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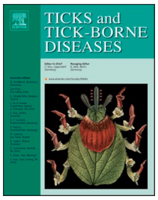

# Environmental and host factors underlying tick infestation in invasive raccoons (*Procyon lotor*) in Hokkaido, Japan

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## ABSTRACT

Revealing interactions between ticks and wild animals is vital for gaining insights into the dynamics of tick-borne pathogens in the natural environment. We aimed to elucidate the factors that determine tick infestation in wild animals by investigating ticks on invasive raccoons (*Procyon lotor*) in Hokkaido, Japan. We first examined the composition, intensity, and seasonal variation of ticks infesting raccoons in six study areas in Hokkaido from March 2022 to August 2023. In one study area, ticks infesting tanukis (raccoon dog, *Nyctereutes procyonoides albus*) were collected in May to July in both 2022 and 2023, and questing ticks were collected from the vegetation by flagging every other week in the same period. Next, we screened 17 environmental and host variables to determine factors that affect the number of ticks infesting raccoons using generalized linear (mixed) models. From 245 raccoons, we identified a total of 3,917 ticks belonging to eight species of two genera: the most prominent species were *Ixodes ovatus* (52.9 %), followed by *Haemaphysalis megaspinosa* (14.4 %), *Ixodes tanuki* (10.6 %), and *Ixodes persulcatus* (9.5 %). *Ixodes ovatus* was also predominant among questing ticks and ticks infesting tanukis. Although *I. tanuki* was frequently collected from raccoons and tanukis, it was rarely collected in the field. The variables that significantly affected the infestation on raccoons differed by genus, species and developmental stage of the tick. For instance, the infestation of adult *I. ovatus* was significantly affected by four

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*Abbreviations:* TBE, tick-borne encephalitis; SFTS, severe fever with thrombocytopenia syndrome; GLMs, generalized linear models; GLMMs, generalized linear mixed models; YEZV, Yezo virus; AIC, Akaike information criterion; IOV, relative Importance Of a Variable; VIFs, Variance Inflation Factors; *ST*, Shintotsukawa Town; *TO*, Tobetsu Town; *SP*, Sapporo City; *EB*, Ebetsu City; *NA*, Nanporo Town; *IW*, Iwamizawa City.

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variables: night-time temperature during nine days before capturing the raccoon, the size of forest area around the capture site, sex of the raccoon, and sampling season. The first two variables were also responsible for the infestation on raccoons of almost all species and stages of ticks. Our study revealed that the number and composition of ticks infesting raccoons can be affected not only by landscape of their habitats but also by weather conditions in several days before capturing.

## 1. Introduction

The occurrence of tick-borne infectious diseases in humans and animals is highly dependent on the local tick fauna. Hokkaido, the northernmost region of Japan, is characterized by a subarctic climate. The tick fauna and tick-borne diseases in Hokkaido are distinct from those on the main island of Japan. *Ixodes* ticks are responsible for most human tick-bite cases in this region (Miyamoto and Nakao, 1991) and transmit Lyme borreliosis spirochetes and tick-borne encephalitis (TBE) virus (Miyamoto et al., 1992; Yoshii et al., 2017). *Haemaphysalis* ticks, which are predominant in the main island, are less prevalent than *Ixodes* ticks in Hokkaido (Miyamoto and Nakao, 1991). Only a single case report of *Amblyomma testudinarium*, one of the major vectors of the severe fever with thrombocytopenia syndrome (SFTS) virus in southwestern Japan (Sato et al., 2021), exists in Hokkaido (Nakao et al., 2021). Accordingly, the number of cases of Lyme borreliosis and TBE are much higher in Hokkaido than in the other areas of Japan, and no SFTS patients have been reported from this region. Evaluating the ecological context forming the local tick fauna will help us to better understand the epidemiology of tick-borne diseases in the region.

The abundance and diversity of wild animal hosts in the environment affect the local tick fauna, thus the prevalence of tick-borne pathogens (Doi et al., 2021a; Gandy et al., 2021, 2022). In Hokkaido, several wild animals including rodents (*Apodemus argenteus, Apodemus speciosus, Myodes rufocanus*, and *Myodes rutilus*), raccoons (*Procyon lotor*), tanukis (raccoon dog, *Nyctereutes procyonoides albus*), sika deer (*Cervus nippon yesoensis*), and brown bear (*Ursus arctos yesoensis*) are the main hosts of ticks (Elbaz et al., 2020; Ozawa and Kadosaki, 1996; Sashika et al., 2011; Taylor et al., 2013; Yamauchi et al., 2012). Raccoons are invasive animals extensively sharing ecological niches with tanukis, which are the predominating native middle-sized animals in Hokkaido (Ikeda, 2015; Saeki, 2015; Saito and Koike, 2013). The population density of raccoons is rapidly increasing in non-native ranges such as Japan and Europe (Beltran-Beck et al., 2012; Ikeda et al., 2015). Generally, invasive species can become new host resources for native parasites and pathogens, potentially altering the dynamics of infectious diseases that threaten public health (Najberek et al., 2022; Zhu et al., 2019). Notably, raccoons inhabit various landscapes and are suspected to contribute to the distribution of various tick species in Japan (e.g. *Haemaphysalis flava, A. testudinarium*, and *Ixodes ovatus*) (Doi et al., 2021a; Osaki et al., 2019). Additionally, raccoons were found to be infected with several tick-borne pathogens of public health concern in Japan, although they are not proven reservoir hosts: *Borrelia garinii* and *Borrelia afzelii* (causative agents of Lyme borreliosis), spotted fever group rickettsiae (Inoue et al., 2011; Sashika et al., 2010), the emerging orthonairovirus Yezo virus (YEZV) (Kodama et al., 2021), and SFTS virus (Tatemoto et al., 2022). The increasing density of invasive raccoons in Hokkaido highlights the need for further investigation into their roles as reservoirs of tick-borne pathogens and hosts for ticks, with possibly important implications for public health.

This study aims to elucidate the role of raccoons as tick hosts and the environmental factors associated with tick infestation on them. We examined the intensity and seasonal dynamics of ticks infesting raccoons which were captured with box traps through the raccoon control programs. We then identified the factors influencing tick infestation on raccoons by examining seasonal, host, landscape, and climatic factors using generalized linear (mixed) models.

