Biology

Microbiology & Immunology fields

Okayama University   *Year* 2008

A stochastic model of Echinococcus multilocularis transmission in Hokkaido, Japan, focusing on the infection process

Tomohiko Nishina*   Hirofumi Ishikawa†

*Department of Human Ecology, Graduate School of Environmental Science, Okayama University

†Department of Human Ecology, Graduate School of Environmental Science, Okayama University

A stochastic model of *Echinococcus multilocularis* transmission in Hokkaido, Japan, focusing on the infection process

Tomohiko Nishina [a, b], Hirofumi Ishikawa [a,*]

[a] Department of Human Ecology, Graduate School of Environmental Science, Okayama University, 700-8530, Japan

[b] Nakashima Propeller Co., Ltd. 700-8691, Okayama, Japan

Running title: Stochastic model of *Echinococcus multilocularis* transmission

[*] Address correspondence to: Hirofumi Ishikawa, Prof. Department of Human Ecology, Graduate School of Environmental Science, Okayama University, 700-8530, Okayama, Japan.
Tel. +81-86-251-8826, Fax. +81-86-251-8837, E-mail: ishikawa@ems.okayama-u.ac.jp

Abstract

Echinococcus multilocularis causes human alveolar echinococcus. In Japan, high prevalence of *E. multilocularis* among the fox population has been reported throughout Hokkaido. Accordingly, control measures, such as fox hunting and the distribution of bait containing Praziquantel, have been conducted. This study developed a transmission model for individuals in the fox population and included a stochastic infection process to assess the prevalence of *E. multilocularis*. To make our model realistic, we used the worm burden for each individual in the fox population. We assumed that the worm burden depends on the number of protoscoleces in a predated vole and the number of infection experiences. We carried out stochastic simulations with 1000 trials for the situations of Koshimizu and Sapporo, Hokkaido, Japan. The distribution of the worm burden among foxes obtained using the model agreed with dissection data. The simulation indicates that a careful choice of season is necessary for an effective distribution of Praziquantel-containing bait. A stochastic model for *E. multilocularis,* which can assess the range of the prevalence in the fox population, would be helpful in analyzing their complex life-cycle and also in designing control strategies.

Keywords: *Echinococcus multilocularis, stochastic model, control strategy, Hokkaido*

1. Introduction

Echinococcus multilocularis is prevalent in the northern part of the northern hemisphere. It causes alveolar echinococcus (AE) in humans. In Japan, AE has spread throughout Hokkaido, where about 10 new human cases have been reported each year since 1982 (Department of Health and Welfare, Hokkaido Government, in the Annual Report of the Council for Alveolar Echinococcus in Hokkaido, 2002–2003). It is difficult to elucidate the source of AE infections because of its long incubation period (more than ten years) (Doi *et al*., 2000).

E. multilocularis maintains its transmission cycle among definitive and intermediate hosts. In Hokkaido, the major definitive host is the red fox (*Vulpes vulpes*); the major intermediate host is the gray-sided vole (*Clethrionomys rufocanus bedfordiae*) (Ohbayashi, 1996). Throughout Hokkaido, the prevalence of *E. multilocularis* in the fox population was reported as 57% in 1998 (Department of Health and Welfare, Hokkaido Government, in the Annual Report of the Council for Alveolar Echinococcus in Hokkaido, 2002–2003) and remains as high as 43% in 2003 (Takahashi *et al*., 2005) in spite of various control measures such as fox hunting and distribution of bait containing Praziquantel (Tsukada *et al*., 2002; Takahashi *et al*., 2002).

Intermediate hosts are infected when they orally ingest *E. multilocularis* eggs, which subsequently develop into hydatid cysts in their livers. Infectious protoscoleces are asexually reproduced within hydatid cysts. Definitive hosts are infected by *E. multilocularis* when they prey on an intermediate host that harbors protoscoleces. The *E. multilocularis* eggs are excreted along with feces of the definitive host after maturation.

A simple deterministic model of *E. multilocularis* was formulated by Roberts and Aubert (1995) to evaluate the effects of control in France. Ishikawa *et al*. (2003) proposed a deterministic model incorporating the dynamics of both the definitive and intermediate hosts and with the seasonal effect on the longevity of *E. multilocularis* eggs for quantitative analysis of the

3

mechanism of seasonal transmission in Hokkaido. Hansen *et al.* (2003; 2004) developed a spatial model based on stochastic methods. Takumi and Giessen (2005) examined the life expectancy of *E. multilocularis* eggs and the density of hosts as variables in their model seeking an effective control method against host animals.

This study is intended to develop the model constructed by Ishikawa *et al.* (2003) into a stochastic model. The foxes are modeled on an individual basis stochastically following the population dynamics and the infection process, so that a simulation using the model will assess the range of prevalence of *E. multilocularis*. In our model, the worm burden incurred by the fox upon infection by the vole is determined based on the distribution of protoscoleces among infected voles and the age distribution of the vole population. Although little is known about how protoscoleces propagate within infected voles, we assume that the number of protoscoleces in voles increases exponentially after a latent period, that the rate of increase declines because of the environmental capacity, and that the number of protoscoleces is eventually saturated. We consider an immune structure in the model based on dissection data showing that juvenile foxes have significantly greater worm burdens than adult foxes (Hofer *et al.*, 2000; Fischer *et al.*, 2005).

Distribution of Praziquantel-containing bait had been undertaken to reduce the prevalence of *E. multilocularis* in the fox population in Koshimizu (Tsukada *et al.*, 2002) and in Nemuro (Takahashi *et al.*, 2002). We performed a follow-up study to the report of Tsukada *et al.* (2002) on the transition of the prevalence in Koshimizu. Furthermore, we compared the effects of bait distribution between the two distribution seasons using our model. The results of simulations suggest a remarkable difference between the effectiveness of early summer and winter baiting programs in terms of prevalence reduction.

Recently, foxes have begun to migrate into urban areas (for example, Sapporo, the capital of Hokkaido), where the risk for AE-infection cannot be disregarded (Uraguchi and Takahashi,

1998). Therefore, immediate control measures against *E. multilocularis* are needed not only for rural but also for urban areas. Our model is inferred to be helpful for design of *E. multilocularis* control strategies.

2. Materials and Methods

2-1. Population dynamics of the hosts

The life-cycle of *E. multilocularis* is maintained through infection of the intermediate host (gray-sided vole, *Clethrionomys rufocanus*) and the definitive host (red fox, *Vulpes vulpes*) (Ohbayashi, 1996). The fox population dynamics are modeled stochastically in this model: each fox i (i an index to the individual numbers of foxes) is assigned a set (F_i) of behavioral characteristic parameters . The elements of F_i are listed in Table 1. The fox breeding season is generally the last third of March–the first third of April; newborns emerge from their dens after wearing, which has been assessed as about 1 month after birth (Uraguchi and Takahashi, 1998). Newborns are exposed to *E. multilocularis* infection after wearing. The average litter size was estimated at 4.0 in Koshimizu (Abe 1971) and 4.05 in Nemuro (Uraguchi and Takahashi, 1998). With regard to elements $F_{i,\,litter\,size}$, $F_{i,\,sex}$ and $F_{i,\,pregnancy}$ of F_i, we presuppose that the litter size follows a Binomial distribution B(7, 0.57) and that the sex ratio in newborns and percentage of breeding females are 0.5 and 90%, respectively. Uraguchi and Takahashi (1991; 1998) reported that only a small minority of juveniles survive their first year. The ratio of the 1-year-old fox population to the 2-year-old fox population was estimated as 2263 : 350 (Uraguchi and Takahashi, 1998). Survival rates of juveniles and adults have been estimated respectively as 0.33 and 0.82 per year (Ishikawa *et al.*, 2003). With regard to an element $F_{i,\,alive}$ (alive or dead), we assume that the daily number of deaths among the fox population follows a Poisson distribution, the parameter of which is applied to an expectation of daily deaths in the population of juveniles or adults followed

5

by each survival rate. Uraguchi and Takahashi (1998) reported that the density/km^2 of the fox population was estimated to be 0.7-1.0 before the breeding season at Nemuro in 1986-96. We adopted about 180 as a population size before the breeding season, which corresponds to 0.9/km^2 in the Koshimizu area (200 km^2).

On the other hand, the gray-sided vole population is modeled deterministically. The gray-sided vole breeds in three seasons of the year (all seasons except winter); the survival rate depends on the season and age (Ota, 1984; Yoccoz *et al.*, 1998). Full details of the dynamics of the vole population were given in Ishikawa *et al.* (2003). The density/km^2 of the vole population has annual and seasonal fluctuations. The census data (The Forest Agency of the Japanese Government) showed 0.30×10^3 in spring (before the breeding season) and 2.84×10^3 in autumn in Nemuro (1984-1993). We adopt about 60,000 as a population size before the breeding season for the situation in the Koshimizu area (200 km^2), which corresponds to 0.3×10^3/km^2.

2-2. Transmission of *E. multilocularis* from foxes to voles and growth of protoscoleces in voles

Voles become infected with *E. multilocularis* when they orally ingest free-living parasite eggs that have been discharged by the definitive hosts. Although no information is available about how often voles ingest eggs under natural conditions, we simply assume that the probability of egg-ingestion by a vole depends on the density of free-living *E. multilocularis* eggs linearly at the simulated time. Therefore, we introduced a transmission factor (*g*) from free-living *E. multilocularis* eggs (the density per km^2) in the environment to voles. Due to a lack of direct survey data, we chose a value of *g* on the basis of the prevalence data for the population of foxes in the next section.

Protoscoleces are found in hydatid cysts of *E. multilocularis* that develop from an ingested egg in the vole about 40 days after infection. A fox can be infected with *E. multilocularis* if a fox

6

preys on a vole that harbors multilocular *Echinococcus* with infectious protoscoleces. Therefore, the number of protoscoleces in voles plays a fundamental role in the transmission of *E. multilocularis*. Yagi and Itoh (1998) reported that experimental infections in 11 voles by ingestion of 200 *E. multilocularis* eggs showed that one necropsied vole started to produce protoscoleces 44 days after infection and another vole had 3,700,000 protoscoleces 142 days after infection. They concluded that the transition of reproduction of protoscoleces traced an S-curve. We assumed that the number of protoscoleces in voles increases exponentially after the latent period, that the rate of increase declines because of environmental factors, and that the number of protoscoleces is eventually saturated. In the model, we approximate the growth of protoscoleces in voles ($P(t_1)$, protoscoleces-day model; t_1, day after a latent period) as a logistic curve.

$$\frac{dP}{dt_1} = r(1 - \frac{P(t_1)}{P_{max}}))P(t_1)$$

Therein, P_{max} and r show the maximum number of protoscoleces (3,700,000) (Yagi and Itoh, 1998) and the growth rate (0.3), respectively, but these figures do not have any reliable grounding in experimental infection.

The vole population is divided into three epidemiological classes: negative, infected but not yet infectious, and infectious holding protoscoleces in hydatid cysts (Fig. 1).

2-3. Transmission of *E. multilocularis* from voles to foxes and growth of worms in foxes

Foxes feed on various foods, but they prefer voles (Yoneda, 1981). The number of voles ingested daily by a fox depends on the vole population density and snowfall (Abe, 1975; Yoneda, 1981), which was formulated as a food habit function by Ishikawa *et al.* (2003). For each fox, the daily number of ingested voles (n) is determined by the Poisson distribution with the estimation obtained from the food habit function at the simulated time. A fox becomes infected if it preys on an infected vole holding protoscoleces. The probability that the fox becomes infected depends on

7

the prevalence of infection among the vole population. More precisely, we assumed that the probability of the number of infected voles ingested by a fox per day follows a Binominal distribution function $B(n, p_{vole})$. Therein, n and p_{vole} stand for the number of voles ingested by a fox per day and the infection prevalence rate among the vole population at the simulated time, respectively. Consequently, the probability that a fox will become infected per day is given as the sum of the probability density function $f(k)$ of $B(n, p_{vole})$ over $k=1,\ldots,n$.

The worm burden in the infected foxes depends on the number of protoscoleces in a predated vole. According to our stochastic system, when a fox ingests an infected vole, we determine the age of the vole (t_{age}) based on the age distribution of voles, which yields the maximum days after infection, and thereafter the precise day after infection (t_1) for the vole within t_{age} uniform-randomly, that indicates the number of worms in the fox ($F_{i,worm}$, an element of F_i). A remarkable difference is apparent in the worm burden between juvenile foxes and adult foxes (from five to ten times on average) from dissection data (Hofer *et al*., 2000). Furthermore, an antibody reaction against *E. multilocularis* in foxes has been confirmed (Kato *et al*., 2005a; 2005b), although it remains unknown whether such a reaction would affect the worm burden in foxes. From the results of these reports, we inferred that the immunity of *E. multilocularis* in foxes is strengthened in proportion to $I^{F_{i,\mathrm{inf}}}$, where we set the constant I as 0.84 and $F_{i,inf}$, as an element of F_i, the number of infection experiences. We further assumed that the worm burden ($F_{i,worm}$) is given as the following formula.

$$F_{i,worm} = P(t_1) \times I^{F_{i,\mathrm{inf}}}$$

Therein, $P(t_1)$ represents the protoscoleces-day model explained in §2-2.

