

## Comparison of Humoral Immune Response Under Low Temperature in Non-breeding Gray Red-backed Voles (*Myodes rufocanus*)

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### Summary

Effects of low temperature on humoral immune function were investigated in non-breeding gray red-backed voles (*Myodes rufocanus*). Twenty-three non-breeding voles were divided into two groups (low-temperature group (5°C,  $n = 12$ ) and room-temperature group (23°C,  $n = 11$ )) with a 12L:12D light cycle. The low-temperature group showed inferior humoral immune responses against a non-pathogenic antigen, sheep red blood cells (SRBCs), compared to the room-temperature group. Food intakes of the low-temperature group increased in comparison with those of the room-temperature group, and fat reserves of the low-temperature group reduced. Kidneys and hearts of the low-temperature group were hypertrophied by approximately 20-25% in comparison with the room-temperature group. These results could be considered as a trade-off between thermoregulation and immunity. Effects of aggregation on humoral immune function were also investigated in non-breeding gray red-backed voles (*M. rufocanus*). Eighteen non-breeding voles were divided into solitary ( $n = 9$ ) and aggregation ( $n = 9$ ) groups. There were no significant effects of aggregation on humoral immune responses of the non-breeding gray red-backed voles (*M. rufocanus*) with a 10L:14D light cycle at 5°C. Effects of a short photoperiod were suggested by a comparison of the humoral immune responses in non-breeding gray red-backed voles (*M. rufocanus*) exposed to low temperature (5°C) under a 10L:14D day length or a 12L:12D day length. The humoral immune responses of the voles with a 10L:14D light cycle were significantly higher than those of the voles with a 12L:12D light cycle at low temperature (5°C). These results suggest that the reduced humoral immune function caused by cold stress could be compensated by short photoperiods.

**Key words:** vole, immune response, photoperiod, aggregation, low temperature

### Introduction

Most of the gray red-backed vole (*Myodes rufocanus*) populations show a density-dependent population decline in winter<sup>24),25),27),28),29)</sup>. In order to explain several factors of the density-dependent population decline in winter, the physiological functions of gray red-backed voles (*M. rufocanus*) were investigated under laboratory conditions<sup>15),16),17)</sup>. According to recent studies, laboratory mice<sup>2)</sup> and gray red-backed voles (*M. rufocanus*)<sup>16)</sup> exposed to low temperature (5°C) showed inferior immune response against SRBCs (sheep red blood cells) to those kept at room temperature (23°C) on a 12L:12D cycle. Immune function competes with other various functions such as thermoregulation for

available resources, and a trade-off exists between thermoregulation and immunity.

Physiological and behavioral adaptations have evolved in nontropical animals to cope with winter conditions because thermoregulatory demands increase when food availability decreases<sup>22</sup>. Immune function is influenced by photoperiods in deer mice (*Peromyscus maniculatus*)<sup>4,22</sup> and in common voles (*Microtus arvalis*)<sup>6</sup>. Adult deer mice (*P. maniculatus*) on an 8L:16D cycle at both room temperature (20°C) and low temperature (8°C) displayed superior immune responses (IgG) in comparison with those on a 16L:8D cycle at low temperature (8°C). Animals on an 8L:16D cycle at low temperature (8°C) showed serum IgG levels which were comparable to those on a 16L:8D cycle at room temperature (20°C)<sup>4</sup>. Furthermore, adult deer mice (*P. maniculatus*)<sup>1</sup> and male common voles (*M. arvalis*)<sup>6</sup> on an 8L:16D cycle showed a greater number of white blood cells than animals on a 16L:8D cycle. In addition, adult deer mice (*P. maniculatus*) on an 8L:16D cycle have higher lymphocyte numbers<sup>1</sup>. Therefore, reductions of immune function caused by cold stress could be compensated by short photoperiods<sup>4,22</sup>.