## 2. Materials and methods

### 2.1. Study areas

This study was conducted in six adjacent municipalities in central Hokkaido, Japan: Shintotsukawa Town, Tobetsu Town, Sapporo City, Ebetsu City, Nanporo Town, and Iwamizawa City (Fig. 1). The climate in these areas is classified as Df according to the Köppen-Geiger classification (Beck et al., 2018): a mean annual temperature of 7.4℃, average annual precipitation of 1094 mm, and an average annual maximum snow depth of 117 cm (National Spatial Planning and Regional Policy Bureau, Japan, 2022). The major large- and medium-sized mammals in this region include brown bears, sika deer, Ezo red foxes (*Vulpes vulpes schrencki*), tanukis, and raccoons (Ministry of the Environment, 2010). Shintotsukawa, Sapporo, Ebetsu, and Iwamizawa are composed of broad-leaved or mixed coniferous forests with croplands, and Nanporo and Tobetsu are occupied by croplands. The forests are dominated by Sakhalin fir (*Abies sachalinensis*), painted maple (*Acer pictum* subsp. *Mono*), water oak (*Quercus mongolica* var. *grosseserratus*), and Manchurian ash (*Fraxinus mandshurica* var. *japonica*) (Ishikawa and Ito, 1988).

### 2.2. Animals

Box traps (Havahart Large Collapsible Pro Cage Model 1089, Wood–stream Corp., Lititz, PA, USA) were used to capture raccoons and tanukis in the forest area of Ebetsu from May to July in 2022 and 2023. Captured raccoons were anesthetized with an intramuscular injection of butorphanol tartrate (Vetorphale 5 mg, 1.2 mg $kg^{-1}$; Meiji Animal Health Co., Ltd., Tokyo, Japan), medetomidine hydrochloride (Dolbene, 40 μg/kg; Kyoritsu Seiyaku Corporation, Tokyo, Japan), and midazolam (Dormicum injection 10 mg, 0.2 mg $kg^{-1}$; Maruishi Pharmaceutical. Co., Ltd., Osaka, Japan). They were euthanized with an intracardiac injection of 1 mL of 20 % potassium chloride. Tanukis were anesthetized using the same method as the raccoons. To avoid repeated sampling of the same individuals, we identified each captured tanuki by subdermally inserting a microchip into the back of the animal. After sample collection, tanukis were revived with an intramuscular injection of naloxone (Naloxone hydrochloride, 0.02 mg $kg^{-1}$; Alfresa Pharma Corporation, Osaka, Japan), atipamezole hydrochloride (Atipame, 0.2 mg $kg^{-1}$; Kyoritsu Seiyaku Corporation), and flumazenil (Flumazenil, 0.5 mg, 0.02 mg $kg^{-1}$; Teva Takeda Pharma Ltd., Aichi, Japan). They were released at the trapping site upon full recovery from anesthesia. Additionally, raccoons captured though the raccoon control programs from January to November in 2022 and 2023 were included. In total, the study employed 245 raccoons (Shintotsukawa: 17, Tobetsu: 21, Sapporo: 1, Ebetsu: 182, Nanporo: 23, and Iwamizawa: 1) and 54 tanukis (all from Ebetsu).

For each animal, we measured the body length [$BL$ (m)] and body weight [$BW$ (kg)] and calculated the body mass index ($BMI$) as an indicator of nutritional condition using the formula: $BMI = BW / BL^2$ (Kato et al., 2012). Based on the closure of root foramina in canines and cranial suture obliteration, the raccoons were classified as under or over one year old (Junge and Hoffmeister, 1980; Kato et al., 2009).

### 2.3. Ticks

Tick sampling was conducted by a single person within 15 min for each raccoon. For tanukis, approximately ten ticks per individual were collected from the head, aiming to minimize anesthesia time. The tanukis which had been captured in the same year were identified by microchips and released without tick sampling. Additionally, questing ticks in the field were collected using a 0.70 m × 1.00 m white flannel fabric at eight sites which were approximately 10–300 m away from the nearest box trap in Ebetsu. Flagging was performed alongside the trails of 200 m for 30 min in the designated direction at one side of the road, and the ticks on the flag were carefully checked every 12 steps. We performed the flagging between 13:00 and 16:00 of every other week in May to July in both 2022 and 2023. As a result, the flagging was performed seven and six times in 2022 and 2023, respectively.

Tick species, developmental stage, and the sex of adult ticks were identified under a stereomicroscope based on their morphological features (Fujita and Takada, 2007; Sasa and Aoki, 1977).

### 2.4. Statistical analysis

The analyses were conducted separately for seven tick species and developmental stages infesting raccoons: adult *I. ovatus*, adult *Ixodes tanuki*, adult *Ixodes persulcatus*, nymphal *I. persulcatus*, nymphal *Haemaphysalis megaspinosa*, nymphal *H. flava*, and nymphal *Haemaphysalis japonica*. Generalized linear (mixed) models (GLMs and GLMMs) were utilized, with the count of each tick species and developmental stage infesting individual raccoons as the response variable. This count was assumed to follow a negative binomial distribution (using a log link function). The explanatory variables for the GLMs and GLMMs included seasonal factors (*day*), host factors (*age, BMI*, and *sex*), landscape factors (*nearforest, forest, grass, wline*, and *fedge*), short-term climatic factors (*ndrain, ndtemp*, and *ndwind*), and long-term climatic factors (*wintdif, maxtemp8, preci6, rad6*, and *snowy*) (Table 1).

### 2.5. Seasonal factors

To reflect the seasonality of tick infestation, we utilized the accumulated days from January 1st (*day*) and its square as explanatory variables in the model: $a[day]^2 + b[day] + c$. Here, *day* represents the accumulated days from January 1st, whereas *a, b*, and *c* are the coefficients in the equation. If *a* is negative, it indicates a descended U-shaped effect of *day* on the number of ticks. Conversely, if *a* is positive, it implies an inverted U-shaped effect.

**Table 1**
Explanatory variables for the GLMMs and GLMs.

| Factor | Variable | Definition |
|---|---|---|
| Seasonal factors | *day*[*] | Accumulated days from January 1st to the date of capture |
| Host factors | *sex* | Sex of the animal: male (baseline: female) |
| | *age* | Age of the animal: over one year (baseline: under one year) |
| | *BMI* | Body Mass Index of the animal as an indicator of nutritional condition |
| Landscape factors | *nearforest* | Distance to the nearest forest |
| | *forest* | Forest area within the buffer zone |
| | *grass* | Grass area within the buffer zone |
| | *wline* | Length of water edge within the buffer zone |
| | *fedge* | Length of forest edge within the buffer zone |
| Short climatic factors | *ndrain* | Total nighttime precipitation before raccoon capture |
| | *ndtemp*[*] | Total nighttime temperature before raccoon capture |
| | *ndwind* | Total nighttime wind speed before raccoon capture |
| Long climatic factors | *wintdif* | Difference between the highest and lowest temperatures in coldest month (January) |
| | *maxtemp8*[*] | Highest temperature in warmest month (August) |
| | *preci6*[*] | Total precipitation in June |
| | *radi6* | Total solar radiation in June |
| | *snowy* | Yearly maximum snow depth |

[*] Variables containing squared terms.

