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ORIGINAL ARTICLE

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Effects of density, season, and food intake on sika deer nutrition on Yakushima Island, Japan

Abstract Sika deer (*Cervus nippon*) suffer severe winter food limitation in northern Japan; however, plant food resources are available during winter in southern Japan and, consequently, deer nutritional status may not decrease there. To test this hypothesis, we measured seasonal changes in Riney's kidney fat index (RKFI) and stomach intake in 74 culled deer individuals from five areas with different deer densities on Yakushima Island, Japan. The average RKFI differed significantly among populations and ranged from 22.91 ± 11.91 to 76.23 ± 15.99. There was no significant correlation between RKFI and deer density. The total fresh weight of rumen contents also differed significantly among populations, ranging from 0.51 to 3.51 kg. Food intake and RKFI exhibited an L-shaped distribution in the vicinity of a town ranch. However, we found that food intake had a significantly positive effect in populations at the other four locations, suggesting that changes in nutritional status were related to the quantity of deer rumen contents. Neither deer density nor season were significantly correlated with RKFI values. The average RKFI values exceeded 20 in all five locations. These findings indicate that the deer were not in a poor nutritional condition even in high density areas of > 70 head/km², which supports the above hypothesis. Notably, food intake varied among individuals in the same area, but was independent of body weight and age, which suggests that food selectivity differs between deer individuals.

Introduction

In herbivores, such as deer, population dynamics are highly dependent upon winter mortality (Clutton-Brock et al. 1985; Loisson and Langvatn 1998; Loison et al. 1999). Deer populations inhabiting high latitudes suffer large fluctuations that reflect severe limitation of food resources and high winter mortality (Hansson and Henttonen 1985; Bjornstad et al. 1995; Kaji et al. 2004); in comparison, populations inhabiting low latitudes, where high fluctuations are rare, exhibit lower and more stable winter mortality rates (Lord 1960; Hansson and Henttonen 1985; Bjornstad et al. 1995). Sika deer (*Cervus nippon*) are a good example of a species living under markedly different food availability at high and low latitudes.

Cervus nippon is widely distributed in the main islands of Japan, from Hokkaido, where the lowlands are snow-covered in winter, to Kyushu district where lowland snow is rare. The sika deer populations have increased rapidly since the 1970s in many regions of Japan, including Hokkaido and Kyushu (Takatsuki 2009a). Vegetation coverage has decreased as a consequence of the increased grazing and browsing pressure in both Hokkaido (Kaji et al. 1988) and the Kyushu district (Yakushima Island; Koda et al. 2008; Onoda and Yahara 2015), leading to less food being available for *C. nippon*.

In Hokkaido, an isolated *C. n. yesoensis* population on Nakanoshima Island, an island in the center of Toya Lake, experienced a large fluctuation and mass winter mortality (Kaji et al. 1988). Three deer individuals were introduced to the island in the 1950s to 1960s. This *C. n. yesoensis* population has grown at a rate of 16% per year for the last 20 years with deer density peaking at 60 head/km² in the autumn of 1983, accompanied with large decrease in vegetation coverage. Consequently, the deer population has suffered mass mortality by which

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the population declined from 299 in the autumn of 1983 to 137 in the winter of 1984 (Kaji et al. 1988). Mass mortality under high density has also been reported in other areas. For instance, the *C. n. yesoensis* population in Shiretoko (Hokkaido, Japan) declined from 592 deer in 1998 to 177 in 1999 (Kaji et al. 2004). In Kinkazan (northern Honshu, Japan), 309 out of 688 *C. n. centralis* individuals starved to death because of food shortages in the cold winter of 1984 (Takatsuki et al. 1994).

In northern Japan, severe winter food limits impact nutritional condition in deer, which leads to mass mortality (Yokoyama et al. 2000; Takahashi and Kaji 2001). In northern Honshu, *C. n. centralis* accumulate kidney fat in the summer and consume it in winter, gradually becoming thinner as winter progresses (Takatsuki 2000). However, no large fluctuations have ever been documented in the Kyushu *C. nippon* populations. For example, in *C. n. mageshimae*, which is endemic to Mageshima Island (south of Kyushu Island, Japan), mortality gradually increased and density reached 52 head/km^2, but the population has not crashed (Tatsuzawa 2004). In *C. n. yakushimae*, endemic to Yakushima Island (adjacent to Mageshima Island), density in the western coastal area increased to 161.2 head/km^2 (Kyushu Regional Environment Office 2013), but no mass mortality has been observed there despite a large decline in vegetation coverage (Koda et al. 2008).

In southern Japan including two islands in the Kyushu district (as mentioned above), deer nutritional status likely does not decrease because food plant resources remain available in winter (Takatsuki 2009b). However, this hypothesis has never been tested. In this study, we examined this hypothesis by measuring the seasonal changes in kidney fat index (Riney 1955) as an indicator of nutritional status in *C. nippon* (Maruyama 1985; Yokoyama et al. 2003). If the kidney fat index does not decrease in winter, the *C. n. yakushimae* population is expected to increase further, implying that it is necessary to implement measures that prevent the further loss of understory vegetation on Yakushima Island.