Arvicoline rodents form communal groups because of environmental stress in winter and/or high population densities<sup>9,12,31</sup>. Winter aggregation in arvicoline rodents might significantly affect their maintenance of body temperature. Does the aggregation display a positive effect on immune function in winter? It is necessary to investigate effects of aggregation on immune function under cold stress conditions. In order to understand physiological reactions and population ecology of the gray red-backed vole (*M. rufocanus*) in winter, laboratory experiments have to be performed on the basis of winter conditions (e.g. temperature and photoperiod).

The first aim of this study was to investigate effects of cold stress on humoral immune responses in gray red-backed voles (*M. rufocanus*). The second aim of this study was to investigate effects of aggregation on humoral immune responses under cold stress conditions; positive effects on the maintenance of body temperature were expected because of communal groups during winter in gray red-backed voles (*M. rufocanus*).<sup>12</sup> The last aim of this study was to describe the possibility of effects of a short photoperiod on immune function at low temperature; data on the immune function of gray red-backed voles (*M. rufocanus*) on a 10L:14D cycle and those on a 12L:12D cycle were compared. These experiments would provide valuable knowledge in order to understand the adaptive physiological reactions of the gray red-backed vole (*M. rufocanus*) in winter.

## Materials and Methods

### 1. Animals and maintenance

All gray red-backed voles (*Myodes rufocanus*) used in this study were collected from a wind shelterbelt in Furen, Hokkaido, Japan (44° 18'N, 142° 25'-142° 28'E) by using Sherman-type live traps. Twenty-three non-breeding voles were caught in September 2003 and were used in experiment 1. Eighteen non-breeding voles were caught in September and in October 2006 and were used in experiment 2.

All animals were housed individually in transparent polymer X (TPX) cage (145 by 290 by 150 mm, SKL-109; Toyoriko, Japan) with 250 ml bedding materials (ALPHA-dri™; Shepherd, USA) under around 23°C with natural light and were allowed free access to water and commercial food (ZC-2 (Funabashi Farms, Chiba, Japan) in experiment 1, ZF (Oriental Yeast, Tokyo, Japan) in experiment 2), and a handful of oats and sunflower seeds until the beginning of experiments after capture. The difference of these commercial foods (ZC-2 and ZF) used in experiment 1 and 2 was caused by sales stop of ZC-2.

## 2. Experimental protocol

All animals were kept in incubators (FMU-130I; FUKUSIMA, Japan). All animals were housed individually in a transparent polymer X (TPX) cage (145 by 290 by 150 mm, SKL-109; Toyoriko, Japan) with 250 ml bedding materials (ALPHA-dri™; Shepherd, USA). All the housed cages were cleaned, and the bedding materials were changed every day. All voles were allowed free access to commercial foods (ZC-2, ZF) and water throughout the experiments. On the 10th day of each experiment, the voles of all groups were immunized with the non-pathogenic antigen, sheep red blood cells (SRBCs), as described below. Antibody production against SRBCs (IgM) was measured according to the standard method by Hemagglutination test<sup>11,15</sup>.

### *Experiment 1:*

Experiment 1 was performed from September to October in 2003. Twenty-three wild voles were divided into low temperature group (WLT;  $n = 12$ , 25.0g - 35.7g) and room temperature group (WRT;  $n = 11$ , 25.8g - 34.7g), after acclimatization to the laboratory. Voles in the low temperature group (WLT) were maintained at 5°C according to Cichoń et al.<sup>2</sup>. Voles in the room temperature group (WRT) were maintained at 23°C, because 23°C can be regarded as the optimal temperature for immune response in gray red-backed voles (*M. rufocanus*)<sup>15</sup>.

### *Experiment 2:*

Experiment 2 was performed from September to October in 2006. Eighteen non-breeding voles were divided into two groups (solitary group (SOL;  $n = 9$ , 21.9g - 28.1g) consisted of one vole per cage and aggregation group consisted of three voles per cage (AGG;  $n = 9$ , 19.1g - 24.7g)) and maintained at 5°C on a 10L:14D cycle (lights on at 0700) designed according to the shortest day length (9L:15D cycle) at the winter solstice on December in Hokkaido.