### 2.6. Landscape factors

Variables of landscape factors were determined based on the landscape surrounding the trap sites. Since it is difficult to directly estimate the spatial scale over which raccoons move and become infested by ticks, we performed a preliminary analysis as follows. The area enclosed

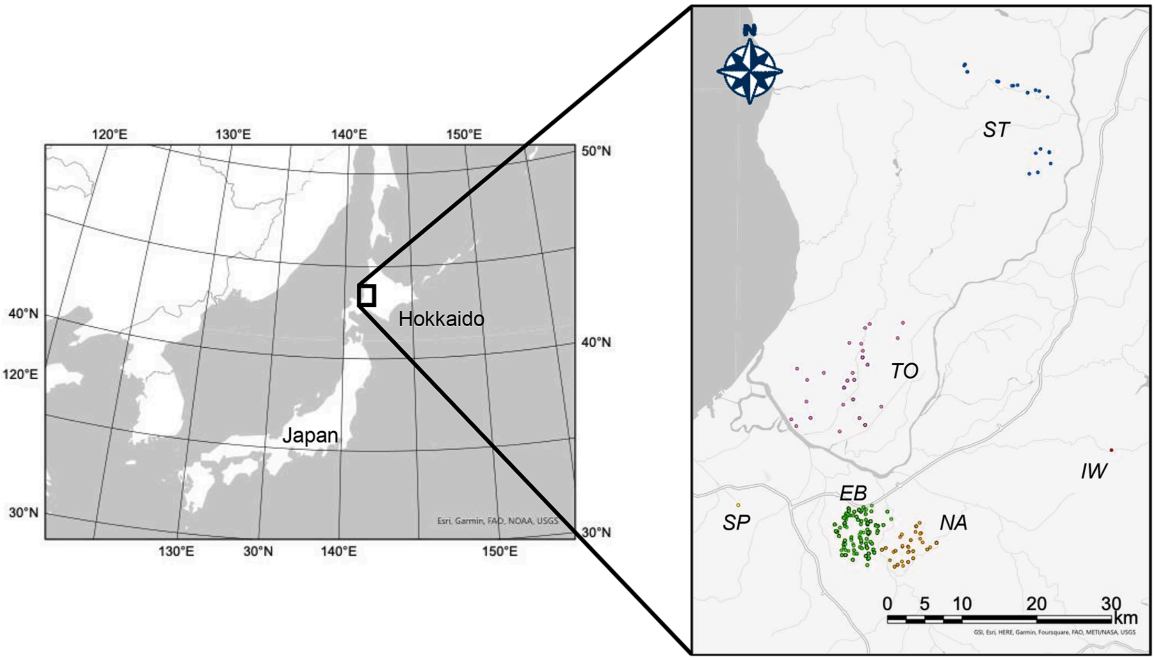

**Fig. 1. Map of study areas.** Each dot represents a site where animals were captured. The dot colors vary according to the study areas; blue: Shintotsukawa Town (ST), pink: Tobetsu Town (TO), red: Iwamizawa City (IW), yellow: Sapporo City (SP), green: Ebetsu City (EB), and orange: Nanporo Town (NA). The base map image was provided by Esri Japan and ZENRIN CO., LTD.

by concentric circles centered on the trap site is referred to as a buffer. Buffer size is defined as the radii of the circle. Eleven buffer sizes (250, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 m) were considered in line with the known home range of raccoons, approximately 500–1000 m in radius (Ikeda et al., 2004; Ishii et al., 2019). The optimal buffer size for each explanatory variable, determined by the smallest Akaike's Information Criterion (AIC) in GLMs for each tick species and developmental stage, was then applied in subsequent analyses. We utilized the High–Resolution Land Use Land Cover Map Ver. 21.11, with a resolution of 10 m, published by the Japan Aerospace Exploration Agency (2023), to extract four landscape variables: *nearforest, forest, grass*, and *fedge*. Additionally, the Digital Map (Basic Geospatial Information) published by the (Geospatial Information Authority of Japan (2023) was used to extract another landscape variable, *wline*. The variable *forest* represents the total surface area of various forest types: deciduous broad-leaf, deciduous needle-leaf, evergreen broad-leaf, and evergreen needle-leaf forests. The variable *grass* accounts for the grassland surface area, *wline* denotes the length of the water edge, and *fedge* represents the total length of forests edge. The distance from the nearest forest to each trap site (*nearforest*) was computed as an indicator of wild animal accessibility to forests. For traps set in the middle of a forest, the *nearforest* was set to zero.

### 2.7. Climatic factors

Climatic data per 1–km$^2$ grid, the mean values obtained during the last 30 years, were sourced from the National Spatial Planning and Regional Policy Bureau, Japan (2022). The data were used for extracting five long-term climatic variables: *wintdif, maxtemp8, preci6, radi6*, and *snowy* (Table 1). The values for the 1–km$^2$ grid at each trap site were used as variables. The variable *wintdif* represents the temperature difference between the highest and lowest temperatures in the coldest month (January), serving as an indicator of overwintering stress for ticks (Herrmann and Gern, 2013). The variable *maxtemp8* indicates the highest temperature in the warmest month (August), reflecting tick activity and heat stress (Nielebeck et al., 2023). Two additional climatic variables in June when the highest number of questing ticks was observed were included: total precipitation (*preci6*) and total solar radiation (*radi6*). The variable *snowy* denotes the yearly maximum snow depth, which enhances the overwintering of ticks (Vollack et al., 2017).

Tick infestation on wild animals may be impacted by short-term climatic conditions through affecting the activity of ticks and/or animals (Kochmann et al., 2021; Nielebeck et al., 2023). Therefore, we adopted the total precipitation (*ndrain*), accumulated mean temperature (*ndtemp*), and accumulated mean wind velocity (*ndwind*) during the 1–10 nights before the day of capture (Table 1). The night was defined as the time between 18:00 and 6:00, which aligns with the nocturnal activity of raccoons in our study areas (Ikeda et al., 2001). Accumulated short-term climatic variables were computed for a range of 1 to 10 nights and the optimal number of night for each tick species and developmental stage was determined based on the smallest AIC in GLMs. Short-term climatic data were obtained from the nearest Japan Meteorological Agency observatory station relative to each trap site (Japan Meteorological Agency, 2023).

Some climatic variables, such as *maxtemp8, preci6*, and *ndtemp*, were hypothesized to exhibit optimal ranges, rather than monotonic positive or negative effects. Hence, their squared values (*maxtemp8*$^2$, *preci6*$^2$, and *ndtemp*$^2$) were also utilized to account for potential descended or inverted U-shaped effects, which align with the methodology for the "*day*" variable.