The fox population is divided into three epidemiological classes: negative, infected but not excreting eggs, infected and excreting eggs (Fig. 1).

2-4. Time-course of egg excretion from infected foxes and egg longevity

In the fox, *E. multilocularis* reaches maturity and lay eggs, beginning about 1 month after infection, for about 2-3 months. Thereafter, a fox shifts from the infected stage to the negative stage. The experimental infection for four foxes (Yagi and Itoh., 1998) where about 150,000 protoscoleces were ingested into each fox orally showed that foxes began to excrete eggs 29-32 days after infection, and that the discharge of eggs per day (EPI) reached a first peak (170,000EPI) and a second peak (45,000 EPI) at 35 and 42 days after infection, respectively, and that the total amount of 5 day-EPIs centered around the days of the first and second peaks were estimated to be 545,000 and 125,000, respectively. Another experimental infection (Kapel *et al.*, 2006) showed that about 84% of 2000-protoscoleces ingested into a fox orally established as worms in the intestine of the fox and that 27 eggs were discharged per worm (95-CI 17–44). Furthermore, Kapel *et al.* (2006) approximately formulated daily fecal egg output for the day after the latent period (t_2) as $M(t_2)$, which is adopted in our model.

$$M(t_2) = \frac{F_{i,worm} \, a \exp(a[b - t_2])}{\{1 + \exp(a[b - t_2])\}^2}$$

Therein, *a* and *b* respectively show a curvature parameter and the day when the excretion of eggs reaches a peak. We set 30 days for the latent period, $a=0.423$ which fulfils the ratio (0.23) between the total amount of 5 day-EPIs centered around the days of the first and second peaks, and $b=5$ (days after the latent period) (Yagi and Itoh., 1998).

The total amount of daily fecal egg output is the sum of daily egg output by each infected fox at the simulated time.

The longevity of *E. multilocularis* eggs is influenced by environmental factors, particularly temperature and humidity (Yagi and Itoh, 1998; 1999). The experimental formula was given as $d = \exp[-0.135(T - 43.49)]$ for the duration (*d* days) of the infection ability of eggs at temperature (T^oC) from experimental infection in mice (Yagi and Itoh, 1998). We assume that the

longevity of eggs (*d*) cannot exceed 100 days due to the experimental report that the infectivity was maintained only for 125 days after excretion (Yagi and Itoh, 1998), as the low temperature in winter will surpass the limit of the formula (Ishikawa *et al*. 2003).

We assume that the number of eggs in the environment that have infectious ability would reduce at the rate of d^{-1} per day where *d* is calculated by the above formula for the mean temperature ($T^o C$) for day at the simulated time.

3. Results

3-1. Stochastic simulations

The stochastic model of *E. multilocularis* transmission was programmed using Fortran90 to work on any computer using the Microsoft Windows ^{TM} platform. With regard to the method of simulation, the time-step of the stochastic process was adopted as a day, and the results in this section were obtained through 1000-iteration simulations, which were carried out after a burn in period of 8 years.

We show the variations in the host populations for two years in Fig. 2 based on the seasonal population models of foxes and voles (§2.1); therein, the area for the simulation was fixed as 200 km^2 for both Koshimizu and Sapporo, and a vole population size before the breeding season for Sapporo (urban) was set to be two-thirds as many as that for Koshimizu (rural). The variation in the fox population was shown as the average with the standard deviation and the maximum-minimum ranges that were obtained though 1000-iteration simulations.

3-2. Prevalence of the host population

We carried out simulations of the model for Koshimizu and Sapporo based on meteorological data (Japan Metrological Agency, 2000–2004) incorporating the growth model of protoscoleces

in voles and the immune response assumptions for foxes (§2-2, 3).We adopted a value of the transmission factor (g) from free-living *E. multilocularis* eggs $/km^2$ in the environment to voles as 1.2×10^{-9} , so that the average prevalence of 1000-iteration simulations showed 24.93±12.15% for the entire fox population in Koshimizu situations (Table 2) (24.6% the average prevalence observed in the non-baited area at Koshimizu for during April, 1998-May, 1999 (Tsukada *et al*. 2003)). Figure 3 (a-c, f-h) and Table 2 show the variations of the average prevalence of *E. multilocularis* with the standard deviation and the maximum-minimum ranges in the entire population and adult population of foxes and the population of voles for Koshimizu and Sapporo. The age category of the fox population is transferred from juveniles to adults on 1st March, causing a discontinuity in the graph of the prevalence in the adult fox population at this time (Fig. 3 (b, g)). Figure 3 (d,i) and (e,j) shows the variations of the average numbers (day) of infected foxes excreting eggs (all ages) and infected voles holding protoscoleces in hydatid cysts, respectively, with the standard deviation and the maximum-minimum ranges for Koshimizu and Sapporo situations.

3-3. Intensity (worm burden) of infected foxes

We examined the distribution of worm burdens during a day in winter derived from the simulation of our model. Figure 4 shows the distribution of worm burdens in 66 infected foxes with *E. multilocularis* and its range was 18–61141 in 21 juveniles and 1–22490 in 45 adults (Table 3). Because we can see that the logarithmically transformed worm burdens almost form a line (Fig. 4), the worm burden would disperse exponentially. Of all foxes, 10 (15%) had more than 10,000 worms; they accounted for 83% of the total worm biomass. On the other hand, 46 foxes (70%) had fewer than 1000 worms, with only 2% of the total worm biomass (Table 4).

3-34. Bait Distribution

We planned a simulation for control measures of the distribution of baits containing Praziquantel in Koshimizu based on a bait uptake rate that was reported by Tsukada *et al.* (2002). The baits had been distributed monthly for a year from the beginning of May, and all baits were set for each fox family on the first day and new baits were added until the fourth day if the original baits had disappeared (Tsukada *et al.*, 2002). The average prevalence among the fox population for 1000-iteration simulations and the observation with 95-CI in Koshimizu are shown in Fig. 5. Next, we compared an effect of three-times-monthly bait distribution per year for 4 years for Koshimizu situation between the two distribution seasons, early summer (May-July) and winter (January-March) on the reduction in the prevalence of *E. multilocularis* after a monthly bait distribution program had been carried out for a year (from May to May of next year). The comparison of the transitions in the prevalence in the entire fox population was shown in Fig. 6, and the comparison of the average prevalence and the number of infected hosts on 1000-iteration simulations were tabulated in Table 6.

4. Discussion

In this article, we constructed a stochastic model of *E. multilocularis* that managed the foxes individually with respect to the population dynamics and the infection process, so that a simulation of the model assessed the range of the prevalence of *E. multilocularis*.

Seasonal forcing was realized in the model through utilization of population dynamics models of the principal definitive and intermediate hosts, foxes and voles, which were assessed by Ishikawa *et al.* (2003), and the food habit function and attention to the longevity of *E. multilocularis* eggs, which reflect climate conditions (temperature, snowfall). Results of the simulation showed that the prevalence of *E. multilocularis* fluctuated seasonally. Fig. 3 (a, f)

12

shows a sharp decline in the prevalence in the entire fox population in April due to an emergence of infants (Fig. 2 (a)); whereas, Fig. 3 (b, g) shows a slight decline in the prevalence in the adult fox population in April. Afterwards, a rapid increase in the prevalence in the entire fox population in May and June (Fig. 3 (a, f)) was caused by a sudden burst of infections in juveniles, because the prevalence in the adult fox population only showed a mild increase at this time (Fig. 3 (b, g)). A continuous increase in the prevalence in the vole population from November to May (Fig. 3 (c, h)) mainly resulted from the reduction in the vole population (Fig. 2 (b)), because the number of infected voles changed little during this period (Fig. 3 (e,j)).

The transmission process from voles to foxes, which is governed by predation, is controlled by the food habit function depending on the vole density and the snowfall in the model, which is adequate to describe the above transmission process (Ishikawa *et al*., 2003). A mystery remains regarding the immune response to *E. multilocularis* in foxes, but results of some studies indicate that foxes might have immunity (Kato *et al*., 2005a; 2005b). The result of dissections of infected foxes showed that juveniles had significantly more worms than adults, which implied the presence of immunity. In the model, an infection experience, which can be inferred to strengthen the immunity to *E. multilocularis* in a fox, is obtained by ingestion of an infected vole without reference to how many protoscoleces are present. Further studies of experimental infection are necessary to elucidate the immune response mechanism against *E. multilocularis* in foxes, but we assumed simply that the immunity would be strengthened in proportion to the infection experiences. Moreover, there are many unknowns regarding multiple infections. The simulation results in Ishikawa *et al.* (2003) reported that the average yearly prevalence in a fox population with multiple infections would be about 1.4-1.8-fold higher than those without multiple infections, but we did not allow for multiple infections of foxes in this study to avoid further complication of the model structure. The worm burden in the infected foxes obtained from the simulation was

distributed exponentially (Fig. 4); the geometric mean of average worm burdens in juveniles were about five times those of adults (Table 3). The range of worm burdens in juvenile and adult foxes agreed with the dissection survey described in Hofer *et al*. (2000); whereas, the geometric mean of worm burdens in the simulation were twice those described in the dissection survey (Table 3). The stochastic system of foxes preying on voles, taking into consideration the age structure of the vole population and the growth of protoscoleces in a vole, generated a reasonable distribution of the worm burden.

Although little is known about the increase of protoscoleces in infected voles due to lack of experimental data, we assumed that the time-course of increasing protoscoleces is applicable to a logistic curve (protoscoleces-day model), which satisfies the result of the experimental infection (Yagi and Itoh, 1998).

For a comparative study of the prevalence of *E. multilocularis* between urban and rural areas, we selected two regions: Sapporo (urban) and Koshimizu (rural). We used the meteorological conditions (temperature and snowfall) that would affect the food habit function and parasite egg longevity, and different conditions of vole density between the regions. Averages of the prevalence in the entire population and the adult population of foxes from the 1000-iteration simulations showed a difference of about 10% between the two areas, which agreed with the actual difference in the average prevalence during 1995–2000 (Department of Health and Welfare, Hokkaido Government, in the Annual Report of the Council for Alveolar Echinococcus in Hokkaido, 2002–2003), although the model did not consider that foxes in urban areas have other available food sources aside from voles compared to foxes in rural areas, or that the home ranges of urban foxes are smaller than those of rural foxes (Contesse *et al*., 2004).

We performed a follow-up study on the transition of prevalence in Koshimizu, where Praziquantel baits had been distributed monthly from May 1998 to May 1999 (Tsukada *et al*., 2002). In each

month from August to May, the range of 1.96 standard deviation around the average prevalence of *E. multilocularis* infection in the entire fox population obtained by 1000-iteration simulation overlapped with the 95% confidence interval of the average obtained by a field survey in Koshimizu (Fig. 5). But it did not match the result of a field survey performed during the first three months (May–July). The observed prevalence had risen during May-July in spite of the distribution of Praziquantel baits; the reason why the prevalence rose was unclear. It is difficult for the simulation of a transmission model to follow the observation of the field survey during the above period. In this model, the prevalence obtained from the model diminished one month after beginning the distribution of baits in May, but it took 3 months until the prevalence decreased in the actual field trial (Tsukada *et al*., 2002).

We compared the effectiveness of three-times-monthly Praziquantel bait distribution per year for 4 years between the two distribution seasons, early summer (May-July) and winter (January-March) on the reduction of prevalence of *E. multilocularis*. The average number of infected foxes (day) and also the average prevalence in the fox population were estimated at 22.2 or 37.7 and 11.04% or 15.48% , respectively, for the bait distribution programs in early summer or winter (Table 5); therefore, the bait distribution program in early summer had a stronger effect on reducing the prevalence of *E. multilocularis* in foxes than that in winter. In winter, the bait smell diffusion is limited by low temperatures and recognition of bait is hindered by snowfall, so foxes are likely to pass by the bait without ingesting it (Tsukada *et al*., 2002). On the other hand, many juveniles born in April become infected with *E. multilocularis* within one month and the density of infection reaches a peak in June (Fig.3 (c); Ishikawa *et al*., 2003); in such a case, the distribution of Praziquantel bait has a good effect on suppression of *E. multilocularis*. Therefore, it is necessary to make a careful choice of season when planning the distribution of bait.