### *In comparison between experiment 1 and experiment 2:*

Two groups, that is, one group maintained at 5°C on a 12L:12D cycle (WLT;  $n = 12$ , 25.0g - 35.7g) and the other group maintained at 5°C on a 10L:14D cycle (SOL;  $n = 9$ , 21.9g - 28.1g) were compared for immune response, body weight, food intake, weights of internal organs, weights of lymphatic organs, and Riney's Kidney Fat Index.

## 3. Body weights, weights of internal organs, lymphatic organs, and food remains, and Riney's Kidney Fat Index

All voles were weighed daily to the nearest 0.1g by an electronic balance. Their in-

ternal and lymphatic organs were weighed in experiment 1 and 2. Liver, kidney and heart were weighed with accuracy to 0.01g in experiment 1 and 2. The lymphatic organs (thymus and spleen) were weighed with accuracy to 1mg in experiment 1 and 2.

Riney's Kidney Fat Index (Riney's KFI) was measured to compare fat reserves in experiment 1 and 2. Food remains were collected daily and were measured after drying at 105°C for 24h in experiment 1 and 2. Food intakes were calculated for 8th-9th days and for 14th-15th days in two groups of experiment 1 and in solitary group of experiment 2, because it was impossible to collect food remains of aggregation group. Those days correspond to the day just before the immunization and the final day of the experiments, respectively. Those days may represent adequate body conditions of voles before and after the immunization treatments.

#### 4. Immunization

The standard non-pathogenic antigen-sterile SRBCs (Inter-Cell Technologies, Florida, America) with 20  $\mu\text{lg}^{-1}$  body weight were injected into peritoneal cavity on the 10th day of the experiments. SRBCs were adjusted to  $6 \times 10^6$  cells  $\text{mm}^{-3}$  in phosphate-buffered saline. On the 6th day after immunization<sup>15)</sup>, the voles were killed by cervical dislocation. Blood collected from their heart using 1 ml or 2 ml syringes was incubated at 37°C for 2 h, and kept at room temperature for 1 h. The blood was centrifuged at 2,500 rpm for 15 min, and the serum was collected. The test serum was heat-inactivated at 56°C for 30 min. Antibody (IgM) production against SRBCs was assessed by the standard method by hemagglutination test<sup>11),15)</sup>. The simplest form of this test involves the aggregation of erythrocytes (as antigens) by increasing dilutions of anti-erythrocyte sera.

#### 5. Hemagglutination test

A hemagglutination was performed with 96 well plates to measure antibody production against SRBCs. First, 20  $\mu\text{l}$  of phosphate-buffered saline (PBS) was added to all the test wells. Second, each test serum was made with a 1: 2 dilution and a 1: 2 dilution with each test serum line was repeated to the end well. Third, 20  $\mu\text{l}$  of 1% SRBCs suspension was added to all the used wells. When the blood cells in normal serum fall to the bottom of the wells, titres of the antibody agglutination are estimated. The number of titres showing positive hemagglutination represents antibody production<sup>11)</sup>. Titres refer to  $\log_2$  antibody concentrations.

#### 6. Statistical analyses

In most analyses, T-test and Paired T-test were used to compare between the two when there were no correlation with body weights. ANCOVA was used for the data that correlated with body weights.

Table 1. Comparison of titres measured in Hemagglutination test between low-temperature group voles and room-temperature group voles.

Factor	<i>df</i>	<i>F</i> -value	Prob.	adjusted $R^2$
Full Model	2	3.972	0.035	0.213
Group	1	6.973	0.016	
Body weight	1	0.311	0.583	

The following numbers of voles were examined: 12 low-temperature group voles and 11 room-temperature group voles in experiment 1.