### 2.8. Model construction and model selection

All continuous explanatory variables were standardized by subtracting each variable's mean and dividing the result by the standard deviation of the variable. We assessed potential collinearities among explanatory variables by calculating variance inflation factors (VIFs). Variables exhibiting multicollinearity, identified by a reference value of 10, were removed prior to model selection. Encompassing all combinations of explanatory variables, we selected the best model based on the AIC. We individually examined trap site, study areas, year, and the interaction of study areas and year as potential random effects, based on AIC and likelihood ratios.

To assess the uncertainty of the best model by AIC, we calculated the relative importance of a variable (IOV) of each variable for each tick species and developmental stage (Burnham and Anderson, 2002). IOV was calculated with the best model by AIC and its competing models, defined as models with an AIC difference of less than two from the best model. The Akaike weight $w$ for the $i^{th}$ model, $w_i$, was calculated as follows:

$$w_i = \frac{\exp\left(-\frac{\Delta_i}{2}\right)}{\sum_{r=1}^{R}\exp\left(-\frac{\Delta_r}{2}\right)}.$$

In the above equation, $\Delta_i$ denotes the difference between the AIC of the best model (AIC$_{min}$) and the AIC of the $i^{th}$ model (AIC$_i$): $\Delta_i = \mathrm{AIC}_i - \mathrm{AIC}_{min}$. The $R$ denotes the number of competing models. IOV for each explanatory variable was the sum of Akaike weights for all competing models including that variable.

The absence of spatial autocorrelation in the residuals of each GLMM or GLM was confirmed by calculating Moran's I Index using the inverse distances between trap sites as a distance-decay function (Moran, 1950). We computed Nagelkerke's $R^2$ for GLMs and both conditional and marginal $R^2$ for GLMMs to evaluate the best models. A p-value $< 0.05$ was considered statistically significant.

For data handling and analysis, we used ArcGIS Pro version 3.1.1 (Esri, 2023) for mapping and extracting environmental variables. Other statistical analyses were conducted in R version 4.2.2 (R Core Team, 2022), employing the MASS (Venables and Ripley, 2002) and lme4 (Bates et al., 2015) packages for fitting GLMs and GLMMs, the MuMIn package (Barton, 2022) for model selection, and the performance package (Lüdecke et al., 2021) for calculating VIFs and $R^2$ values.

## 3. Results

### 3.1. Intensity, composition, and seasonal variation of ticks infesting raccoons

A total of 3917 ticks were collected from 245 raccoons (Table 2). Among these raccoons, 204 raccoons were infested with at least one tick. The number of ticks collected ranged from 0 to 99, with the mean, median, and standard deviation of 16, 9, and 19, respectively. From six raccoons, one could not collect all attached ticks within the sampling time (15 min). Ticks infested various parts of the raccoons' bodies, predominantly on the head and shoulders, especially around the ears, eyes, and under the chin. Nymphal *Haemaphysalis* spp. were sometimes found on the abdomen, around the anus, and on the testes (data not shown).

Among the ticks collected from racoons throughout the study period, adult *I. ovatus* ($n$ = 2046) was the most predominant, followed by nymphal *H. megaspinosa* ($n$ = 345), adult *I. tanuki* ($n$ = 335), larval *H. megaspinosa* ($n$ = 217), adult *I. persulcatus* ($n$ = 212), nymphal *H. flava* ($n$ = 199), nymphal *I. persulcatus* ($n$ = 144), and nymphal *H. japonica* ($n$ = 69) (Table 2). Most adult ticks belonged to the genus *Ixodes*, whereas nymphal and larval ticks predominantly belonged to *Haemaphysalis* (Supplement A: Figs. S1 and S2). The number of raccoons infested by specific tick species/stage was the highest for adult *I. ovatus* ($n$ = 169), followed by adult *I. tanuki* ($n$ = 99), adult *I. persulcatus* ($n$ = 98), nymphal *H. megaspinosa* ($n$ = 89), nymphal *H. flava* ($n$ = 75), nymphal *I. persulcatus* ($n$ = 60), larval *H. megaspinosa* ($n$ = 42), and nymphal *H. japonica* ($n$ = 35) (Table 2).

**Table 2**
Proportion and frequency of ticks infesting raccoons.

| Tick species | Developmental stage | Number of ticks (Proportion* (%)) | Number of infested raccoons (Frequency** (%)) |
|---|---|---|---|
| *I. ovatus* | Adult | 2046 (52.2) | 169 (69.0) |
| | Nymph | 3 (0.1) | 3 (1.2) |
| | Larva | 22 (0.6) | 1 (0.4) |
| *I. tanuki* | Adult | 335 (8.6) | 99 (40.4) |
| | Nymph | 41 (1.0) | 21 (8.6) |
| | Larva | 41 (1.0) | 21 (8.6) |
| *I. persulcatus* | Adult | 212 (5.4) | 98 (40.0) |
| | Nymph | 144 (3.7) | 60 (24.5) |
| | Larva | 16 (0.4) | 13 (5.3) |
| *I. pavlovskyi* | Adult | 26 (0.7) | 17 (6.9) |
| | Nymph | 17 (0.4) | 13 (5.3) |
| | Larva | 17 (0.4) | 13 (5.3) |
| *Ixodes* spp.*** | Adult | 0 (0.0) | 0 (0.0) |
| | Nymph | 5 (0.1) | 4 (1.6) |
| | Larva | 6 (0.2) | 5 (2.0) |
| *H. megaspinosa* | Adult | 1 (0.0) | 1 (0.4) |
| | Nymph | 345 (8.8) | 89 (36.3) |
| | Larva | 217 (5.5) | 42 (17.1) |
| *H. flava* | Adult | 50 (1.3) | 25 (10.2) |
| | Nymph | 199 (5.1) | 75 (30.6) |
| | Larva | 11 (0.3) | 7 (2.9) |
| *H. japonica* | Adult | 34 (0.9) | 18 (7.3) |
| | Nymph | 69 (1.8) | 35 (14.3) |
| | Larva | 45 (1.1) | 14 (5.7) |
| *H. longicornis* | Adult | 0 (0.0) | 0 (0.0) |
| | Nymph | 1 (0.0) | 1 (0.4) |
| | Larva | 3 (0.1) | 1 (0.4) |
| *Haemaphysalis* spp.*** | Adult | 0 (0.0) | 0 (0.0) |
| | Nymph | 5 (0.1) | 3 (1.2) |
| | Larva | 0 (0.0) | 0 (0.0) |
| Unknown | | 6 (0.2) | 5 (2.0) |
| Total | | 3917 (100.0) | 204 (83.3) |

[*] Proportion refers to the ratio of each tick species and developmental stage to the total collected ticks ($n = 3917$).