This *E. multilocularis* transmission model, which has been evolved realistically in

consideration of protoscoleces growth in voles and the immune response based on infection experiences, can be helpful for designing control strategies.

References

Abe H (1971) Studies on the ecology of a red fox in the eastern part of Hokkaido (in Japanese). Department of Health and Welfare, Hokkaido Government, Sapporo

Abe H (1975) Winter food of red fox, *Vulpes vulpes* schrencki Kishida, in Hokkaido, with special reference to vole population. *Appl Entomol Zool* **10**:40–51

Contesse P, Hegglin D, Gloor S, Bontadina F and Deplazes P (2004) The diet of urban foxes (*Vulpes vulpes*) and the availability of anthropogenic food in the city of Zurich, Switzerland. *Mamm Biol* **69**: 81-95

Doi R, Nakao M, Nihei N, Kutsumi H (2000) Epidemiology of alveolar hydatid disease (AHD) and estimation of infected period of AHD in Rebun Island, Hokkaido. *Jpn J Public Health* **47**: 145-152 [in Japanese with English abstract]

Fischer C, Reperant LA, Weber JM, Hegglin D, Deplazes P (2005) *Echinococcus multilocularis* infections of rural, residential and urban foxes (*Vulpes vulpes*) in the canton of Geneva, Switzerland. *Parasite* **12**:339-346.

Hansen F, Tackmann K, Jeltsch F, Wissel C, Thulke HH (2003) Controlling *Echinococcus multilocularis* ecological implications of field trials. *Prev Vet Med* **60**:91–105

Hansen F, Jeltsch F, Tackmann K, Staubach C, Thulk HH (2004) Processes leading to a spatial aggregation of *Echinococcus multilocularis* in its natural intermediate host *Microtus arvalis. Int J Parasitol* **34:**37–44

Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P (2000) High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. *Parasitology* **120**:135–142

Ishikawa H, Ohga Y, Doi R (2003) A model for transmission of *Echinococcus multilocularis* in Hokkaido, Japan. *Parasitol Res* **91**:444-451

Kapel CM, Torgerson PR, Thompson RC, Deplazes P (2005) Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. *Int J Parasitol* **36**:79-86

Kato N, Nonaka N, Oku Y, Kamiya M (2005a) Modified cellular immune responses in dogs infected with *Echinococcus multilocularis. Parasitol Res* **95**:339-45

Kato N, Nonaka N, Oku Y, Kamiya M (2005b) Immune responses to oral infection with *Echinococcus multilocularis* protoscoleces in gerbils: modified lymphocyte responses due to the parasite antigen. *Parasitol Res* **96:**12-17.

Ohbayashi M (1996) Host of *Echinococcus multilocularis* in Hokkaido. In: Uchino J, Sato N (eds) Alveolar echinococcosis: strategy for eradication of alveolar echinococcosis of the liver. Fujishoin, Sapporo, pp 59–64

Ota K (1984) Study on wild murid rodents in Hokkaido (in Japanese). Hokkaido Univ Press, Sapporo.

Roberts MG, Aubert MFA (1995) A model for the control of *Echinococcus multilocularis* in France. *Vet Parasitol* **56**:67-74

Takahashi K, Uraguchi K, Romig T, Hatakeyama H, Tamura M (2002) Preliminary report on *Echinococcus multilocularis* control by fox baiting with Praziquantel. *Rep Hokkaido Inst Public Health* **52:**61-63

Takahashi K, Uraguchi K, Kudo S (2005) The epidemiological status of *Echinococcus multilocularis* in animals in Hokkaido, Japan. *Mammal Study* **30**(Suppl.), S101-S105

Takumi K and Van Der Giessen J (2005) Transmission dynamics of *Echinococcus multilocularis*; its reproduction number, persistence in an area of low rodent prevalence, and effectiveness of control. *Parasitology*. **131**:133-140

Tsukada H, Hamazaki K, Ganzorig S, Iwaki T, Konno K, Lagapa JT, Matsuo K, Ono A, Shimizu M, Sakai H, Morishima Y, Nonaka N, Oku Y, Kamiya M (2002) Potential remedy against *Echinococcus multilocularis* in wild red foxes using baits with anthelmintic distributed around fox breeding dens in Hokkaido, Japan. *Parasitology*. **125**:119-129

Uraguchi K, Takahashi K (1991) Wildlife conservation. In: Maruyama N, Bobek B, Ono Y, Regelin W, Bartos L, Ratcliffe PR (eds). Proceedings of the International Symposium on Wildlife Conservation in Tsukuba and Yokohama, Japan 1990, p 228

Uraguchi K, Takahashi K (1998) Ecology of the red fox in Hokkaido. In: Tsuduki T, Yano S, Furuya K, Takahashi K, Kawase S, Yagi K, Sudo S (eds) Alveolar echinococcus in Hokkaido (in Japanese). Hokkaido Inst Public Health, Sapporo, pp 39-48

Yagi K, Itoh T (1998) Biological characteristics of *Echinococcus multilocularis* clarified in experimental infections (in Japanese). In: Tsuduki T, Yano S, Furuya K, Takahashi K, Kawase S, Yagi K, Sudo S (eds) Alveolar echinococcus in Hokkaido (in Japanese). Hokkaido Inst Public Health, Sapporo, pp 51- 63.

Yagi K, Itoh T (1999) Influence of low temperature and desiccation on the infectivity of *Echinococcus multilocularis* eggs (in Japanese). *Rep Hokkaido Inst Public Health* **49**:167–168

Yoccoz NG, Nakata K, Stenseth NC, Saitoh T (1998) The demography of *Clethrionomys rufocanus*: from mathematical and statistical models to further field studies. *Res Popul Ecol* **40**:107-121

Yoneda M (1981) Red fox, *Vulpes vulpes* schrencki Kishida: population and their food habits, 2 (in Japanese). *Hoppou Ringyo Northern Forestry* **33**:285–288

Table 1 The list of behavioral characteristics of foxes in the model.

Identity	Status of infection
alive or dead	number of infection experiences
sex	days after infection
age	number of worms
pregnancy or not (only female)	
litter size (only female)	

Table 2 Comparison of the simulated prevalence and number of infected hosts in rural and urban areas.

	Koshimizu (rural)					Sapporo (urban)				
	Prevalence (%)			Number of infected hosts (day)		Prevalence (%)			Number of infected hosts (day)	
	Fox		Vole	Fox	Vole	Fox		Vole	Fox	Vole
	Entire	Adults		Entire		Entire	Adults		Entire	
Average in 1000 trials	24.93	35.61	1.05	58.8	1444.0	21.77	31.70	0.93	52.2	851.2
Standard deviation	12.15	7.72	0.59	44.3	339.3	10.31	6.69	0.55	39.0	227.0
Maximum of trials	70.94	68.50	4.31	315.0	2720.0	65.19	61.15	4.49	298.0	1856.0
Minimum of trials	1.72	4.76	0.07	2.0	177.6	1.16	2.08	0.07	1.0	54.1
Maximum of average	56.05	50.74	2.75	178.4	1961.6	47.92	42.41	2.44	155.3	1193.8
Minimum of average	9.37	24.87	0.36	34.0	1120.0	10.60	27.23	0.29	29.4	687.3

Table 3 Geometric mean worm burden and range of *E. multilocularis* in foxes from the results of simulations.

	No. of infected foxes	Worm number range	*E. m.* biomass (%)	Mean worm burden
Adult	45	1-22489	27.1	1803
Juvenile	21	17-61141	72.9	10373
Total	66	1-61141	100	4530

Table 4 Worm burdens and biomass of *E. multilocularis* in infected foxes obtained using simulations.

Worm burden	Number of foxes	Adult	Juvenile	Biomass (%)
-10000	10	3	7	83.5
1000-10000	10	7	3	13.9
100-1000	9	5	4	2.3
1-100	37	30	7	0.3
Total	66	45	21	100

Table 5 Comparison of the simulated prevalence and number of infected hosts between three-times-monthly bait distribution programs in early summer and winter seasons.

	Early summer (May-July)					Winter (January-March)				
	Prevalence (%)			Number of infected hosts (day)		Prevalence (%)			Number of infected hosts (day)	
	Fox		Vole	Fox	Vole	Fox		Vole	Fox	Vole
	Entire	Adults		Entire		Entire	Adults		Entire	
Average in 1000 trials	11.04	17.45	0.60	22.2	693.7	15.48	24.20	0.72	37.7	905.1
Standard deviation	8.87	12.67	0.48	17.5	322.9	10.33	12.49	0.46	37.0	350.8
Maximum of trials	51.09	65.44	3.74	106.0	2210.0	66.17	68.90	3.66	294.0	2458.0
Minimum of trials	0.00	0.00	0.00	0.0	0.0	0.00	0.00	0.00	0.0	0.0
Maximum of average	29.63	42.91	1.96	49.9	951.8	47.20	45.24	1.92	148.2	1444.8
Minimum of average	0.11	0.24	0.12	0.3	324.2	0.87	1.30	0.19	3.2	387.0

Legends

Fig. 1 The basic model scheme of transmission dynamics of *E. multilocularis*

Fig. 2 The dynamics of the host populations for two years.

    (a) Fox population. The black line shows the average in 1000 trials. The dark gray and light gray zones show the standard deviation range and the whole range, respectively.

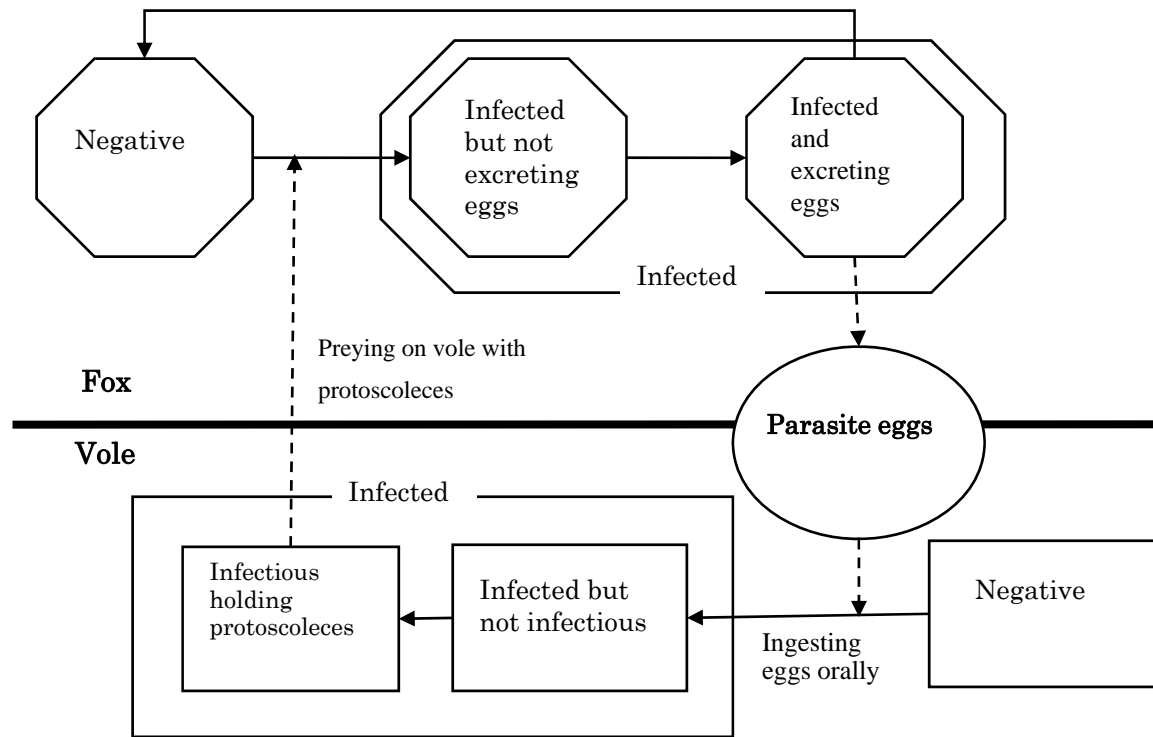   **(b)** Vole population. The black and gray lines show variations for Koshimizu and Sapporo, respectively.

Fig. 3 Variations in the prevalence of *E. multilocularis* and the number of infected foxes for two years obtained through 1000 trial simulations.

  The black line shows the average in 1000 trials. The dark gray and light gray zones show the standard deviation range and the whole range, respectively.

    (a) Prevalence among the entire fox population in Koshimizu.

    (b) Prevalence among the adult fox population in Koshimizu.

