly mortalities

The number of monthly mortalities showed a temporal increase during the study period, and this could be ascribed to a change in the principal habitat of the Japanese serow. Firstly, the temporal increase in mortality was not due to population growth in all local

Fig. 2. The seasonal component estimated by the state space model for the number of mortality records of Japanese serow in Gifu prefecture, from March 2006 to December 2018; (a) All causes, (b) Disease, (c) Accident, and (d) Vehicle collision. Black line represents the changes in seasonality over time and blue line is the mean of the estimates within each month. Disease: diseases except for parapoxvirus infection, Accident: accidents except for vehicle collision, Vehicle collision: vehicle collision.

serow populations in Gifu prefecture because the serow population size has been decreasing in all conservation areas established in mountainous regions [26, 47]. Furthermore, the proportion of the human settlement area around the carcass detection point has increased over time, although there was almost no change in the proportion of the human settlement area in the whole area of Gifu prefecture during the study period [17]. Because the buffer area approximated the home range of the Japanese serow, the result indicated that the occupancy rate of human settlement has increased within the habitat of the Japanese serow. Taking these factors into consideration, the possibility is that the serow population has increased near human settlements, and the likelihood of detecting carcasses has become greater because of this change in Japanese serow habitat.

There was seasonality in the number of mortality records, and the phenology of the Japanese serow could be responsible for this. The spring peak in all-cause mortality reflected the seasonality of Accident and Vehicle collision. This trend could be explained by a seasonal increase in the daily moving distances of the Japanese serow [24], resulting in increased opportunities for appearance in the human activity area, being detected (alive or dead), and meeting with an accident. The small number of mortalities in winter (all-cause mortality, Accident, and Vehicle collision) could be ascribed to a seasonal reduction in daily human activity, resulting in a decreased

likelihood of detecting carcasses [32]. Meanwhile, Disease did not show a notable reduction in observations from January to March compared to Accident and Vehicle collision. The mortality related to starvation increases in winter or following spring in wild ungulates [9, 46], and therefore the number succumbing to Disease should increase in winter. The increased number for Disease in February 2015 (Fig. 1b) contributed to the irregular peak in all-cause mortality at the same month (Fig. 1a). It should have also been influenced by the harsh winter because the winter of 2014–2015 recorded the deepest snow during the study period [21].
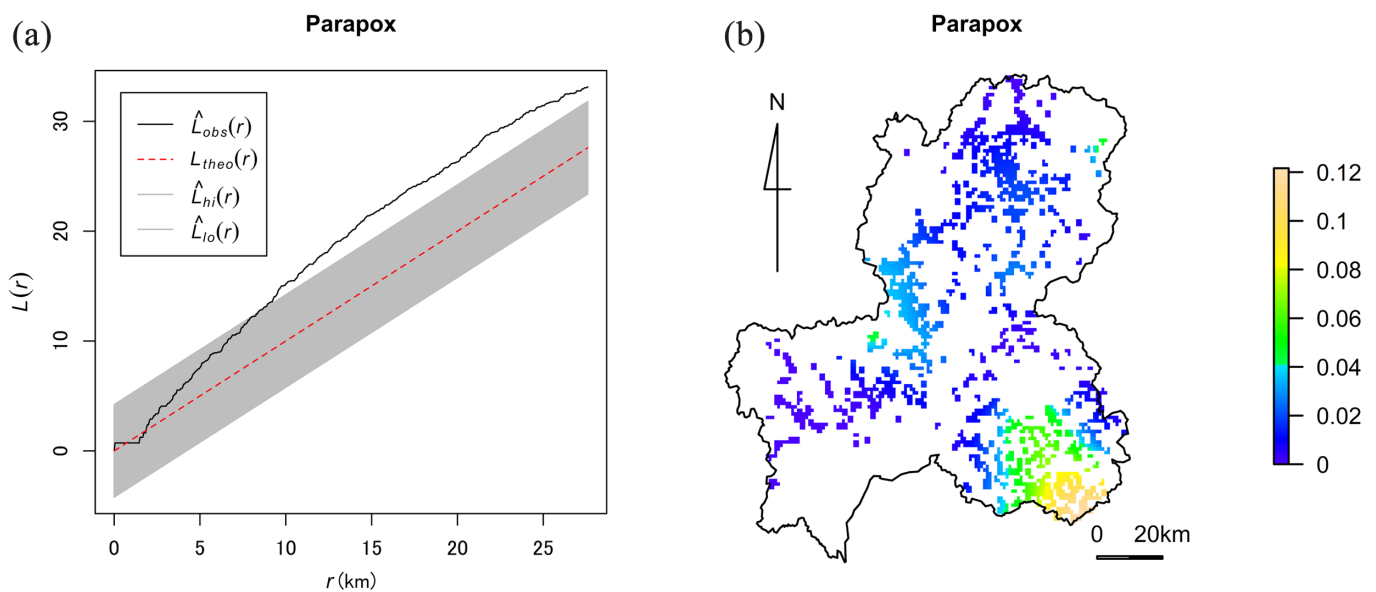
Spatial clustering of parapoxvirus infection cases