The *C. n. yakushimae* population density on Yakushima Island varies from 4.6 to 161.2 head/km^2 in different areas of the island (Kyushu Regional Environment Office 2013). The nutritional condition of individual deer is expected to vary with deer density because food plant availability is lower in higher density habitats. More specifically, we expected that deer nutritional condition would be low in high-density areas of Yakushima Island, where vegetation cover has been lost under heavy grazing and limited food availability (Onoda and Yahara 2015). Moreover, kidney fat index and the quantity and quality of rumen contents are likely associated, although the effect of the latter on the former may be delayed. We tested this hypothesis by examining rumen contents in terms of total weight, percentage nitrogen content, and the proportion of green to non-green leaves and addressed the following questions: (1) Are kidney fat index values low under high deer density and vice versa? (2) Are kidney fat index values lower in winter than in summer? (3) Are kidney fat index values correlated with the quantity and quality of rumen contents?

Materials and methods

Study animals

Yakushika, *Cervus nippon yakushimae*, is a subspecies endemic to Yakushima and Kuchinoerabu-jima Islands. It is the smallest of the seven *C. nippon* subspecies found in Japan (Takatsuki 1990). Its home range (17.6 ha for females and 65.9 ha for males) is also the smallest of any *C. nippon* subspecies (Kyushu Regional Forest Office 2012). *C. n. yakushimae* inhabits a wide area from the lowlands to the summits (1836 m) of Yakushima Island. *C. n. yakushimae* are browser in the lowlands and feed on evergreen trees and understory herbs (Takatsuki 1990; Terada et al. 2010; Agetsuma et al. 2011), whereas they are grazer in the highlands and feed on dwarf bamboo (*Pseudosasa owararii*) (Takatsuki 1990, 2009b). In the western part of Yakushima Island, 45.6–59.8% of a deer's diet comprises fallen leaves (Agetsuma et al. 2011). Kawamura et al. (2013) reported that *C. n. yakushimae* graze on grass at a town ranch in Koseda.

C. n. yakushimae abundance decreased as a result of overhunting in the 1950s, and the population size was estimated to be 1900–3000 during 1967–1969 (Kagoshima Nature Conservation Association, 1981). Thereafter, the population subsequently increased under protection. According to light censuses carried out at night in the western, northern, and eastern areas the number of sighted deer individuals increased from 2–4 head/km^2 in 1995 to > 33 in 2004 (Yahara 2006). According to daytime censuses, the estimated population size in the western area was 2.55 head/km^2 in 1988 and 40.74 in 2001 (Tsujino et al. 2004), 43–78 head/km^2 in 2001 (Agetsuma et al. 2003), 51.5–63.8 in 2002 (Tsujino and Yumoto 2004), and 95.70–111.65 in 2007 (Koda et al. 2011). According to a more recent estimation, local deer density varied among populations from 4.6 to 161.2 head/km^2, and total population size was 18,677 with a 95% confidence interval of 16,402–21,088 (Kyushu Regional Environment Office 2013). Additionally, according to Kawamura et al. (2013), average deer density at a town ranch in Koseda was 420 head/km^2, where *C. n. yakushimae* grazed on grass because the deer fence was not always effective in preventing them from entering the pasture.

Mean retention time through the entire digestive tract varies seasonally from 22.5 h in February to 45.7 h in July (Odajima et al. 1991). Therefore, the gastric contents of deer captured reflect consumption over for the previous few days.