    (c) Prevalence among the vole population in Koshimizu.

    (d) Number of infected foxes in Koshimizu.

    (e) Number of infected voles holding protoscoleces in hydatid cysts in Koshimizu.

    (f) Prevalence among the entire fox population in Sapporo.

    (g) Prevalence among the adult fox population in Sapporo.

    (h) Prevalence among the vole population in Sapporo.

    (i) Number of infected foxes in Sapporo.

    (j) Number of infected voles holding protoscoleces in hydatid cysts in Sapporo.

Fig. 4 Distribution of worm burdens in infected foxes obtained by simulation. The number of worms (worm burden) and the log number of the worm burden are shown by dots and circles, respectively.

Fig. 5 Variance of the prevalence of *E. multilocularis* in the fox population when Praziquantel-containing bait was distributed monthly from May to May, derived from 1000 trial simulations and the observations with 95-CI in Koshimizu by Tsukada *et al*., (2002). The dark gray and light gray zones respectively show the range of 1.96 standard derivation around the average and the whole range, respectively. The dashed line and the box with marble pattern show the observed prevalence and 95-CI,

respectively.

Fig. 6 Comparison of the transitions in prevalence of *E. multilocularis* in the fox population between two distribution times, (a) early summer (May-July) and (b) winter (January-March), derived from 1000 trial simulations. The black line shows the average prevalence in 1000 trials. The dark gray and light gray zones show standard deviation range and the whole range, respectively.
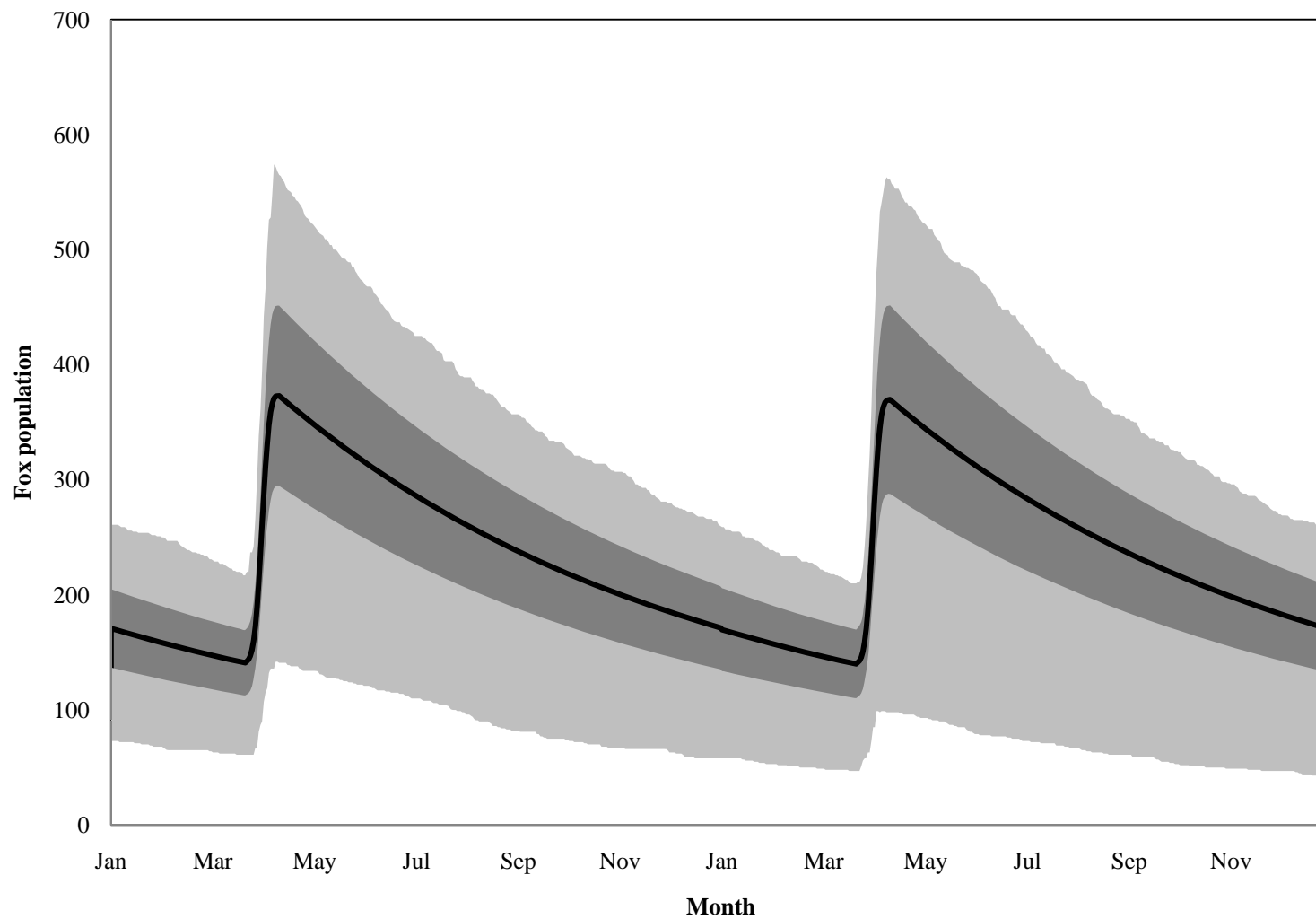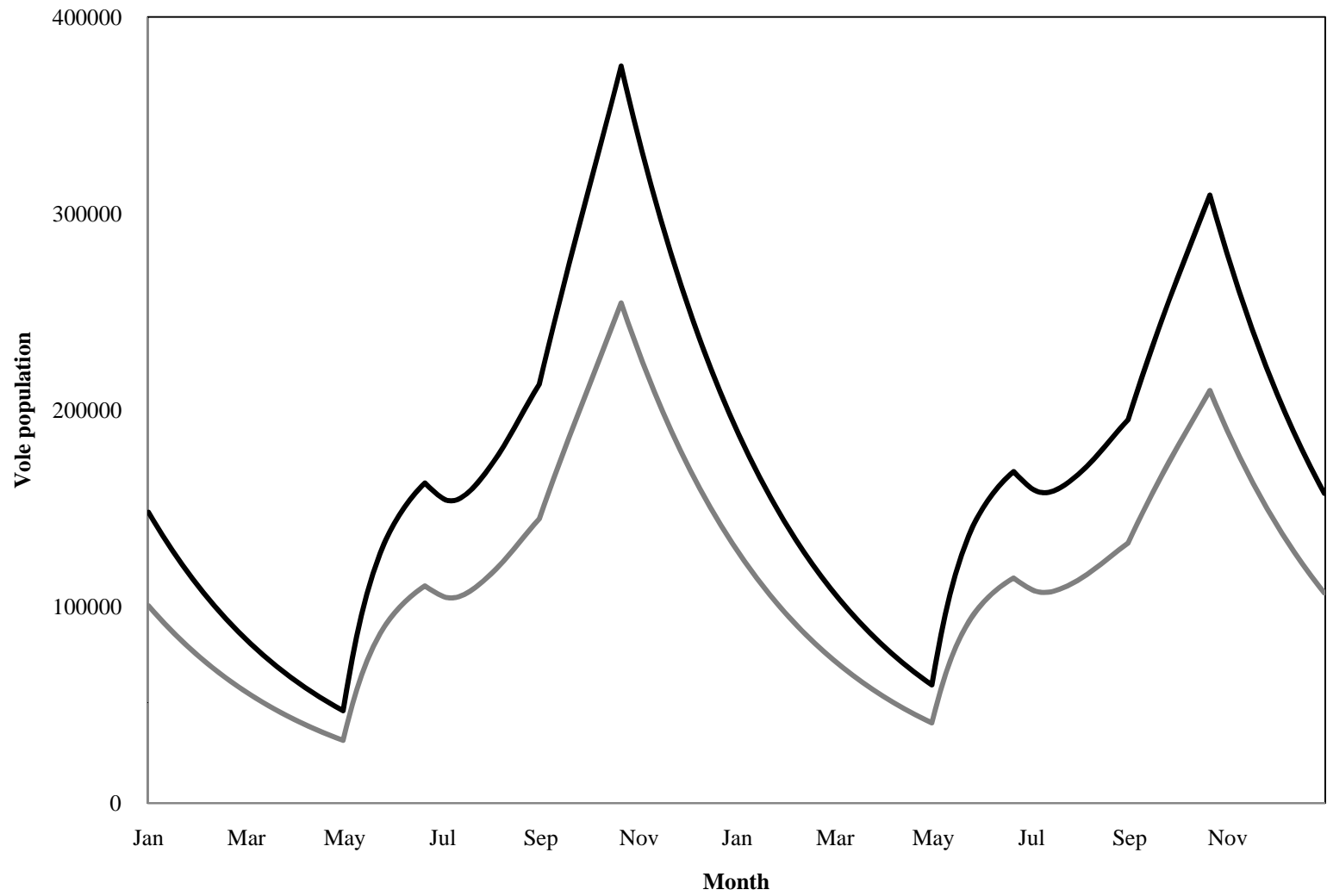
Negative

Infected
but not
excreting
eggs

Infected
and
excreting
eggs

Infected

Preying on vole with
protoscoleces

Fox

Vole

Parasite eggs

Infected

Infectious
holding
protoscoleces

Infected but
not infectious

Ingesting
eggs orally

Negative

Fig. 1

Fig. 2 (a)