[**] Frequency denotes the ratio of raccoons infested with the tick(s) to the total captured raccoons ($n = 245$).

[***] Ticks not identified at the species level were categorized as *Ixodes* spp. or *Haemaphysalis* spp.

The number of ticks collected from each raccoon was visualized for seven predominant tick species/stages in Fig. 2. The number of ticks infesting raccoons exhibited one or two peaks when graphed against the accumulated days starting from January 1st. Adult *I. ovatus* and both adult and nymphal *I. persulcatus* showed peak infestations from spring to early summer. Adult *I. tanuki* displayed two peaks: first in spring to early summer and second in autumn. Nymphal *H. megaspinosa, H. flava*, and *H. japonica* were present from spring through autumn.

### 3.2. Questing ticks and ticks infesting raccoons and tanukis

In one study area (Ebetsu), tanukis were captured and subjected to tick counting. Questing ticks were also collected by flagging at the predetermined areas nearby trap sites. We collected a total of 1845 adult ticks from raccoons ($n = 95$), 704 from tanukis ($n = 54$), and 945 from the field (Supplement A: Fig. S3). The average number of ticks collected from individual raccoons and tanukis was 19 and 13, respectively. *Ixodes ovatus* was the predominant species on raccoons (78.3 %), tanukis (91.8 %,), and the field (81.5 %) (Table 3). The second and third most common species were *I. persulcatus* and *Ixodes pavlovskyi* in the field, and *I. tanuki* and *I. persulcatus* in both raccoons and tanukis, respectively. The percentage of *I. tanuki* among the total adult ticks collected from tanukis was less than half of that from raccoons (4.0 % in tanukis versus 10.9 % in raccoons). *Ixodes tanuki* was rarely collected from the field (0.1 %).

**Table 3**
Proportion (%) of adult ticks collected from raccoons, Tanukis, and the field.

| Tick species | Raccoons | Tanukis | Field |
|---|---|---|---|
| *I. ovatus* | 78.3 | 91.8 | 81.5 |
| *I. tanuki* | 10.9 | 4.0 | 0.1 |
| *I. persulcatus* | 7.8 | 3.8 | 12.6 |
| *I. pavlovskyi* | 1.0 | 0.0 | 2.8 |
| *H. megaspinosa* | 0.0 | 0.0 | 1.1 |
| *H. flava* | 1.4 | 0.1 | 1.5 |
| *H. japonica* | 0.7 | 0.3 | 0.4 |
| *H. longicornis* | 0.0 | 0.0 | 0.1 |

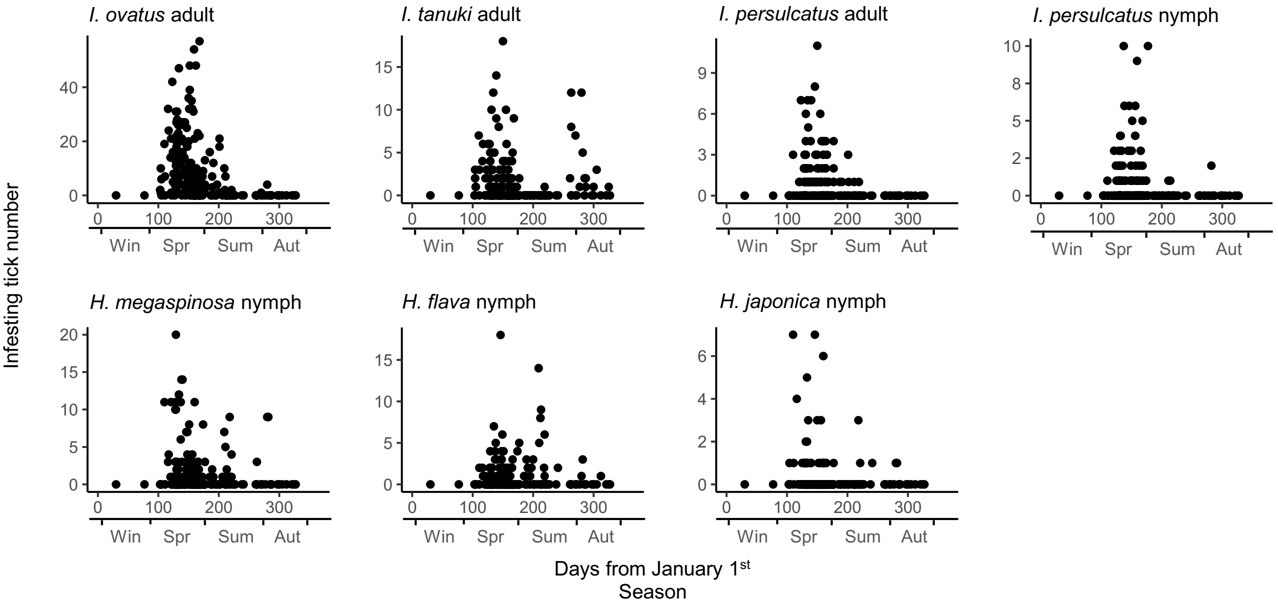

**Fig. 2. Seasonal variation of tick infestations on Raccoons.** Each dot indicates an individual raccoon. The x-axis represents accumulated days from January 1st (*day*) to the date when the raccoon was captured, and the y-axis shows the count of each tick species and developmental stage of the individual animal. In Hokkaido, Japan, the seasons are defined as follows: winter (Win) from December to March (*day*: 1–90, 335–365), spring (Spr) from April to June (*day*: 91–181), summer (Sum) from July to September (*day*: 182–273), and autumn (Aut) from October to November (*day*: 274–334).

### 3.3. Factors determining tick infestation on raccoons

We conducted the statistical analyses to identify the factors related to the infestation of the seven most predominant tick species/stages on raccoons: adult *I. ovatus*, adult *I. tanuki*, adult *I. persulcatus*, nymphal *I. persulcatus*, nymphal *H. megaspinosa*, nymphal *H. flava*, and nymphal *H. japonica*. The optimal buffer size and the number of nights before the day of capture for each variable were determined for each tick species/stage and used in the following analyses (Table 4 and Table S1 in Supplement A).

Explanatory variables with high IOV were typically included in the model with the smallest AIC and exhibited a significant impact on the response variable. This trend was noted in adult *I. ovatus*, adult *I. tanuki*, adult *I. persulcatus*, nymphal *H. megaspinosa*, and nymphal *H. flava* (Supplement A: Table S2). In contrast, for nymphal *I. persulcatus* and nymphal *H. japonica*, a discrepancy between the IOV and their importance in the best model existed, particularly with variables such as *radi6* and *wintdif* for nymphal *I. persulcatus* (Supplement A: Table S2). The principal findings from the best model for each tick species/stage are outlined below (Table 5 and Supplement A: Fig. S4). Additionally, response curves of five top variables included in the best model for each tick species/stage are provided in Fig. 3.