Study area

Yakushima Island (30°20′N, 131°30′E) is 70 km south of Kyushu Island, Japan (Fig. 1). According to the re-
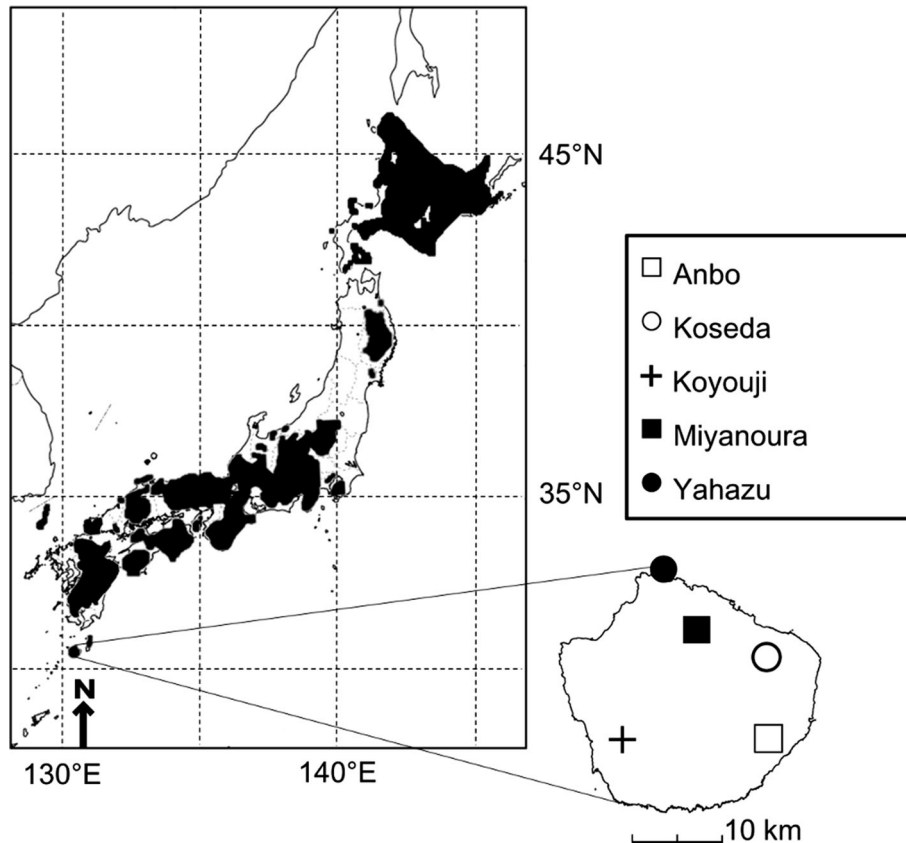
Fig. 1 *Cervus nippon* distribution in Japan and the sampling locations of *C. n. yakushimae* in Yakushima Island. The *black area* indicates the *C. nippon* range. *Redrawn* from a figure in Ministry of the Environment (2016)

cords from Koseda station located at 37.0 m asl (above sea level) on the eastern coast of Yakushima Island, the average temperature from 1981 to 2010 was 19.4 °C and the average annual total rainfall for the same period was 4477 mm (Japan Meteorological Agency, http://www.data.jma.go.jp/obd/stats/etrn/view/ nml_sfc_ym.php?prec_no=88&block_no=47836&year=&month=&day=&view=p1). The total area of the island is 504 km²; 107 km² of which was registered as a Natural World Heritage in 1993 by UNESCO. The island is mountainous and the highest peak reaches 1836 m asl. Altitudinal vegetation changes from broad-leaved evergreen forest in the lowlands, to conifer-broad leaf mixed forest in the middle elevation, conifer forest dominated by *Cryptomeria japonica* at higher elevations, and dwarf bamboo grassland at the summits (Yumoto 1987, 1988).

The sika deer, *C. n. yakushimae*, population has increased since the 1990s and caused serious damage to agriculture, forestry, and the natural forest ecosystem on Yakushima Island (Onoda and Yahara, 2015). To mitigate further damage, 3811 and 4556 individuals were culled by trapping and shooting in 2012 and 2013, respectively (Kyushu Regional Forest Office 2014).

We recorded the capture locations by GPS and determined the elevation and slope of each location using a 10-m digital elevation model (Geospatial Information Authority of Japan, Tsukuba, Japan 2005). The slope of

the capture location, as an indicator of habitat topography, was determined by considering the differences in home range area between the sexes; 300 m for females and 500 m for males (Kyushu Regional Forest Office 2012). First, we specified grids (10 × 10 m each) located within a circle centered at the capture location with a radius of either 300 m for females or 500 m for males. Second, for each of those grids, a slope value was determined as the maximum difference among nine grids in a 30 × 30 m area including the target grid and its eight neighboring grids. Finally, the slope values of all grids in a circle were averaged. All of the geoprocessing steps and analyses were performed in ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, CA, USA).

Kidney and rumen content sampling

We examined 74 culled deer individuals from five areas that differed in deer density: Anbo (7.1 and 6.9 head/km² in 2008–2009 and 2012, respectively), Miyanoura (40.1, 69.8), Koyouji (71.7, 161.2; both estimates from Kurio, a location near Koyouji), Yahazu Head (52.4 at Yahazu, 73.2 from Isso Forest Road near Yahazu), and Koseda (92.5, 93.5) (Fig. 1). To examine the sensitivity of our results to variability in deer density, we compared the results from statistical analysis using two sets of deer

density estimates from 2008 to 2009 and 2012. The culled individuals were hunted and provided by two hunting communities, Yakushima Forestry Ecosystem Conservation Center and Yakushima Forestry Administration Station. We recorded cull date, sex, and location, which we georeferenced using a Garmin GPS, eTrex Vista (Garmin Ltd., Olathe, KS, USA).

Deer density data were obtained from Kyushu Regional Environment Office (2013) and estimated by the improved pellet count method (Iwamoto et al. 2000) in winter. Pellets were counted along 220-m transects at each location (Dr. Shioya, Kagoshima Environmental Research and Service, pers. comm.). To determine deer nutritional condition, we collected kidney fat and stomach contents from culled deer within 3 h of death. The kidneys and surrounding fat were removed, measured, and weighed. We also weighed the rumen, sampled 20 g of the rumen contents, mixed it well by stirring with a spoon, and packed it in a zip-lock plastic bag. The samples were then stored at $-20\ °C$ until analysis.