Parapox points were clustered moderately in the middle-west area and densely in the south-east area of the Gifu prefecture, indicating a 'hot spot'. The densely clustered area corresponds to the district where morbidity of the disease was highest in 1984–1985, when an outbreak of parapoxvirus infection was observed in Japanese serow in Gifu prefecture [42]. Parapoxvirus is transmitted by direct contact to cutaneous lesions or on fomites (e.g., grove with the virus attached) through their social behavior, such as territory marking, feeding, breeding, or suckling [19, 42], and has long-term infectivity in the environment [29]. Diseases that are transmitted directly between hosts typically follow a density-dependent transmission process [5]. The contact rate between susceptible and infected animals and the number of new susceptible animals is a determining factor in the persistence of infection [43]. Extrapolating these knowledges to the present case, spatial continuity of territory, and constant recruitment of susceptible individuals as newborn calves would be significant factors in maintaining the infection in the south-east area of the Gifu prefecture. Transmission of the disease could also be affected by community structure, landscape [5], and virulence that could be changed during evolutionary interplay between the host and pathogen [49]. More detailed surveys on this topic are required.

On the other hand, interpretation of the cluster should be considered in the context of any observational bias that may be present. There was no common protocol to diagnose the parapoxvirus infection, and the diagnostic criteria may differ in each municipality.
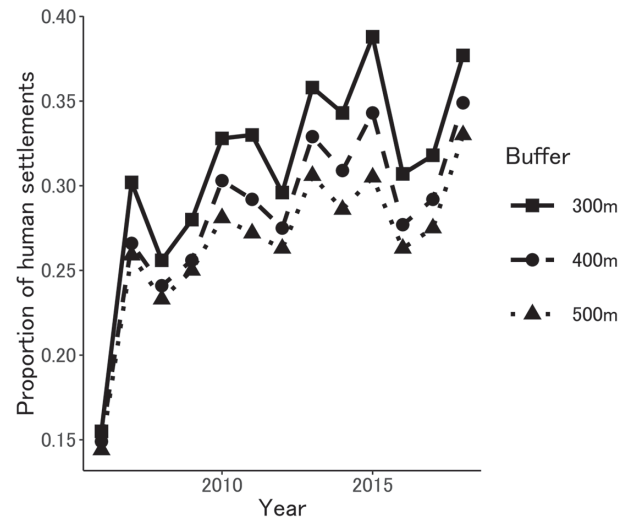
Fig. 3. The proportion of human settlements in each tested buffer area centered at the detection point of Japanese serow carcass. Filled square with solid line, filled circle with dashed line, and filled triangle with dotted line stand for 300 m, 400 m, and 500 m buffer zones, respectively, used in the calculations. Mortality record data were collected in Gifu prefecture from March 2006 to December 2018.

Fig. 4. (a) Spatial pattern analysis using *L*-function for distribution of parapoxvirus infection cases in Japanese serow. $\hat{L}_{obs}(r)$ is the estimated *L*-function for the data point pattern, $L_{theo}(r)$ is the theoretical *L*-function under a homogeneous Poisson point process (CSR), $\hat{L}_{hi}(r)$ and $\hat{L}_{lo}(r)$ are upper and lower global envelopes, respectively. All functions have been estimated at each scaled distance *r*. Mortality record data were collected in Gifu prefecture from March 2006 to December 2018. (b) The kernel density estimation on point patterns of parapoxvirus infection cases in Japanese serow. Estimated density is indicated as intensity values (counts per square kilometer). An outline map of Gifu prefecture is shown by black line. Mortality record data were collected in Gifu prefecture from March 2006 to December 2018.

Conservation implications

Mortality records can be used in the development of conservation plans and the determination of future monitoring plan. The distribution of parapoxvirus infection from an epidemiological perspective is important, and more precise conservation plans at a regional scale are required. In addition, the risk of disease transmission to humans and livestock in high occurrence areas should be considered because parapoxvirus infection is a zoonotic disease and infectious to multiple species [19]. The number of mortalities should be monitored over time and could be used to verify the hypothesis that the habitat area of the Japanese serow has been changing.

Our results suggest the necessity of monitoring anthropogenic causes. Anthropogenic effects on Japanese serow have been recognized as a significant problem [48]. Mortality records could offer both qualitative and quantitative information about the anthropogenic causes of mortality and are therefore valuable material for monitoring the effects of human activities on local serow populations.

Several improvements in data-collecting systems are imperative for precise understanding and appropriate management. Data accumulation and integration would help us to understand the habitat status of each population group. Although the serow population cannot be separated by artificial borders such as the prefectural boundary, the mortality records were accumulated at the prefectural level. A cross-jurisdictional analysis would enable us to detect extraordinary events at the local population level.

"Abnormal findings on the carcass", as well as "cause of mortality", should be reported in order not to overlook the clinically important cases. Institutionally, only "cause of mortality" is required. However, diseases, which in themselves are not fatal, can still have a significant impact on the serow local population, especially in the case of endangered.

To ensure accurate diagnoses, it is essential to standardize the diagnostic protocol, so that it is comprehensible to non-experts, and to conduct post-mortem examinations by veterinarians as much as possible. Furthermore, we recommend that the diagnosis of parapoxvirus infection should be based on a scientific virus detection method implementable on-site [20]. The long-term collection of mortality data would not only offer insights into the current ecology of the Japanese serow, but would also be a guide for future conservation plans.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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