The major variables influencing the tick infestation on raccoons were landscape features associated with forests, particularly the size of forest area within the buffer (*forest*), which had a positive effect on almost all tick species and developmental stages. The forest edge length in the buffer (*fedge*) positively impacted the best models for adult *I. tanuki* and nymphal stages of three *Haemaphysalis* ticks. Additionally, *fedge* had significant positive effects on all analyzed tick species/stages in the single regression analyses (data not shown). In terms of host factors, the infestation intensities of all adult *Ixodes* ticks (*I. ovatus, I. persulcatus*, and *I. tanuki*) were influenced by the sex of the raccoon; males had higher infestation than females. Climatic factors also had significant effects on tick infestation on raccoons. For three tick species/stages, the difference between the highest and lowest temperatures in the coldest month (*wintdif*) negatively impacted the number of ticks on raccoons. The highest temperature in the warmest month (*maxtemp8*) exhibited an inverted U-shaped effect or positive effect on four tick species/stages. The yearly maximum snow depth (*snowy*) exhibited a negative impact on the infestation numbers of all species of *Haemaphysalis* nymphs on raccoons. In addition to the long-term climatic factors, the weather conditions during several nights before the day of capture had significant effects on tick infestation on raccoons. Notably, the accumulated mean temperature (*ndtemp*) during several nights before the day of capture influenced the numbers of almost all species/stages of ticks. The total precipitation (*ndrain*) was also included in the best models for four tick species/stages with significant effects in two of them.

## 4. Discussion

We explored the intensity, composition, and seasonal dynamics of tick infestations on raccoons in the subarctic region in Japan, i.e. Hokkaido. The most predominant tick species/stage infesting raccoons was adult *I. ovatus*, followed by adult *I. tanuki*, adult *I. persulcatus*, and nymphal stage of three *Haemaphysalis* species. *Ixodes ovatus* was also predominant among questing ticks in the field and ticks infesting tanukis. Though *I. tanuki* was frequently collected from raccoons and tanukis, it was rarely collected in the field. Our results indicate that tanukis and raccoons can host the similar repertoire of tick species/stage. *Ixodes tanuki* was originally described using the specimens collected from tanukis (Saito, 1964) and tanuki has been considered as a primary host of the tick species. Higher frequency of *I. tanuki* infestation on raccoons than tanukis suggests that *I. tanuki* may prefer raccoons over native tanukis.

The abundance and species composition of ticks infesting raccoons vary based on the regions where studies were conducted (Doi et al., 2021b, 2018; Sanjuán et al., 2022; Smith et al., 2019; Yamauchi et al., 2012). Tick species observed in the present study differ from those previously conducted in the humid subtropical region in Japan (i.e., Kanagawa Prefecture in the main island of Japan) (Doi et al., 2021b, 2018). In those previous studies, almost all ticks infesting raccoons were *H. flava* (97 %), with the mean numbers of ticks collected per individual being 306 and 139. *Haemaphysalis flava* is one of the predominant species in Kanagawa prefecture (Doi et al., 2021a). Thus, it is evident that the abundance and species composition of ticks infesting raccoons are largely influenced by the local tick fauna.

We utilized GLMMs to examine the environmental and host factors responsible for the tick infestation on raccoons. Our data indicated that in addition to the size of forest area within the buffer (*forest*), other landscape features associated with forests, such as the length of forest edges, an indicator of ecotones, also significantly affect the number of ticks infesting raccoons. These findings align with previous observations indicating that forest areas and ecotones may increase tick abundance in the field (Doi et al., 2021a; Peralbo-Moreno et al., 2022) and also exposure to tick-borne diseases (Liu et al., 2023). Additionally, for all analyzed tick species and stages, including those where *fedge* was not included in their best model, *fedge* had significant positive effects in the single regressions (data not shown), suggesting its effect on wide range of tick species and stages.

The optimal buffer sizes for *fedge* were broader than those for other landscape variables and also than the estimated range of raccoon activity in our study areas, i.e. approximately 500 to 1000 m in radius (Ikeda et al., 2004; Saito and Koike 2013). Our results may imply that the tick infestation intensity on raccoons is affected by the forest edges outside the estimated raccoon activity range. In fact, Doi et al. (2021a) reported that the presence of questing ticks in the field is positively influenced by surrounding forests, which act as corridors for wild animals. Therefore, forest edges may increase the risk of tick infestation by facilitating passive migration of ticks by wild animals. Given that the risk of tick infestation at a site is affected by its surrounding area, the impact of landscape factors in broader areas should be considered to examine the risk of tick-borne diseases.

In addition to the landscape factors, host and climatic factors were found to affect tick infestation on raccoons; male raccoons were infested by more adult *Ixodes* ticks than females, which may be related to sexually dimorphic traits in males including higher activity as suggested elsewhere (Hughes and Randolph., 2001; Ruiz-Fons et al., 2013). Climatic factors relating to temperature were shown to significantly affect tick infestation, in line with previous studies both in the field and laboratory settings demonstrating temperature is crucial for the questing activity and survival of ticks (Doi et al., 2021a; Herrmann and Gern, 2013; Nielebeck et al., 2023). Interestingly, in the present study, the yearly maximum snow depth negatively impacted the infestation numbers of nymphal *Haemaphysalis* ticks on raccoons. Since snow depth indirectly decreases the abundance of *Haemaphysalis longicornis* in the field via the decreasing abundance of sika deer (Iijima et al., 2022; Kaji et al., 2000; Tsukada et al., 2014), the negative effects of snow depth on the infestation intensity of *Haemaphysalis* ticks on raccoons might be

**Table 4**
Optimal buffer size (m) for each variable of each tick species and developmental stage.

| Response variable | Explanatory variables | | | |
|---|---|---|---|---|
| | *fedge* | *forest* | *grass* | *wline* |
| *I. ovatus* adult | 3500 | 1500 | 1000 | 2500 |
| *I. tanuki* adult | 4500 | 500 | 1500 | 250 |
| *I. persulcatus* adult | 5000 | 250 | 1500 | 2000 |
| *I. persulcatus* nymph | 4000 | 250 | 250 | 1500 |
| *H. megaspinosa* nymph | 4000 | 500 | 1500 | 1000 |
| *H. flava* nymph | 1000 | 2000 | 1500 | 5000 |
| *H. japonica* nymph | 2000 | 1500 | 1500 | 2000 |