Determining kidney fat index and food intake quantity and quality

For each kidney sample, both ends of the fat tissue were trimmed with scissors. The excised fat and kidney tissues were then weighed with an accuracy of 0.01 g. Riney's kidney fat index (RKFI; Riney 1955) was then calculated by dividing the weight of kidney fat remaining after trimming by kidney weight. The RKFI values of both right and left kidneys were measured and averaged for each individual. A higher RKFI value is considered an indicator of better nutritional status in deer.

To quantify food intake, we measured the dry weight of rumen contents. Total rumen content fresh weight was also obtained. A 50-g sample of rumen contents was transferred to a 50-ml centrifuge tube with a spoon, stored at $-20\ °C$ at the Kyushu University, Yakushima Island field station, freeze-dried (VD-250R; TAITEC Co., Saitama, Japan) in the laboratory, and weighed. Total rumen content dry weight was then calculated as follows: total fresh weight × dry:fresh weight ratio. Rumen content dry weight is referred to as food intake hereafter.

We measured nitrogen in the stomach contents as an indicator of nutritional value. The rumen contents were washed with distilled water to rinse off rumen bacteria, freeze-dried, and crushed with a TissueLyser (Qiagen, Hilden, Germany). The percentage of nitrogen was determined by the Service Centre of the Elementary Analysis of Organic Compounds, Faculty of Science, Kyushu University. We determined the percentage of non-green leaves as an indicator of food quality, because cellular nitrogen is transported to other organs during leaf senescence; therefore, non-green leaves have less nitrogen (Hörtensteiner and Feller 2002). The rumen contents were spread on the bottom of a container (445 × 325 × 70 mm) with 1-cm square grids (25 × 20 grids) and the leaf fragments were assigned one by one to squares. The green and non-green leaf fragments were then individually counted and the percentage of non-green leaf fragments was determined.

Statistical analysis

To determine if the RKFI values and the quantity and quality of rumen contents differed among the five study locations, we employed the Welch's ANOVA using an option that does not assume homogeneity of variances and made multiple comparisons following Holm (1979). To examine the relationship between food intake and either body weight or age, we employed a generalized linear mixed model (GLMM) with a normal distribution and an identity link function, where body weight and age (three categories; 1, 2, and 3+ years) were dependent variables and area was a random factor. We examined the relationship between RKFI and density using Spearman's rank correlation tests. To determine the factors that significantly affected RKFI, we employed a GLMM with a normal distribution and an identity link function with five dependent variables (sex, season, food intake, percentage of nitrogen, and percentage of non-green leaves) and area as a random factor. All of the statistical analyses were performed in R 3.3.1 (R Development Core Team, Vienna, Austria 2016). The GLMMs were fitted in R using the *lme4* package with the *glmer* function (Bates et al. 2014).

Results

Deer density and sampling location altitudes and slopes

On Yakushima Island, the deer sampling locations varied in altitude from 2 to 658 m (average ± SD; 125.88 ± 153.47 m) asl and the slopes ranged from 4.28 to 27.65 m (16.18 ± 5.47 m) (Table 1). Seventy-four culled deer comprised 36 male (body length, 108.80 ± 14.95 cm; body weight, 25.72 ± 11.30 kg) and 38 female individuals (body length, 106.97 ± 10.67 cm; body weight, 23.36 ± 7.52 kg); 31 individuals were collected in summer (June, July, and August) and 43 in winter (November, December, January, and February) (Table 2).

RKFI and rumen content quantity and quality

The average RKFI value varied from 22.91 ± 11.91 in Miyanoura to 76.23 ± 15.99 in Anbo (Fig. 2; Table 3). We found a significant difference in the average RKFI value among populations (Welch's ANOVA: $F = 7.9637$, $P < 0.01$); RFKI was significantly lower in Miyanoura than in either Anbo or Yahazu Head (pairwise *t* test: all $P < 0.01$; Table 3). There was no significant correlation between RKFI and density in either 2008–2009 or 2012 (Spearman's rank correlation test: $S = 72{,}052$, $P = 0.57$; $S = 72{,}083$, $P = 0.57$). Total rumen content fresh weight