Fig. 2 (b)
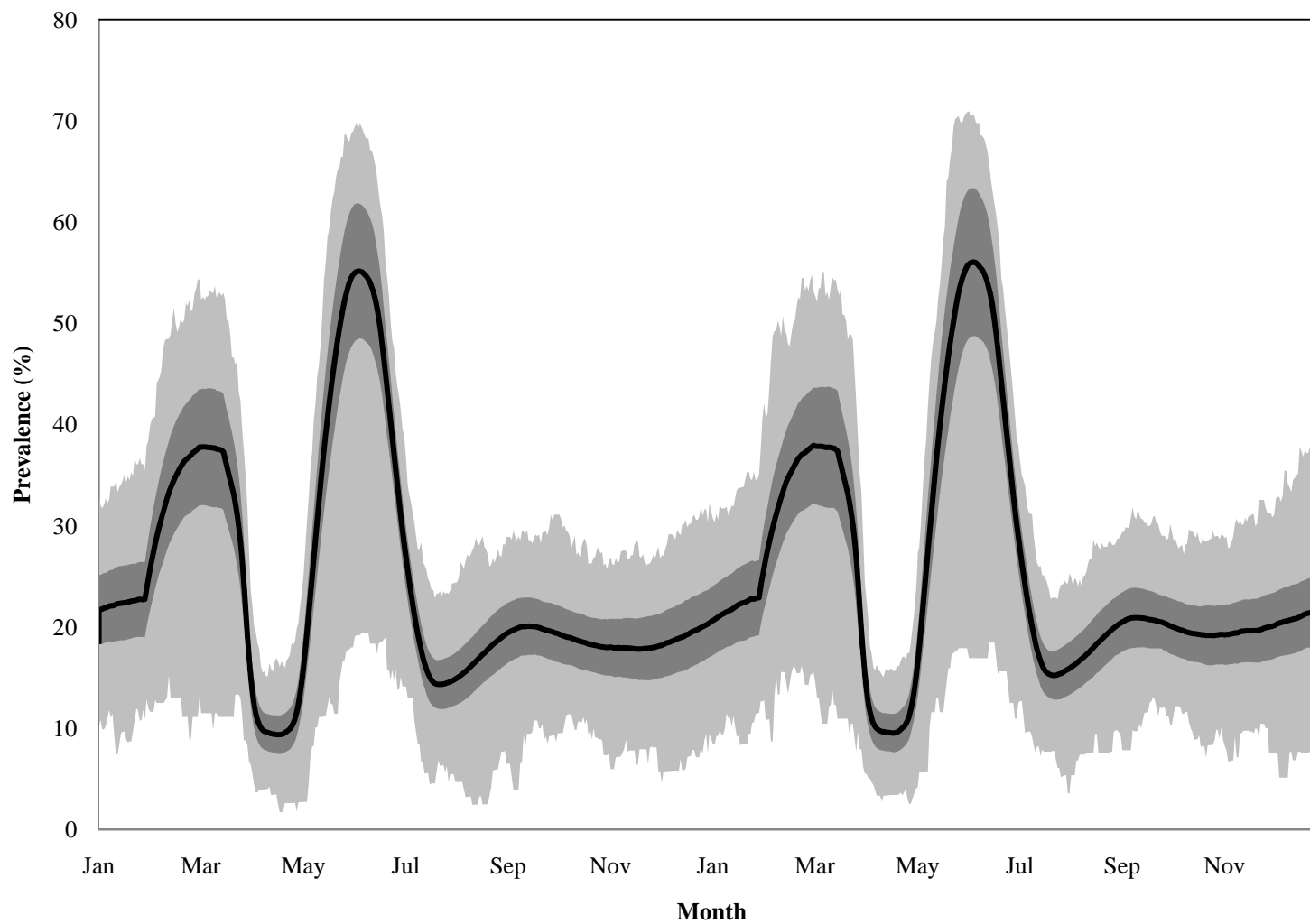
Fig. 3 (a)
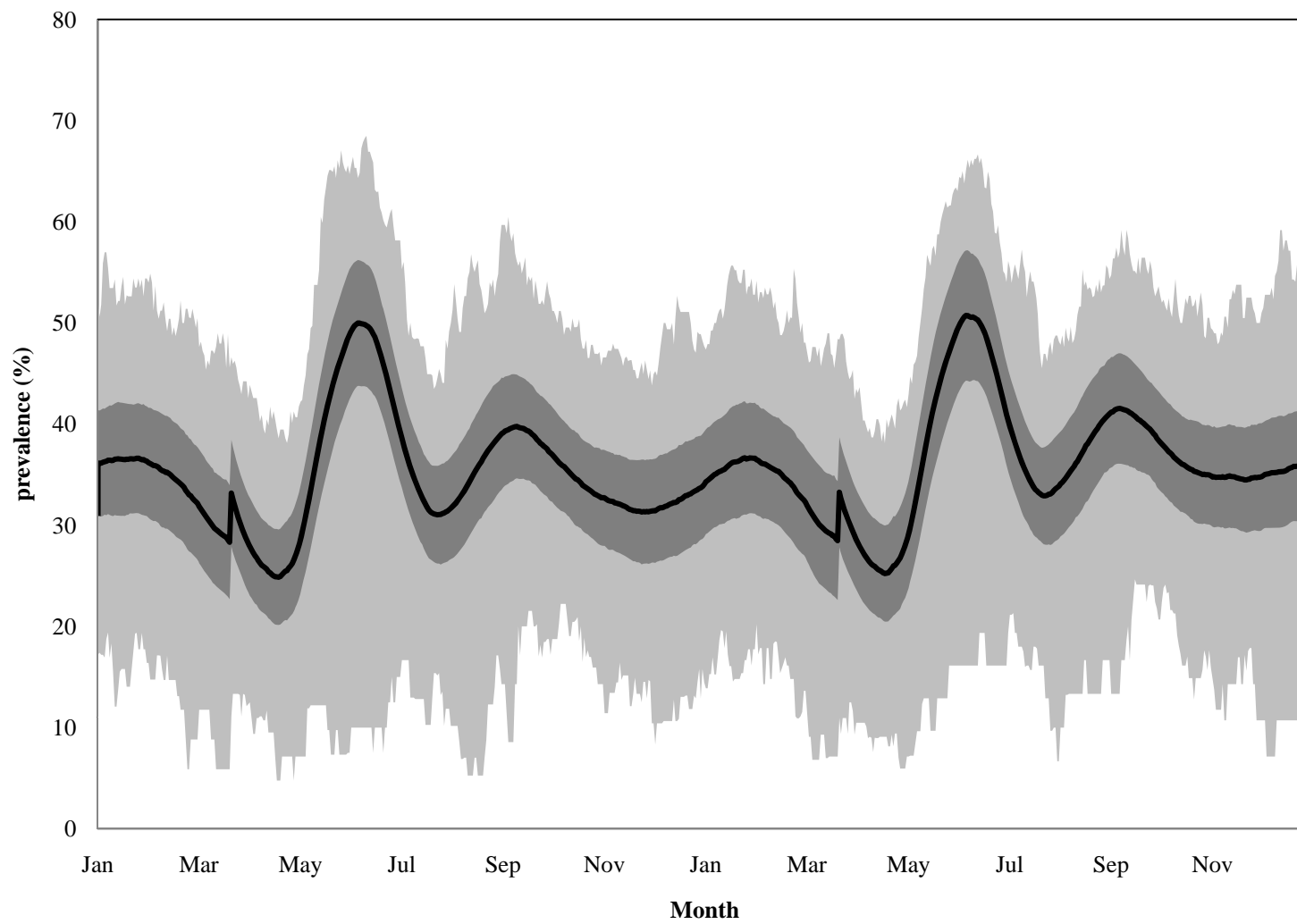
Fig. 3 (b)
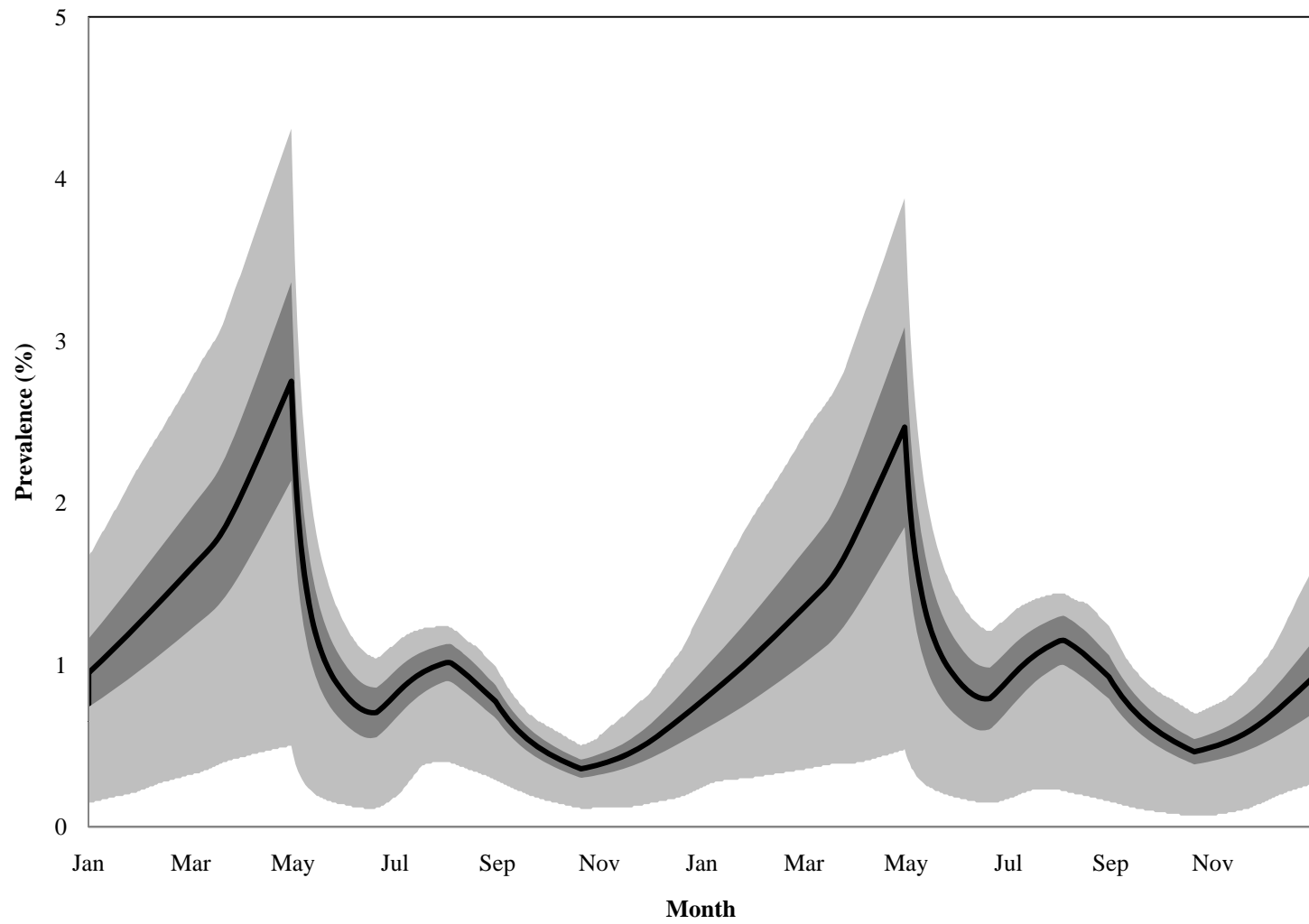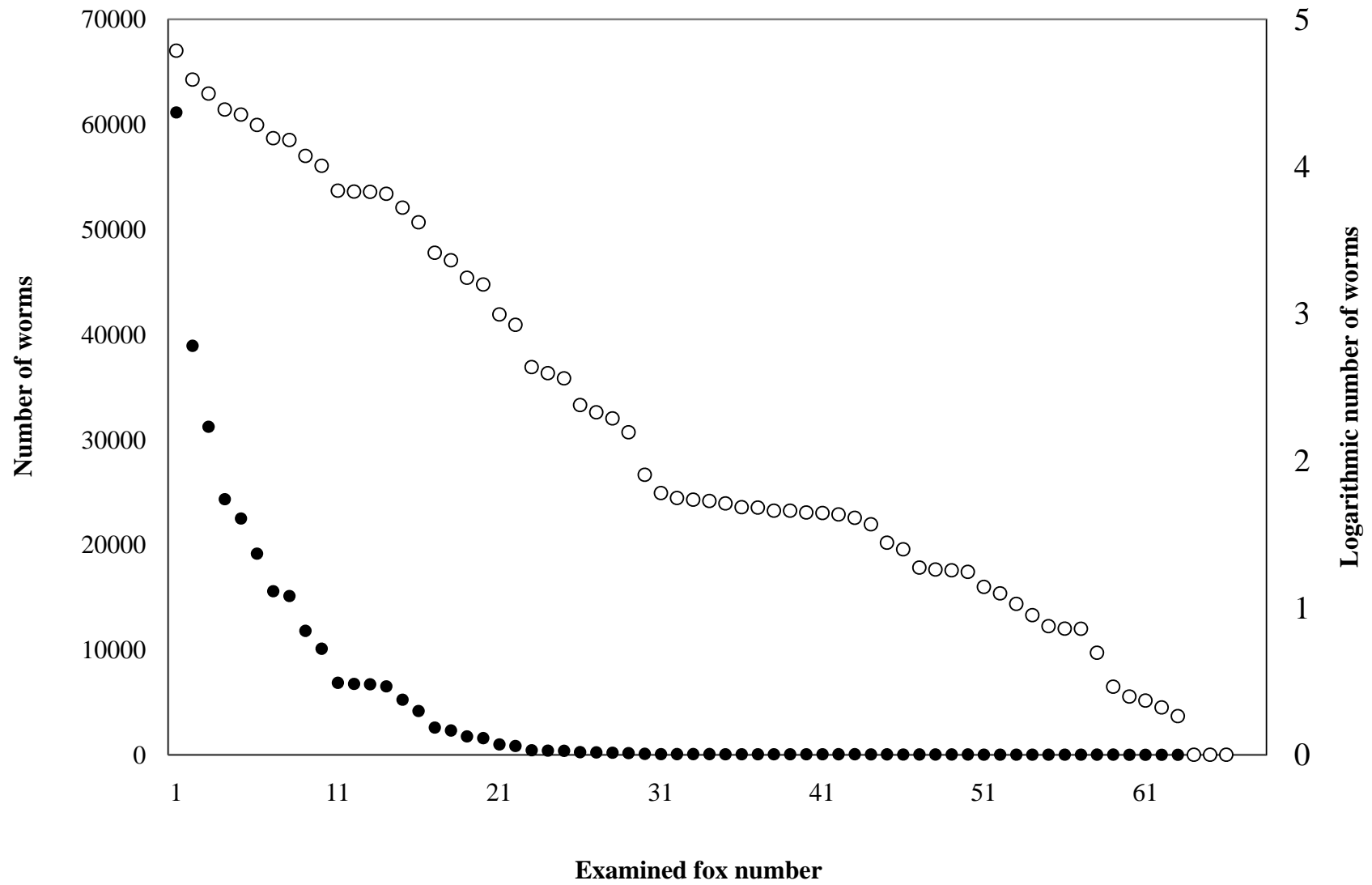