**Table 5**
Outputs from the best GLM(M) for each tick species and developmental stage.

| Response variable | Explanatory variable | Estimate | SE | z–value | Pr (> \|z\|) | Significance[a] | $R^2$ |
|---|---|---|---|---|---|---|---|
| *I. ovatus* | *Intercept* | 1.433 | 0.151 | 9.495 | <0.001 | *** | 0.817[b] |
| adult | *day* | –1.282 | 0.202 | –6.339 | <0.001 | *** | 0.757[c] |
| | *sex* | 0.507 | 0.151 | 3.351 | <0.001 | *** | |
| | *forest* | 0.986 | 0.148 | 6.664 | <0.001 | *** | |
| | *wline* | 0.183 | 0.140 | 1.307 | 0.191 | | |
| | *ndtemp* | 0.541 | 0.173 | 3.125 | 0.002 | ** | |
| | *ndtemp*$^{2}$ | –0.534 | 0.117 | –4.565 | <0.001 | *** | |
| | *wintdif* | –0.207 | 0.111 | –1.870 | 0.062 | | |
| *I. tanuki* | *Intercept* | –0.123 | 0.222 | –0.555 | 0.579 | | 0.507[d] |
| adult | *day* | –0.559 | 0.237 | –2.362 | 0.018 | * | |
| | *day*$^{2}$ | 0.540 | 0.137 | 3.932 | <0.001 | *** | |
| | *sex* | 0.685 | 0.230 | 2.974 | 0.003 | ** | |
| | *fedge* | 0.485 | 0.188 | 2.574 | 0.010 | ** | |
| | *forest* | 0.658 | 0.182 | 3.621 | <0.001 | *** | |
| | *wline* | 0.306 | 0.150 | 2.041 | 0.041 | * | |
| | *nearforest* | –0.223 | 0.158 | –1.409 | 0.159 | | |
| | *ndrain* | 0.255 | 0.102 | 2.512 | 0.012 | * | |
| | *ndtemp*$^{2}$ | –0.726 | 0.182 | –3.992 | <0.001 | *** | |
| | *maxtemp8*$^{2}$ | –0.202 | 0.093 | –2.170 | 0.030 | * | |
| | *radi6* | 0.396 | 0.139 | 2.843 | 0.004 | ** | |
| | *snowy* | –0.241 | 0.123 | –1.956 | 0.050 | | |
| *I. persulcatus* | *Intercept* | –0.231 | 0.195 | –1.182 | 0.237 | | 0.601[d] |
| adult | *day* | –0.877 | 0.312 | –2.806 | 0.005 | ** | |
| | *day*$^{2}$ | –1.739 | 0.478 | –3.641 | <0.001 | *** | |
| | *sex* | 0.679 | 0.197 | 3.452 | <0.001 | *** | |
| | *BMI* | –0.319 | 0.127 | –2.518 | 0.012 | * | |
| | *fedge* | 0.194 | 0.125 | 1.549 | 0.121 | | |
| | *forest* | 0.701 | 0.134 | 5.249 | <0.001 | *** | |
| | *maxtemp8* | 0.362 | 0.132 | 2.736 | 0.006 | ** | |
| | *preci6*$^{2}$ | –0.373 | 0.140 | –2.670 | 0.008 | ** | |
| *I. persulcatus* | *Intercept* | –0.709 | 0.310 | –2.289 | 0.022 | * | 0.503[d] |
| nymph | *sex* | –0.483 | 0.294 | –1.642 | 0.101 | | |
| | *BMI* | 0.403 | 0.163 | 2.473 | 0.013 | * | |
| | *forest* | 0.767 | 0.195 | 3.929 | <0.001 | *** | |
| | *nearforest* | 0.249 | 0.168 | 1.477 | 0.140 | | |
| | *ndrain* | –1.280 | 0.900 | –1.422 | 0.155 | | |
| | *ndtemp*$^{2}$ | –0.583 | 0.240 | –2.428 | 0.015 | * | |
| | *ndwind* | 0.350 | 0.146 | 2.399 | 0.017 | * | |
| | *radi6* | –0.653 | 0.259 | –2.524 | 0.012 | * | |
| | *wintdif* | –0.694 | 0.297 | –2.340 | 0.019 | * | |
| *H. megaspinosa* | *Intercept* | 0.779 | 0.381 | 2.046 | 0.041 | * | 0.684[b] |
| nymph | *day* | 1.143 | 0.393 | 2.911 | 0.004 | ** | 0.653[c] |
| | *day*$^{2}$ | –0.687 | 0.253 | –2.716 | 0.007 | ** | |
| | *sex* | 0.302 | 0.270 | 1.117 | 0.264 | | |
| | *fedge* | 0.547 | 0.244 | 2.242 | 0.025 | * | |
| | *nearforest* | –0.558 | 0.260 | –2.145 | 0.032 | * | |
| | *ndtemp* | –0.584 | 0.238 | –2.454 | 0.014 | * | |
| | *maxtemp8*$^{2}$ | –0.575 | 0.143 | –4.026 | <0.001 | *** | |
| | *snowy* | –0.778 | 0.251 | –3.095 | 0.002 | ** | |
| | *wintdif* | –0.870 | 0.222 | –3.927 | <0.001 | *** | |
| *H. flava* | *Intercept* | –0.351 | 0.391 | –0.899 | 0.369 | | 0.577[b] |
| nymph | *day* | 0.852 | 0.269 | 3.170 | 0.002 | ** | 0.505[c] |
| | *day*$^{2}$ | –0.362 | 0.186 | –1.945 | 0.052 | | |
| | *fedge* | 0.690 | 0.191 | 3.622 | <0.001 | *** | |
| | *forest* | 0.599 | 0.217 | 2.760 | 0.006 | ** | |
| | *ndrain* | –0.166 | 0.150 | –1.106 | 0.269 | | |
| | *maxtemp8*$^{2}$ | –0.164 | 0.079 | –2.079 | 0.038 | | |
| | *snowy* | –0.656 | 0.200 | –3.285 | 0.001 | ** | |
| | *wintdif* | –0.456 | 0.208 | –2.191 | 0.028 | | |
| *H. japonica* | *Intercept* | –2.834 | 0.769 | –3.687 | <0.001 | *** | 0.812[b] |
| nymph | *day* | –0.911 | 0.409 | –2.227 | 0.026 | * | 0.761[c] |
| | *day*$^{2}$ | 0.595 | 0.265 | 2.242 | 0.025 | * | |
| | *sex* | 0.638 | 0.419 | 1.524 | 0.127 | | |
| | *fedge* | 0.680 | 0.339 | 2.009 | 0.045 | * | |
| | *forest* | 0.983 | 0.290 | 3.392 | <0.001 | *** | |
| | *ndrain* | 0.708 | 0.218 | 3.253 | 0.001 | ** | |
| | *ndtemp*$^{2}$ | –0.442 | 0.331 | –1.335 | 0.182 | | |
| | *ndwind* | 0.301 | 0.216 | 1.395 | 0.163 | | |
| | *preci6*$^{2}$ | –0.379 | 0.292 | –1.297 | 0.195 | | |
| | *radi6* | 0.834 | 0.433 | 1.929 | 0.054 | | |
| | *snowy* | –1.563 | 0.721 | –2.168 | 0.030 | * | |

[a] Significance codes are as follows: ***, <0.001; **, <0.01; *, <0.05.
[b] Conditional $R^2$.
[c] Marginal $R^2$.
[d] Nagelkerke's $R^2$.