Table 1 The geographical variable values for each sampling area

Area	Deer density (head km^{-2})	Elevation (m)			Slope (°)			Number of samples
		Range	Average	SD	Range	Average	SD	
Koyouji	161.2	238–658	440.86	168.99	16.03–25.67	21.12	3.57	6
Koseda	93.5	36–112	96.29	20.97	5.00–14.20	11.87	2.74	21
Yahazu Head	73.2	2.00	2.00	0.00	17.48	17.48	0.00	16
Miyanoura	69.8	9–390	139.17	123.81	7.13–27.65	18.81	6.44	23
Anbo	6.9	8–607	135.88	196.51	4.28–22.34	12.71	5.45	8
Total		2–658	125.88	153.47	4.28–27.65	16.18	5.47	74

varied from 0.51 to 3.51 kg, and differed significantly among populations (Welch's ANOVA: $F = 4.148$, $P < 0.01$); this was significantly higher in Yahazu Head than in Koseda, Koyouji, and Miyanoura (pairwise t test: all $P < 0.05$, Table 4). Food intake did not differ significantly between summer and winter (Welch's t test: t value $= -0.18$, $P = 0.43$). Food intake did not correlate with body weight (GLMM: $\beta \pm SE = 0.0060 \pm 0.0090$, t value $= 0.66$, $P = 0.51$) or age (GLMM: $\beta \pm SE = 0.14 \pm 0.082$, t value $= 1.67$, $P = 0.10$). The ratio of dry to fresh weight ranged from 0.34 to 0.49, and total dry weight varied from 0.2 to 1.71 kg. Nitrogen percentage varied from 1.98 to 3.33, and differed significantly among populations (Welch's ANOVA: $F = 3.2262$, $P = 0.03$), and it was significantly higher in Yahazu Head than in Miyanoura (pairwise t test: $P < 0.01$, Table 4). The percentage of non-green leaves varied from 1.0 to 88.1, differed significantly among populations (ANOVA: $F = 5.7515$, $P < 0.01$), and was significantly higher in Yahazu Head and Miyanoura than in Koseda and Anbo (pairwise t test: all $P < 0.05$, Table 4).

Variables affecting RKFI

Both food intake and RKFI exhibited an L-shaped relationship in Koseda; nine individuals with a food intake of < 0.81 exhibited highly variable RKFI values, and five of nine had RKFI values of > 50. However, in 12 individuals that exhibited a food intake ranging from 0.93 to 2.67, RKFI was < 50 (Fig. 3). The relationship between food intake and RKFI was not significant if we included the data from Koseda ($\beta \pm SE = 0.14 \pm 0.17$, t value $= 0.832$, $P = 0.405$). However, we found a common significant relationship in the other four study locations: of the five variables in the GLMM, food intake had a significant positive effect ($\beta \pm SE = 0.53 \pm 0.14$, t value $= 3.70$, $P < 0.01$; Fig. 4), while the other four variables (sex, season, percentage of nitrogen, and percentage of non-green leaves) had no significant effects according to the GLMM analysis (Fig. 5; Table 5).

Discussion

The key finding of this study is that food intake was the only candidate factor that correlated with RKFI. Neither density nor season significantly affected RKFI. The correlation between food intake and RKFI was not significant. However, it was significant in the other four areas when Koseda where food intake and RKFI exhibited an L-shaped relationship was excluded. In Koseda, deer can graze pasture grass in the town ranch (Kawamura et al. 2013). Therefore, deer individuals with low food intake but high RKFI values may have grazed in nutrient-rich pasture grass. There was no significant correlation between nitrogen content and RKFI in this study, suggesting that fat storage in the kidneys is highly dependent on carbohydrate availability.

We expected RKFI values to be lower in higher deer density areas where understory plants have been heavily browsed (e.g., Koseda and Yahazu); however, there was no significant correlation between deer density and RKFI. The average RKFI value at each location exceeded a threshold value of 20, below which the nutritional condition of *C. nippon* is considered poor (Maruyama 1985; Torii and Fujishita 1998). This threshold value is based on the relationship between RKFI and femur marrow fat (FMF) percentage: the percentage of FMF remains above 50 when RKFI > 20, but rapidly decreases when RKFI < 20, suggesting that both kidney and femur marrow fats are consumed if RKFI < 20 (Torii and Fujishita 1998). Our finding that the average RKFI values exceeded 20 in all five locations indicates that none of the five populations, including the high density populations in Koseda and Yahazu, are malnourished. In fact, fresh rumen contents in Koseda and Yahazu weighed as much as 2.3 and 3.8 kg, respectively, indicating that deer individuals in those populations are getting good amounts of plant food.

Season was not significantly correlated with RKFI values. These results are consistent with our expectation that, even in winter, the nutritional status does not decrease in southern Japan, including Yakushima Island. This expectation is based on the fact that most understory plants are evergreen in the lower elevations of Yakushima Island and *C. n. yakushimae* can consume them during winter (Takatsuki 2009b). A high, stable supply of evergreen plants is also available throughout the year in Chiba Prefecture, central Japan (Asada and Ochiai 1996) and Yamaguchi Prefecture, western Japan (Jayasekara and Takatsuki 2000).