Fig. 3 (c)
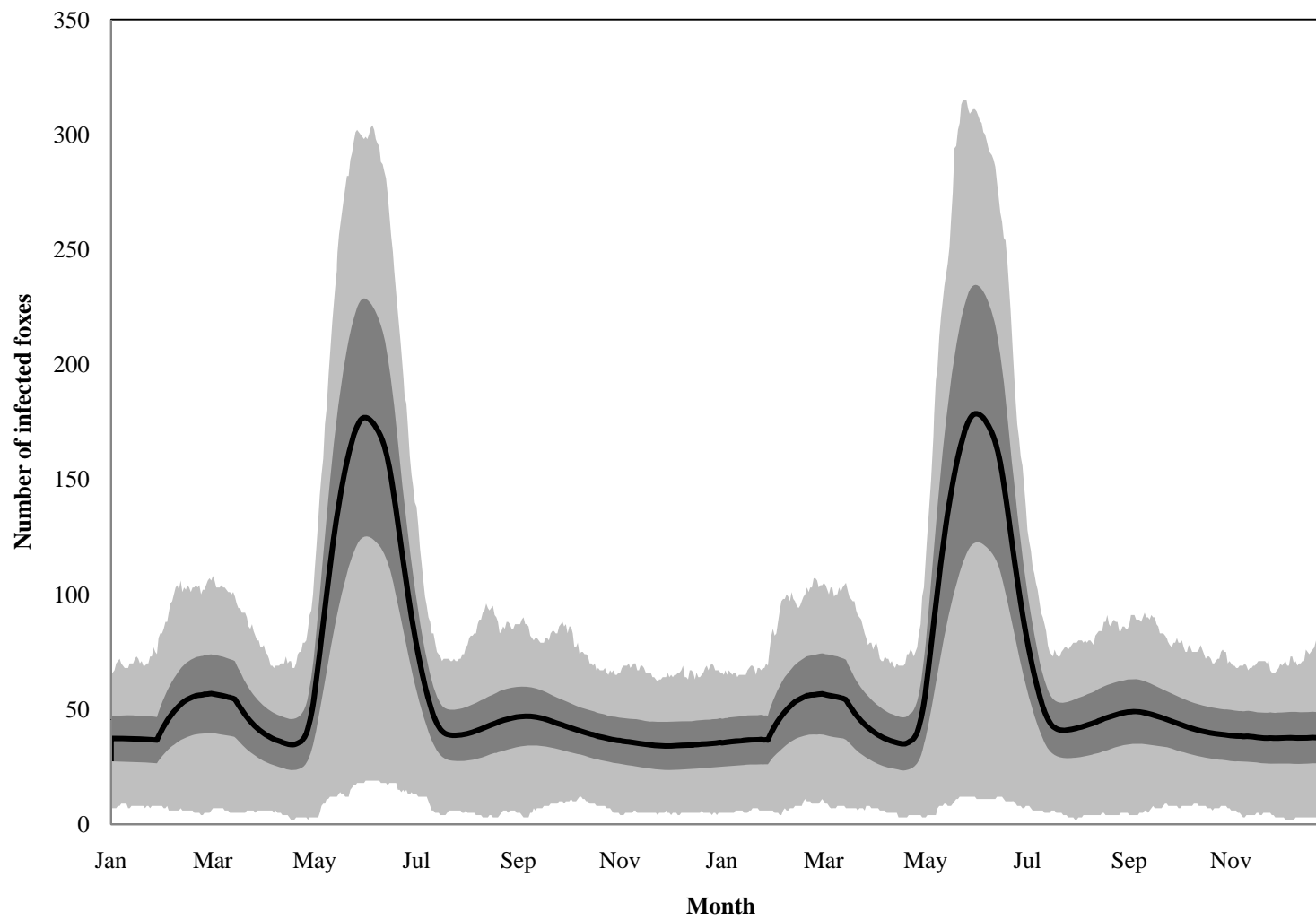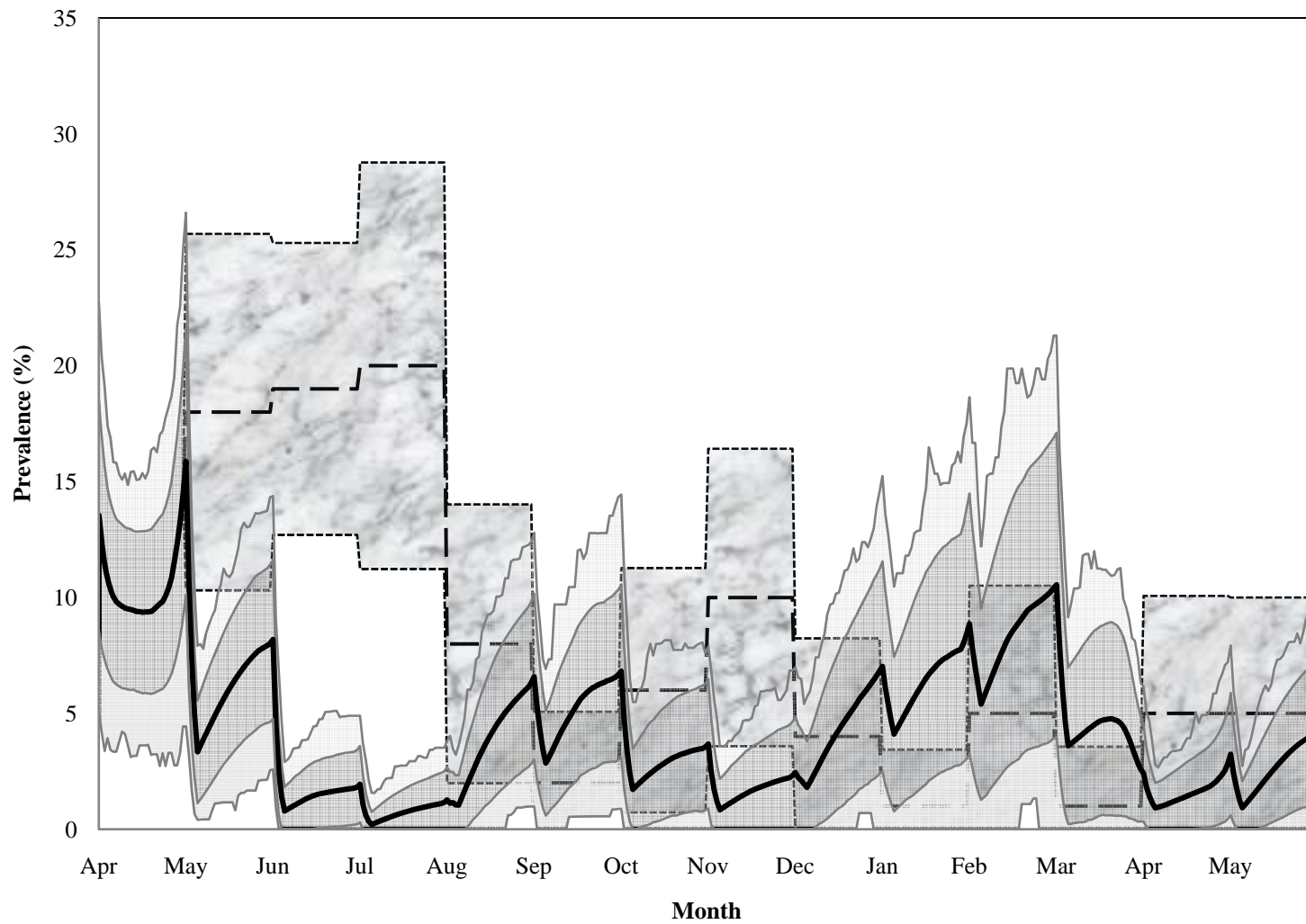
Fig. 3 (d)
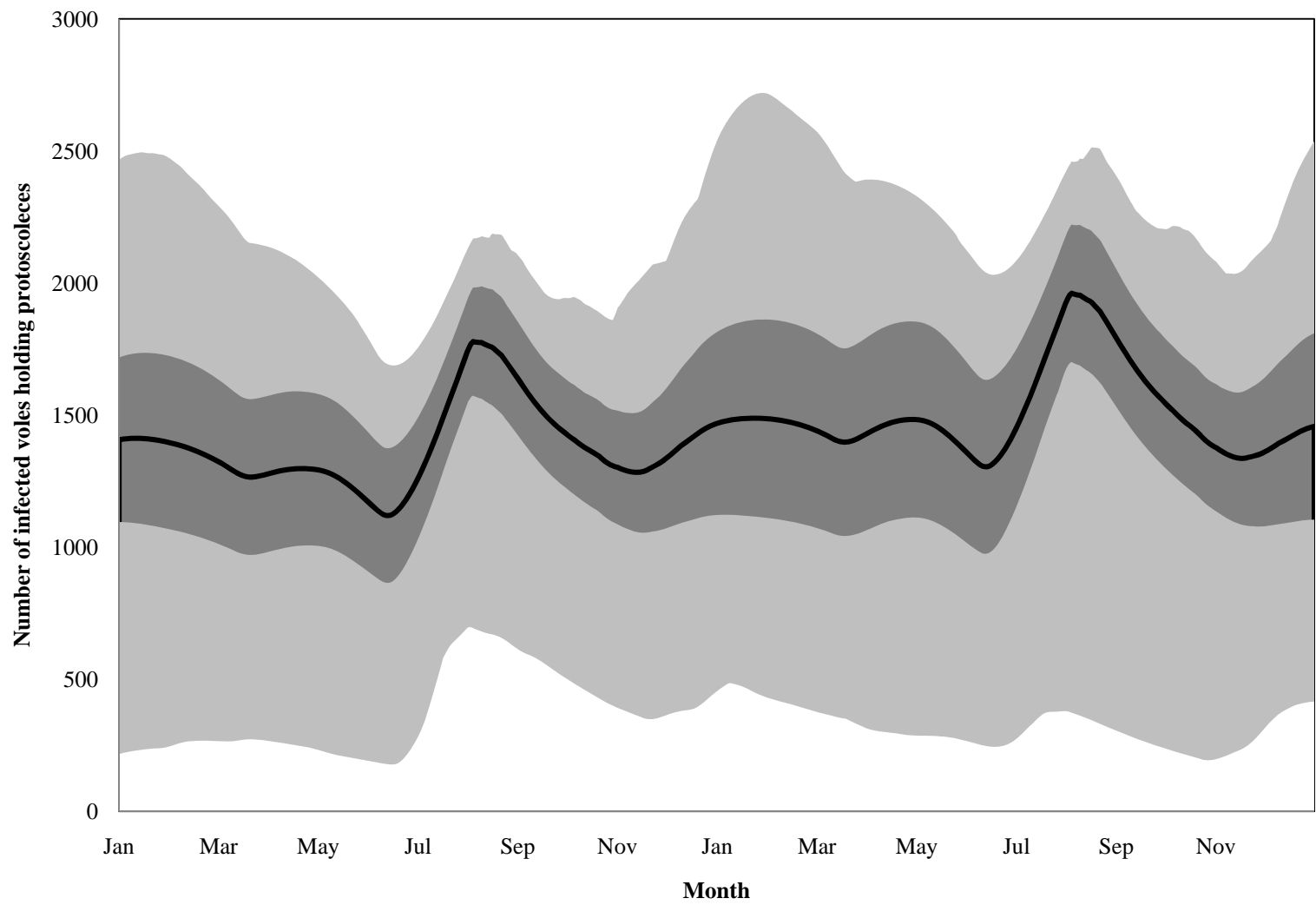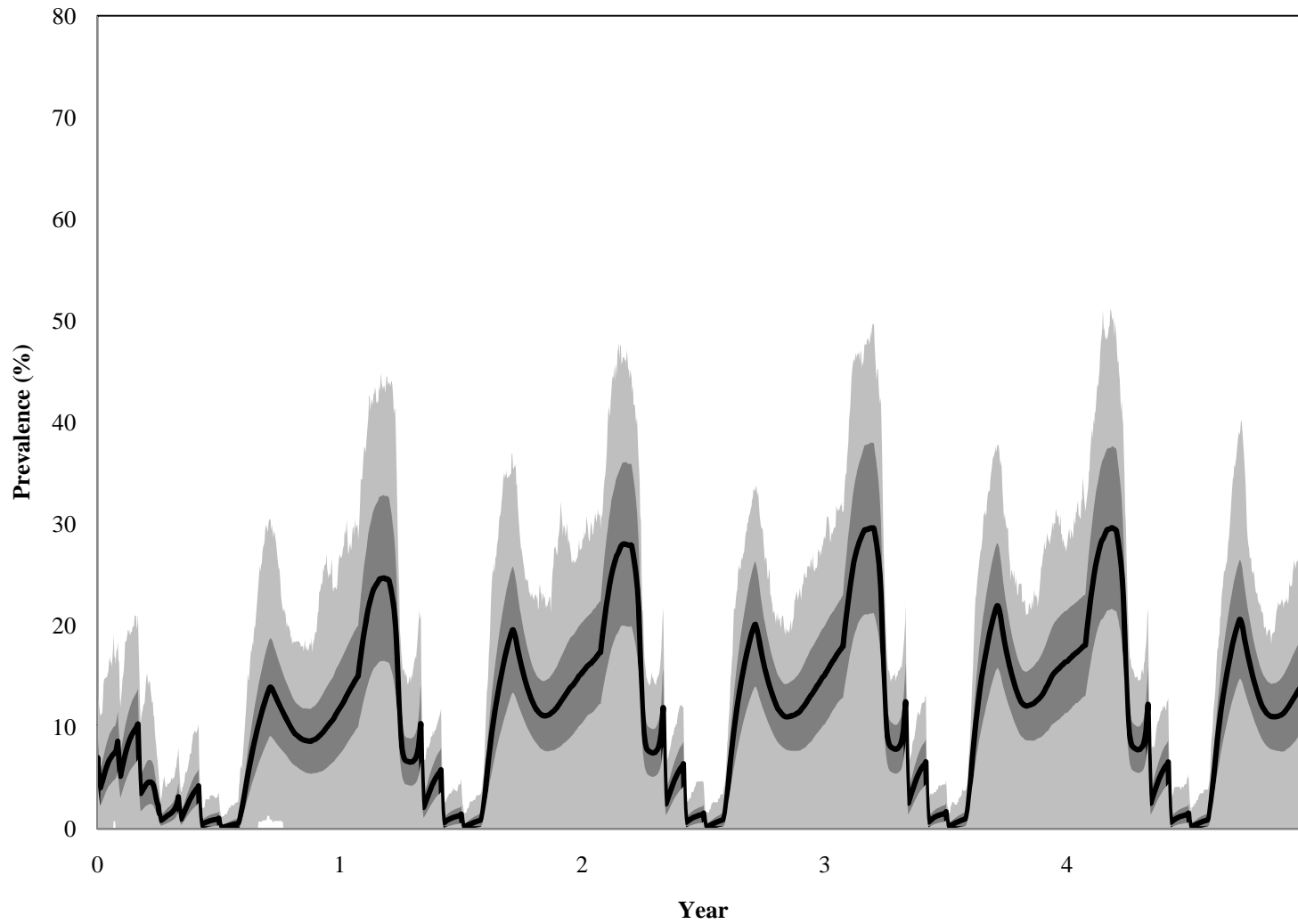