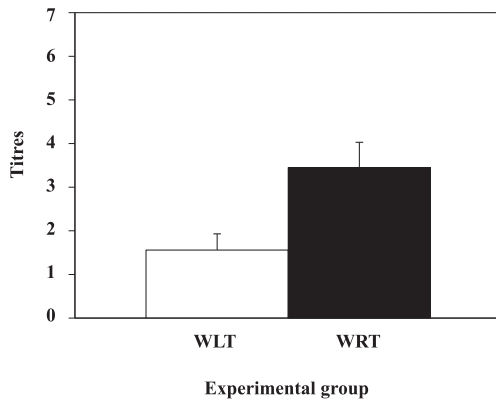


Fig. 1. Titres of immune response against sheep red blood cells (SRBCs) in gray red-backed voles (*Myodes rufocanus*) exposed to cold stress (5°C) on a 12L:12D cycle in experiment 1. White bar: low-temperature (5°C) group (WLT) kept on a 12 L:12D cycle; black bar: room-temperature group (23°C) kept on a 12L:12D cycle.

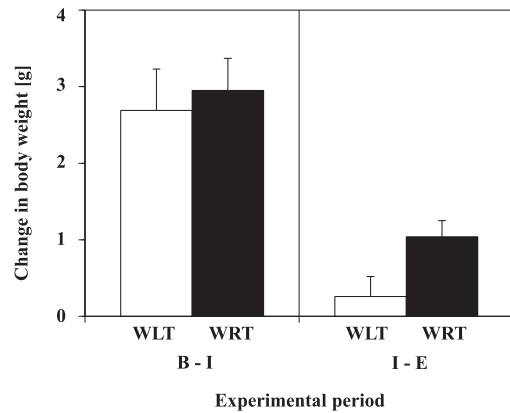


Fig. 2. Change in body weight of gray red-backed voles (*Myodes rufocanus*) during the 16-day experimental period (B-I: 10 days from the beginning of the experiment to immunization, I-E: 6 days from immunization to the end of the experiment). White bar: low-temperature group voles (WLT) exposed to 5°C on a 12L:12D cycle; black bar: room-temperature group voles (WRT) exposed to 23°C on a 12L:12D cycle.

## Results

### 1. Experiment 1

#### *Immune response and body weight:*

The wild voles exposed to low temperature (WLT;  $n = 12$ ) showed inferior immune response in comparison with the wild voles at room temperature (WRT;  $n = 11$ , Fig. 1, Table 1). The difference in titres between the groups was large, that is, the mean titres (SE) of WLT and WRT were 1.56 (0.37) and 3.45 (0.58), respectively (ANOVA;  $F = 7.89$ ,  $P = 0.011$ ).

Body weights were adjusted to be even between the groups (WRT and WLT) at the beginning of the experiment (the 1st day, mean  $\pm$  SE [g];  $30.0 \pm 0.94$  for WRT,  $29.8 \pm 0.90$  for WLT. T-test;  $t = 0.17$ ,  $P = 0.864$ ). Body weight of WRT significantly increased during the experimental period (Fig. 2): from  $30.0 \pm 0.94$  (mean  $\pm$  SE [g]) to  $33.0 \pm 1.11$  for the first 10 days (Paired T-test;  $t = 7.06$ ,  $P < 0.001$ ); from  $33.0 \pm 1.11$  to  $34.0 \pm 1.20$  for 6 days after immunization (Paired T-test;  $t = 5.01$ ,  $P < 0.001$ ). Body weight of WLT also in-

Table 2. Weights of Riney's KFI, internal and lymphatic organs of wild voles for the two experimental groups (room-temperature [WRT] and low-temperature [WLT] group).