However, the evergreen plants consistently declined under continued heavy browsing pressure in areas with high deer density such as Koseda and Yahazu. *C. n.*

Table 2 Collection data for culled deer (*Cervus nippon yakushimae*)

Area	Latitude	Longitude	Deer density in 2012 (head km^{-2})	Season	Sex	Age	Length (cm)	Weight (kg)
Koyouji	30.39503	130.5509	161.2	Summer	Female	3 +	116.5	30.3
Koyouji	30.29403	130.4481	161.2	Summer	Female	1	97	15
Koyouji	30.29194	130.4302	161.2	Summer	Female	2	108	24.6
Koyouji	30.29411	130.4236	161.2	Summer	Female	2	112	23.4
Koyouji	30.29411	130.4236	161.2	Summer	Female	1	95	26.5
Koyouji	30.29416	130.4479	161.2	Summer	Female	1	108	19.5
Koseda	30.38011	130.6409	93.5	Summer	Male	1	88	17
Koseda	30.38253	130.6421	93.5	Summer	Female	1	103	20
Koseda	30.38011	130.6409	93.5	Summer	Female	1	109	18.2
Koseda	30.37995	130.6415	93.5	Summer	Female	1	94	10
Koseda	30.37976	130.6421	93.5	Summer	Male	3 +	108	20
Koseda	30.37976	130.6421	93.5	Summer	Male	1	94	13
Koseda	30.37995	130.6415	93.5	Summer	Female	1	88	10
Koseda	30.38232	130.642	93.5	Summer	Female	1	94	12
Koseda	30.38253	130.6421	93.5	Summer	Male	1	89	12
Koseda	30.37573	130.6612	93.5	Summer	Male	3 +	135	49
Miyanoura	30.43631	130.5542	69.8	Summer	Female	3 +	113	23.5
Miyanoura	30.43611	130.5544	69.8	Summer	Female	1	98	15.5
Miyanoura	30.41121	130.526	69.8	Summer	Male	1	103	16.6
Miyanoura	30.40169	130.5118	69.8	Summer	Male	1	93.5	14.6
Miyanoura	30.41439	130.5399	69.8	Summer	Female	1	96	12.2
Miyanoura	30.41176	130.531	69.8	Summer	Male	1	90.5	10.9
Miyanoura	30.4032	130.519	69.8	Summer	Female	1	98.5	14.4
Anbo	30.31642	130.646	6.9	Summer	Female	3 +	120	41.5
Anbo	30.3147	130.6469	6.9	Summer	Female	3 +	112	31
Anbo	30.31466	130.6469	6.9	Summer	Male	3 +	127	44.5
Anbo	30.3067	130.6374	6.9	Summer	Male	1	110	27
Anbo	30.30229	130.6424	6.9	Summer	Female	3 +	114	28
Anbo	30.29397	130.6352	6.9	Summer	Female	2	104	23.5
Anbo	30.28032	130.6239	6.9	Summer	Male	3	131.5	54
Anbo	30.28434	130.6018	6.9	Summer	Male	1	96.5	13.6
Koseda	30.38162	130.6353	93.5	Winter	Male	1	107	19
Koseda	30.38162	130.6353	93.5	Winter	Female	3 +	120	30
Koseda	30.38162	130.6353	93.5	Winter	Female	3 +	125	30
Koseda	30.38162	130.6353	93.5	Winter	Female	3 +	120	31
Koseda	30.38162	130.6353	93.5	Winter	Female	1	91	16
Koseda	30.38162	130.6353	93.5	Winter	Male	1	91	16
Koseda	30.38162	130.6353	93.5	Winter	Female	3 +	117	28
Koseda	30.38162	130.6353	93.5	Winter	Male	3 +	121	34
Koseda	30.38162	130.6353	93.5	Winter	Male	3 +	111	32
Koseda	30.38162	130.6353	93.5	Winter	Male	2	110	25
Koseda	30.38357	130.6405	93.5	Winter	Female	2	120	27
Miyanoura	30.40159	130.5122	69.8	Winter	Male	3 +	129	40
Miyanoura	30.41179	130.5604	69.8	Winter	Male	3 +	123	32
Miyanoura	30.41459	130.5333	69.8	Winter	Male	2	119	28
Miyanoura	30.40785	130.5199	69.8	Winter	Female	1	94	17
Miyanoura	30.41446	130.5731	69.8	Winter	Male	1	94	14
Miyanoura	30.40985	130.5488	69.8	Winter	Female	1	88	11
Miyanoura	30.41075	130.5499	69.8	Winter	Male	1	94	14
Miyanoura	30.40914	130.5482	69.8	Winter	Male	3 +	125	30
Miyanoura	30.41021	130.5509	69.8	Winter	Male	2	115	24
Miyanoura	30.4047	130.5169	69.8	Winter	Female	1	89	10
Miyanoura	30.41026	130.525	69.8	Winter	Female	1	93	10.5
Miyanoura	30.40899	130.5651	69.8	Winter	Male	2	99	23.5
Miyanoura	30.41102	130.5508	69.8	Winter	Male	3 +	121	31
Miyanoura	30.41099	130.5509	69.8	Winter	Male	3 +	122	30
Miyanoura	30.40914	130.5482	69.8	Winter	Female	2	108	20
Miyanoura	30.40978	130.5653	69.8	Winter	Male	1	83	10.5
Yahazu	30.46372	130.4959	73.2	Winter	Male	2	110	25.6
Yahazu	30.46372	130.4959	73.2	Winter	Male	3 +	122	38.2
Yahazu	30.46372	130.4959	73.2	Winter	Female	2	113	26.2
Yahazu	30.46372	130.4959	73.2	Winter	Female	1	91	14.4
Yahazu	30.46372	130.4959	73.2	Winter	Female	2	109	27.6
Yahazu	30.46372	130.4959	73.2	Winter	Male	2	110	20.7
Yahazu	30.46372	130.4959	73.2	Winter	Male	3 +	130	39.7
Yahazu	30.46372	130.4959	73.2	Winter	Male	1	80	11