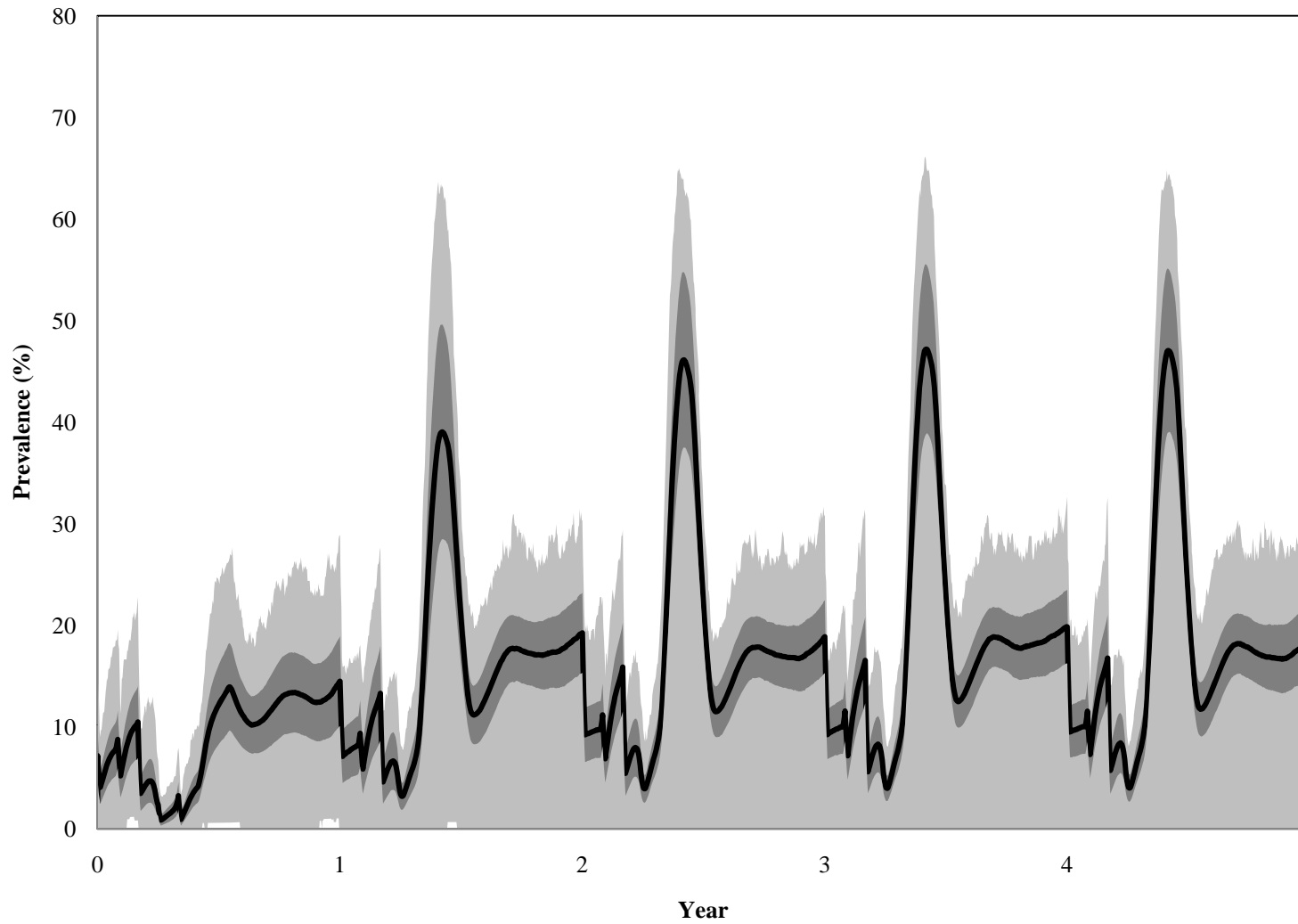Fig. 3 (e)
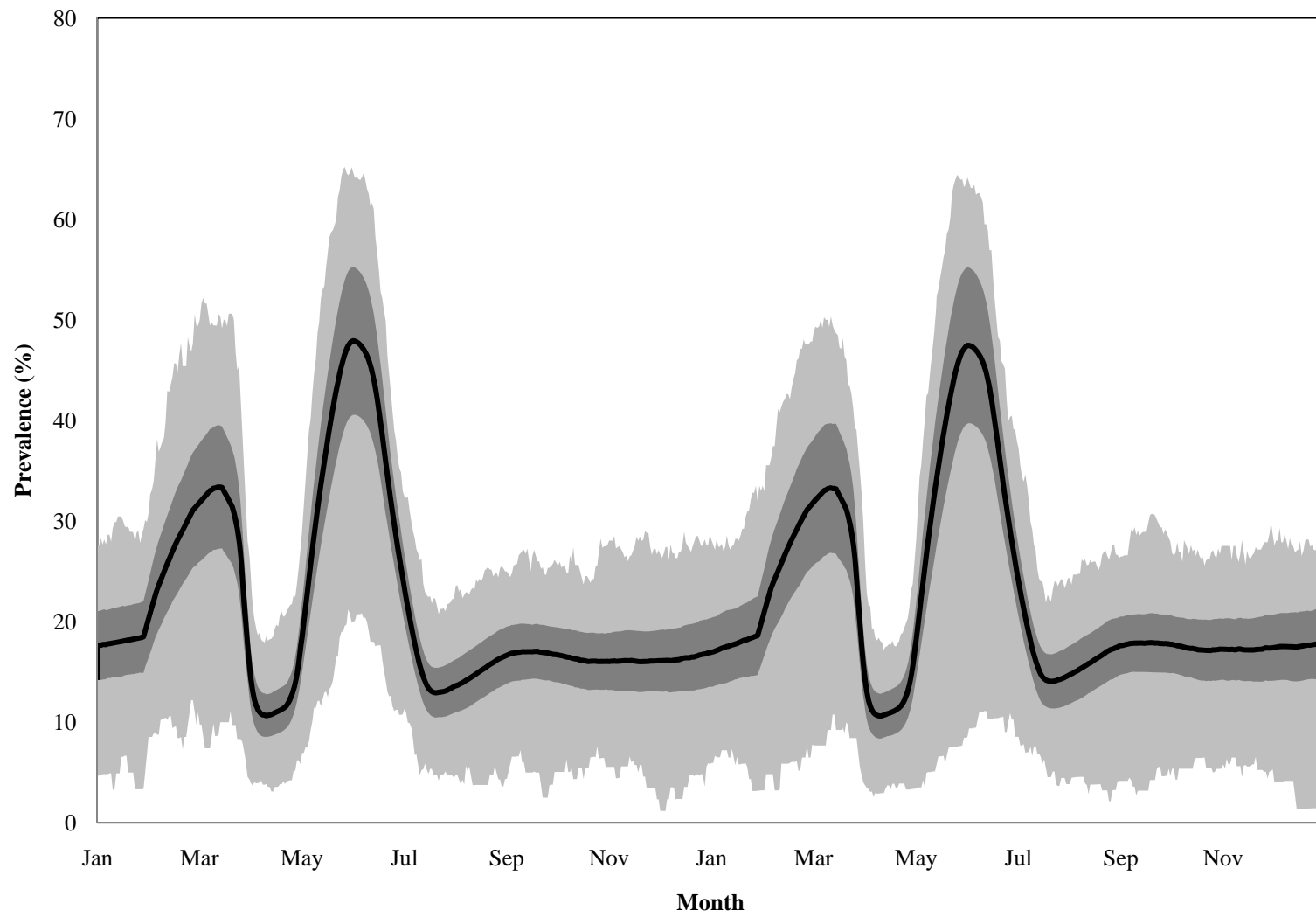
Fig. 3 (f)
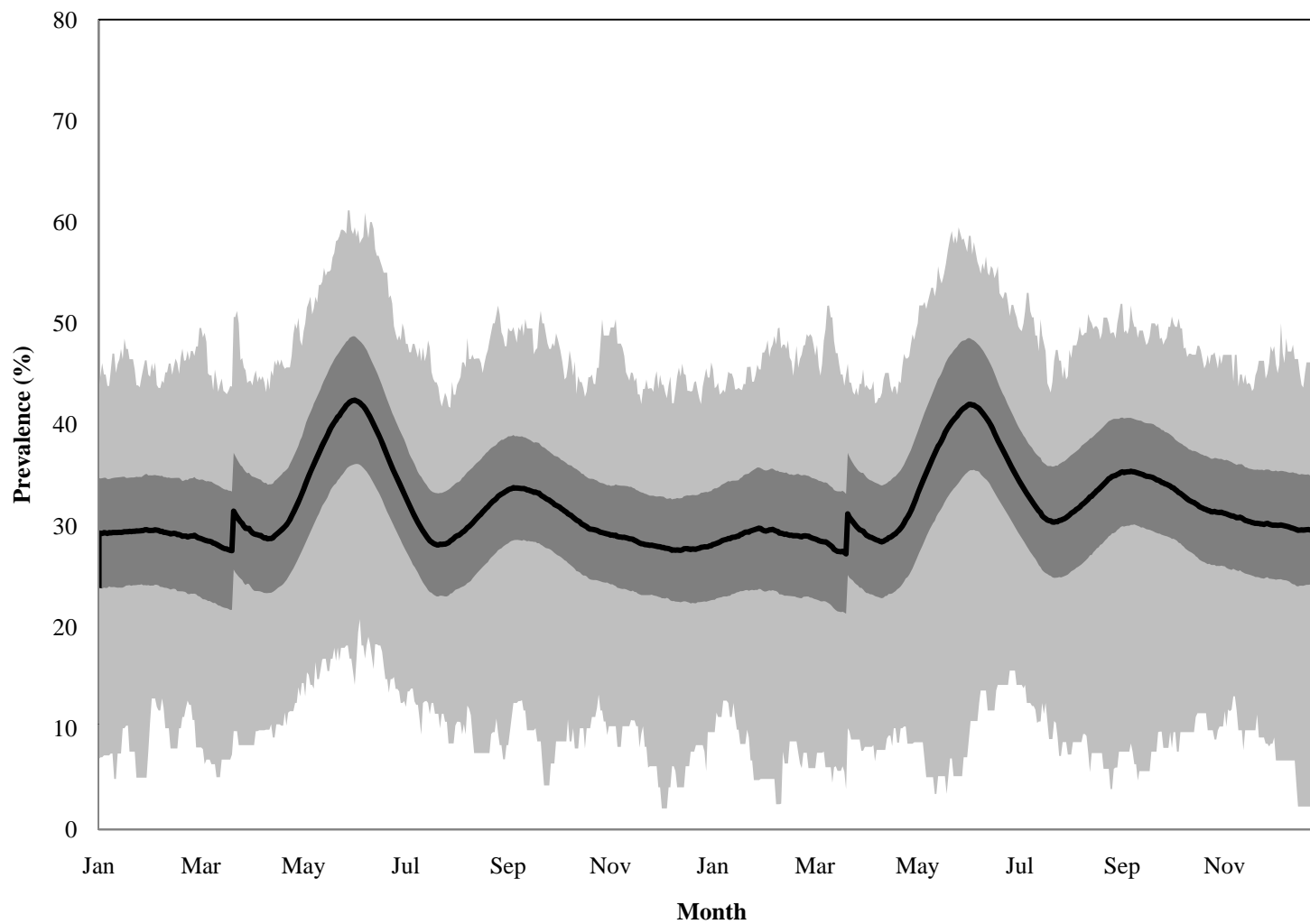
Fig. 3 (g)