	Group				ANCOVA	
	WRT [Mean±SE]	<i>n</i>	WLT [Mean±SE]	<i>n</i>	<i>F</i> -value	<i>P</i>
RKFI [%]	32.8±3.79	11	16.9±2.87	12	10.91	0.004
Kidney [g]	0.17±0.01	11	0.21±0.01	12	17.06	< 0.001
Liver [g]	1.76±0.11	11	1.83±0.11	12	2.468	0.132
Heart [g]	0.15±0.01	11	0.18±0.01	12	21.67	< 0.001
Spleen [mg]	56±6.1	11	60±6.4	12	1.266	0.274
Thymus [mg]	15±2.4	11	12±3.9	12	0.105	0.75

Those weights were compared between experimental groups by ANCOVA using body weight as covariance. Effects of body weight were significantly positive ( $P < .05$ ) for the index and all organs. *F*-value and probability (*P*) indicate effects of experimental group.

creased from  $29.8 \pm 0.90$  to  $32.5 \pm 1.18$  for the first 10 days (Paired T-test;  $t = 4.94$ ,  $P < 0.001$ ), but that did not change thereafter ( $32.7 \pm 1.23$  at the end of the experiment, Paired T-test;  $t = 1.01$ ,  $P = 0.336$ ). In spite of the difference in the pattern of body weight changes between WRT and WLT, the differences in body weights at the immunization (T-test;  $t = 0.29$ ,  $P = 0.771$ ) and at the end of the experiment (T-test;  $t = 0.73$ ,  $P = 0.474$ ) were not statistically significant.

#### Food intake:

Food intake of WLT was significantly higher than that of WRT in the both period of 8th-9th days and 14th-15th days (8th-9th days [before immunization], mean  $\pm$  SE [g];  $6.43 \pm 0.21$  for WRT,  $9.80 \pm 0.29$  for WLT, T-test,  $t = 8.99$ ,  $P < 0.001$ ; 14th-15th days [after immunization],  $6.01 \pm 0.25$  for WRT,  $9.16 \pm 0.26$  for WLT,  $t = 8.68$ ,  $P < 0.001$ ). Food intakes of wild voles decreased from 8th-9th days to 14th-15th days (Paired T-test;  $t = 3.24$ ,  $P = 0.012$  for WRT;  $t = 4.05$ ,  $P = 0.002$  for WLT).

#### Riney's KFI, internal and lymphatic organs:

Riney's KFI of WLT was significantly lower than that of WRT (Table 2). Weights of kidney and heart in WLT were heavier than those of WRT, but liver weights of WLT were similar to those of WRT (Table 2). There were no significant differences of lymphatic organs (spleen and thymus) weights between WRT and WLT (Table 2).

## 2. Experiment 2

#### Immune response and body weight:

In immune response, there was not a significant difference between solitary group

Table 3. Comparison of titres measured in Hemagglutination test between solitary group voles and aggregation group voles.

Factor	<i>df</i>	<i>F</i> -value	Prob.	adjusted R <sup>2</sup>
Full Model	2	0.629	0.547	-0.046
Group	1	0.467	0.505	
Body weight	1	0.438	0.518	

The following numbers of voles were examined: 9 solitary group voles and 9 aggregation group voles.

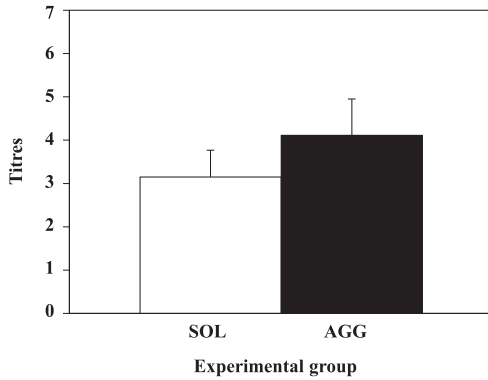


Fig. 3. Titres of immune response against sheep red blood cells (SRBCs) in gray red-backed voles (*Myodes rufocanus*) exposed to cold stress (5°C) on a 10L:14D cycle in experiment 2. White bar: solitary group (SOL) exposed to low temperature (5°C) on a 10L:14D cycle; black bar: aggregation group (AGG) exposed to low temperature (5°C) on a 10L:14D cycle.