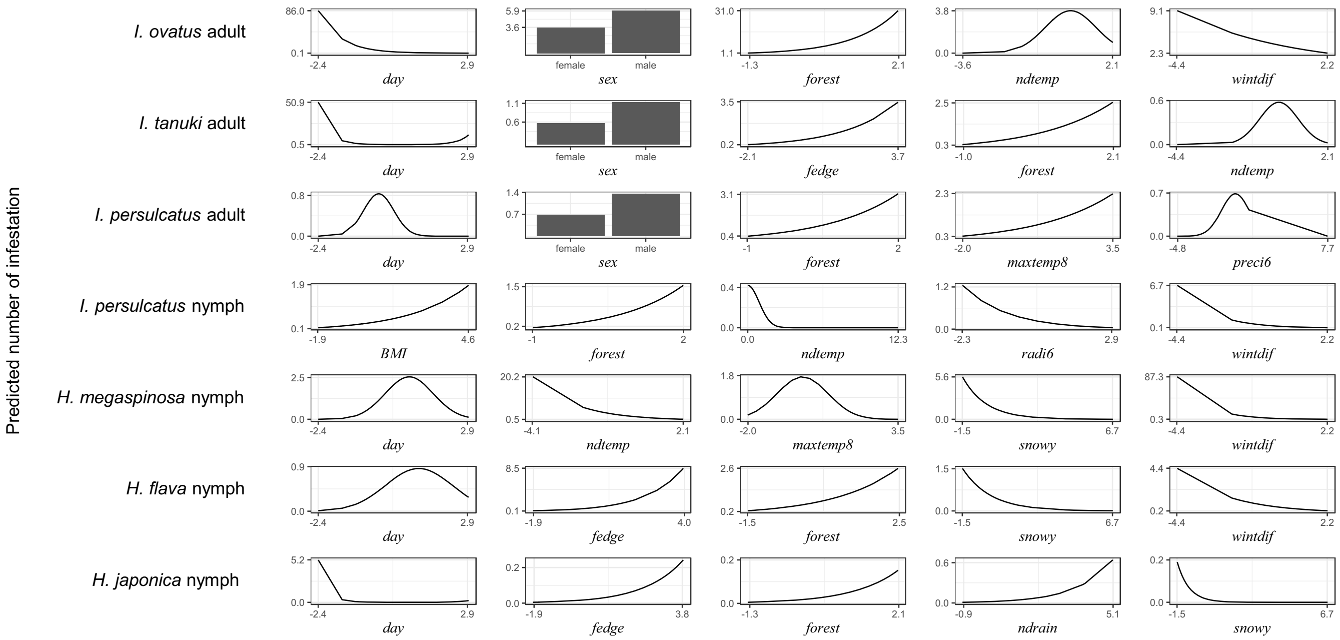

**Fig. 3. Response curves of the seven most predominant tick species/stages for explanatory variables.** This figure displays response curves for the five top variables that contributed substantially to the best model for the seven most predominant tick species/stages.

mediated by the decreased density of sika deer by snow depth. The indirect effects of factors on tick infestation suggested in the present study should be carefully examined.

Our findings also indicate that short-term weather conditions, such as total temperature during 1–10 nights before the day of capture, significantly influence the number of ticks infesting wild animals depending on tick species and developmental stages. In addition to affecting the questing activity of ticks (Nielebeck et al., 2023), temperature is essential to determine the distribution and activity of raccoons (Kochmann et al., 2021). Although previous studies of tick infestation on wild animals have generally considered long-term climatic factors (Sherrard-Smith et al., 2012; Sobrino et al., 2012), our results emphasize the importance of the short-term climatic parameters to investigate their tick infestation status.

## 5. Conclusions

We collected 3917 ticks from 245 raccoons captured in central Hokkaido, and revealed that the most predominant tick species/stage on raccoons is adult *I. ovatus*, followed by adult *I. tanuki*, adult *I. persulcatus*, and nymphal *Haemaphysalis* ticks. Complex interactions of environmental and host factors influence the intensity of tick infestation on raccoons. The key determinants of tick infestation on raccoons identified in this study are as follows: adult *Ixodes* ticks infest males more than females while the infestation intensity of nymphal *Haemaphysalis* ticks is not affected by the sex of raccoons, the forest edges increases tick infestations, weather conditions during several nights before the day of capturing raccoons influence infestation, and snow depth negatively affects the infestation intensity by nymphal *Haemaphysalis* ticks. These environmental and host factors can affect the dynamics of tick-borne pathogens by changing the intensity of tick infestation on raccoons.

## Ethical approval

All procedures, including sample collection from live animals, were conducted in accordance with the Guidelines for Animal Care and Use of Hokkaido University. The study was approved by the Animal Care and Use Committee of the Graduate School of Veterinary Medicine, Hokkaido University (permit numbers: 18–0001 and 23–0030). Permission to capture raccoons in Ebetsu was granted by the Ministry of the Environment under the raccoon control program. Additionally, authorization to capture tanukis for academic research was obtained from the Hokkaido Government.

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## CRediT authorship contribution statement

**Mebuki Ito:** Funding acquisition, Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization. **Miku Minamikawa:** Resources, Investigation. **Anastasiia Kovba:** Resources. **Hideka Numata:** Resources, Investigation. **Tetsuji Itoh:** Resources. **Yuki Katada:** Resources, Investigation. **Shiho Niwa:** Resources, Investigation. **Yurie Taya:** Resources, Investigation. **Yuto Shiraki:** Resources, Investigation. **Gita Sadaula Pandey:** Resources. **Samuel Kelava:** Writing – review & editing. **Nariaki Nonaka:** Writing – review & editing. **Ryo Nakao:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Ryosuke Omori:** Writing – review & editing, Methodology. **Yuma Ohari:** Resources, Methodology, Funding acquisition. **Norikazu Isoda:** Writing – review & editing, Resources. **Michito Shimozuru:** Writing – review & editing. **Toshio Tsubota:** Writing – review & editing. **Keita Matsuno:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Mariko Sashika:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no competing interests.

## Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2024.102389.

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