Table 2 continued

Area	Latitude	Longitude	Deer density in 2012 (head km^{-2})	Season	Sex	Age	Length (cm)	Weight (kg)
Yahazu	30.46372	130.4959	73.2	Winter	Female	2	94	19.8
Yahazu	30.46372	130.4959	73.2	Winter	Female	2	107	19.6
Yahazu	30.46372	130.4959	73.2	Winter	Male	2	100	24.5
Yahazu	30.46372	130.4959	73.2	Winter	Male	3+	115	33
Yahazu	30.46372	130.4959	73.2	Winter	Male	3+	120	38
Yahazu	30.46372	130.4959	73.2	Winter	Female	3+	113	27
Yahazu	30.46372	130.4959	73.2	Winter	Female	2	105	20
Yahazu	30.46372	130.4959	73.2	Winter	Female	3+	116	27.5

Summer: June, July, and August. Winter: November, December, January, and February
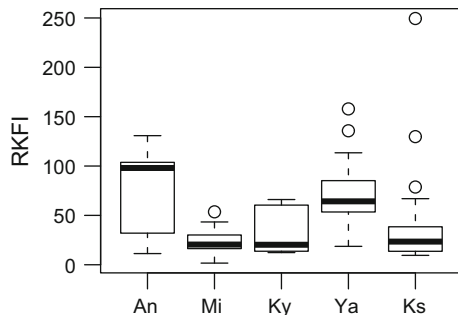
Fig. 2 Variation in Riney's kidney fat index in *Cervus nippon yakushimae* at five locations on Yakushima Island (Anbo: An, Miyanoura: Mi, Koyouji: Ky, Yahazu: Ya, Koseda: Ks). *Each box* indicates an interquartile range (IQR) with a median, a *pair of whiskers* indicate a range of IQR ± 1.5 IQR, and *open circles* represent outliers

Table 3 The Riney's kidney fat index (RKFI) values at each sampling location

Area	Deer density (head km^{-2})	RKFI Range	Average	SD
Koyouji	161.2	2.00–66.05	32.16[ab]	22.30
Koseda	93.5	9.49–249.50	42.94[ab]	55.73
Yahazu Head	73.2	18.63–157.81	71.16[a]	38.17
Miyanoura	69.8	1.67–53.50	22.91[b]	12.19
Anbo	6.9	11.31–130.78	76.2[a]	45.23
Total		1.67–249.50	45.54	42.80

[a] and [b] represent significant differences in multiple comparison tests ($P < 0.05$)

yakushimae had RKFI values >20; however, how the deer obtain nutritious food in these areas remains unclear (Table 4). We suggest that the deer populations might obtain sufficient nutrition from fallen leaves in high deer density areas, as has been previously reported (Takahashi and Kaji 2001; Miyaki and Kaji 2004). Consumption of fallen leaves in high density areas has been observed in Toya Lake, Hokkaido (Takahashi and Kaji 2001; Miyaki and Kaji 2004). In the western part of Yakushima Island, 45.6–59.8% of the deer diet comprises fallen leaves (Agetsuma et al. 2011). In our results, the average proportion of non-green leaves in the rumen contents (Table 4) varied from 30% in Anbo, where deer density was the lowest (6.9 head/km^2), to 58% in Yahazu Head, where deer density was as high as 73.2 head/km^2, and the difference between these two populations was significant. We therefore conclude that the high density deer population in Yahazu Head is more dependent on fallen leaves than that in Anbo. In the Miyanoura deer population, the percentage of non-green leaves in the rumen contents was as high as that in Yahazu Head (55%, Table 4). However, the RKFI values and nitrogen percentage were significantly lower than in Yahazu Head (Table 3). These results suggest that not only quantity of fallen leaves but also quality of leaves that vary among plant species may affect the value of RKFI. Variation in the species composition of diets between localities, may explain why neither the percentage of non-green leaves nor nitrogen was significantly correlated with RKFI. DNA analysis, e.g., DNA barcoding, of rumen contents could be useful to identify the plant species composition of the deer diet.