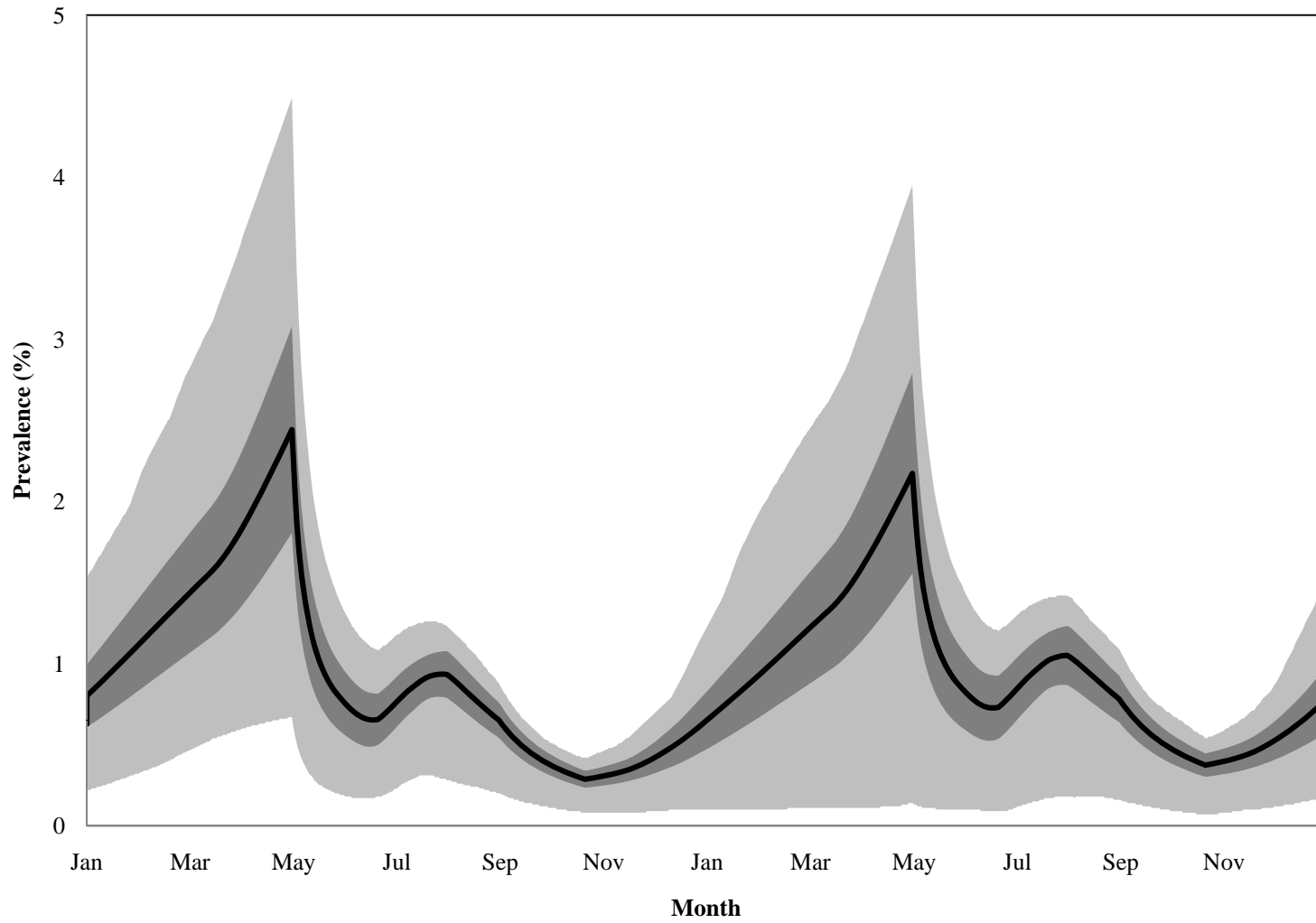
Fig. 3 (h)
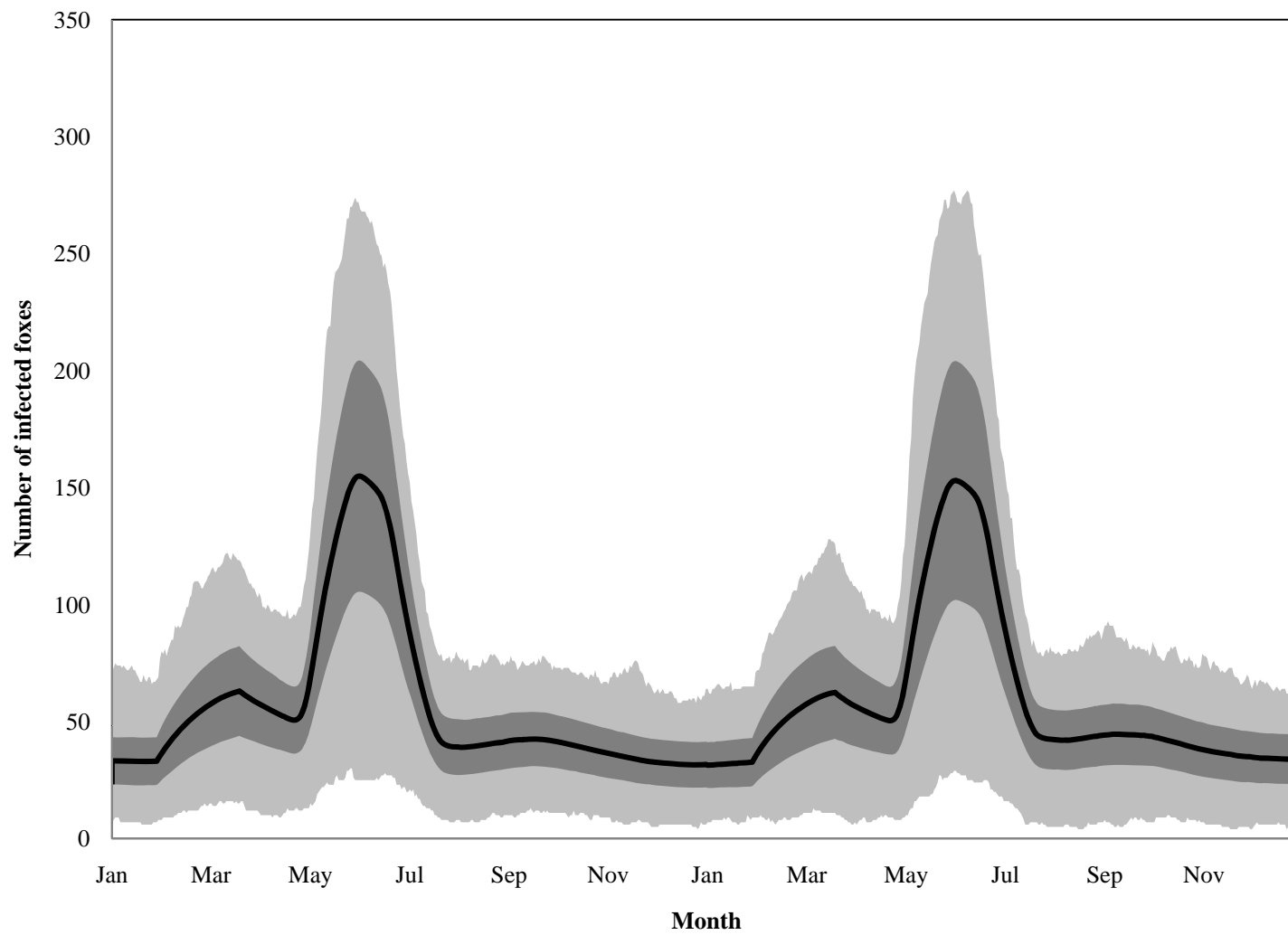
Fig. 3 (i)
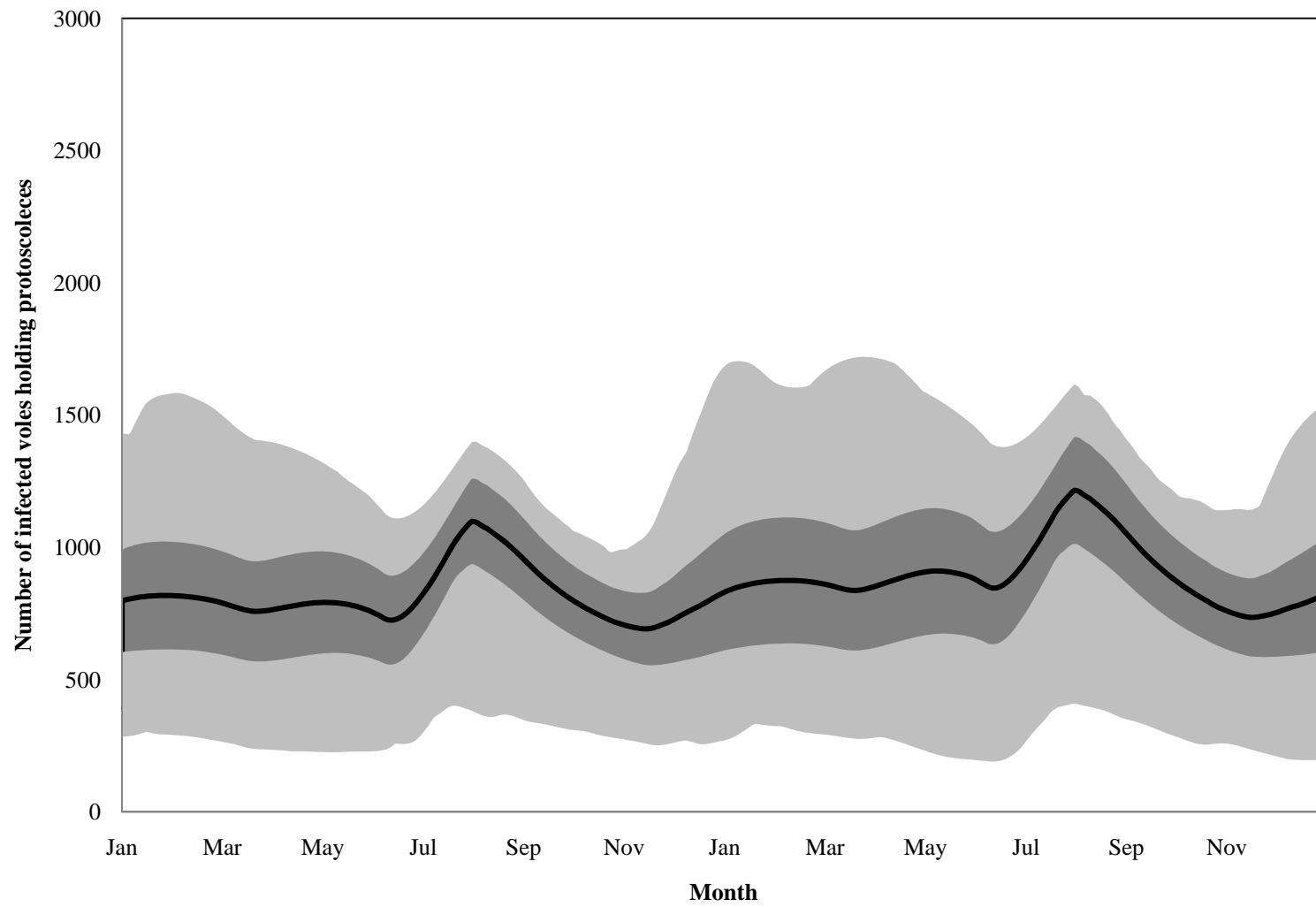
Fig. 3 (j)

Fig. 4

Fig. 5

Fig. 6 (a)

Fig. 6 (b)