Table 4 The rumen content variable values at each sampling location

Area	Deer density (head km^{-2})	Intake (kg) Range	Average	SD	Nitrogen content (%) Range	Average	SD	Non-green leaves (%) Range	Average	SD
Koyouji	161.2	0.21–1.94	1.13[b]	0.41	1.89–2.77	2.52[ab]	0.30	8.0–75.0	40.67[ab]	25.06
Koseda	93.5	0.63–2.67	1.25[b]	0.64	1.98–3.23	2.44[ab]	0.37	3.0–71.0	33.76[b]	15.67
Yahazu Head	73.2	0.63–3.51	1.89[a]	0.86	2.10–3.33	2.76[a]	0.38	26.0–82.0	58.38[a]	18.43
Miyanoura	69.8	0.29–2.40	1.10[b]	0.65	1.51–2.98	2.32[b]	0.33	1.0–88.0	53.78[a]	22.33
Anbo	6.9	0.62–1.88	1.23[ab]	0.41	1.75–3.01	2.46[ab]	0.46	4.0–82.0	30.00[b]	26.31
Total		0.21–3.51	1.33	0.71	1.98–3.33	2.48	0.39	1.0–88.0	45.26	25.03

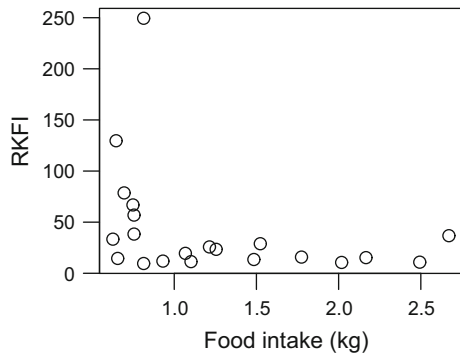[a] and [b] represent significant differences by multiple comparison tests ($P < 0.05$)

Fig. 3 Relationship between *Cervus nippon yakushimae* Riney's kidney fat index and food intake in Koseda
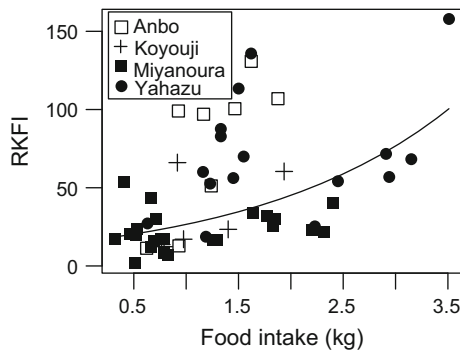

Fig. 4 Relationship between *Cervus nippon yakushimae* Riney's kidney fat index (RKFI) and food intake in four locations. The regression line is based on the generalized linear mixed model analysis with a logarithmic transformation of RKFI values
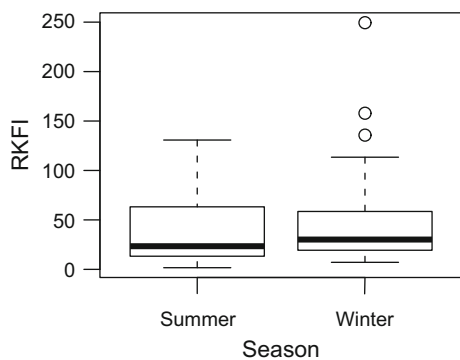

Fig. 5 Variation in *Cervus nippon yakushimae* Riney's kidney fat index in Summer (June, July, and August) and Winter (November, December, January, and February). *Each box* indicates an interquartile range (IQR) with a median, a *pair of whiskers* indicate a range of IQR $\pm$ 1.5 IQR, and *open circles* represent outliers

It is worth noting that food intake varied, even among individuals in the same area (Fig. 3), whereas the total rumen content fresh weight was correlated with neither body weight nor age. This finding suggests that food selectivity differs between individuals. Bergvall (2007) examined fallow deer feeding selectivity for food with tannins and found that tannin intake differed significantly among ten individuals. Further studies are

Table 5 Generalized linear mixed model (GLMM) results

	Estimate	SE	*t* value	*P*
(Intercept)	2.75	0.66	4.17	*< 0.001*
Sex	0.10	0.21	0.50	0.61
Season	0.12	0.37	0.32	0.74
Non-green leaves (%)	0.0080	0.0044	0.20	0.84
Nitrogen content (%)	−0.034	0.031	−0.112	0.91
Intake	0.53	0.14	3.70	*< 0.001*

We employed GLMM with five dependent variables (sex, season, food intake, nitrogen percentage, and percentage of non-green leaves), Riney's kidney fat index (RKFI) was the response variable, and area was a random factor, using a normal distribution with a log link function

Significant effects are shown in italics

needed to determine if the *C. n. yakushimae* population exhibit any selectivity based on food type.

In conclusion, our findings show that *C. n. yakushimae* is not in a poor nutritional condition despite living in a high density of > 70 head/km^2; this is likely because of the high plant productivity on Yakushima Island. Furthermore, this result indicates that the *C. n. yakushimae* population will likely continue to increase and, thus, is expected to further reduce the understory vegetation on Yakushima Island. Therefore, implementing a strategic management program for both deer population size and the occurrence of threatened plant species in the Yakushima Island should be a priority as suggested by Fujimaki et al. (2016).

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