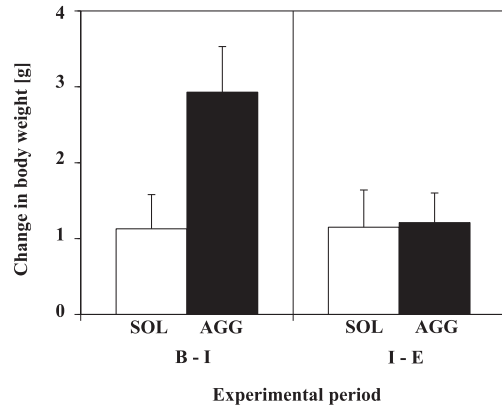


Fig. 4. Change in body weight of gray red-backed voles (*Myodes rufocanus*) during the 16-day experimental period (B-I: 10 days from the beginning of the experiment to immunization, I-E: 6 days from immunization to the end of the experiment). White bar: solitary group voles (SOL) exposed to 5°C on a 10L:14D cycle; black bar: aggregation group voles (AGG) exposed to 5°C on a 10L:14D cycle.

Table 4. Weights of Riney's KFI, internal and lymphatic organs of wild voles for the two experimental groups (the solitary [SOL] and aggregation [AGG] group).

	Group				ANCOVA	
	SOL [Mean±SE]	n	AGG [Mean±SE]	n	F-value	P
RKFI [%]	8.30±1.27	9	11.6±2.17	9	1.254	0.280
Kidney [g]	0.17±0.01	9	0.16±0.01	9	0.594	0.453
Liver [g]	1.49±0.06	9	1.48±0.13	9	1.613	0.223
Heart [g]	0.16±0.01	9	0.15±0.01	9	0.697	0.417
Spleen [mg]	44±6.6	9	42±4.0	9	0.089	0.770
Thymus [mg]	17±3.5	9	28±4.8	9	4.608	0.049

Those weights were compared between experimental groups by ANCOVA using body weight as covariance. Effects of body weight were significantly positive ( $P < .05$ ) for the index and all organs.  $F$ -value and probability ( $P$ ) indicate effects of experimental group.

(SOL) and aggregation group (AGG) on a 10L:14D cycle (Fig.3, Table 3, mean  $\pm$  SE of titres;  $3.15 \pm 0.62$  ( $n = 9$ ) for SOL,  $4.11 \pm 0.84$  ( $n = 9$ ) for AGG, ANOVA;  $F = 0.850$ ,  $P = 0.370$ ).

Mean body weight of AGG was lighter than SOL at the 1st day of the experiment (mean  $\pm$  SE [g];  $24.9 \pm 0.83$  for SOL,  $21.4 \pm 0.62$  for AGG, T-test;  $t = 3.38$ ,  $P = 0.004$ ), however, after then, there were not significant differences in body weights (at the immunization;  $26.0 \pm 0.87$  for SOL,  $24.3 \pm 1.01$  for AGG,  $t = 1.26$ ,  $P = 0.225$ , at the end of experiments;  $27.2 \pm 0.69$  for SOL,  $25.5 \pm 1.21$  for AGG, T-test;  $t = 1.17$ ,  $P = 0.259$ ). Body weight of SOL increased for the first 10 days (Fig.4, Paired T-test;  $t = 2.52$ ,  $P = 0.036$ ) and also increased thereafter (Paired T-test;  $t = 2.34$ ,  $P = 0.048$ ). As same as SOL, body weight of AGG increased for the first 10 days (Fig.4, Paired T-test;  $t = 4.92$ ,  $P = 0.001$ ) and also increased thereafter (Paired T-test;  $t = 3.13$ ,  $P = 0.014$ ).

*Riney's KFI, internal and lymphatic organs:*

Although thymus weights of AGG were significantly heavier than those of SOL, there were not significant differences between SOL and AGG in Riney's KFI, kidney, heart, liver, and spleen (Table 4).

**3. In comparison between experiment 1 and experiment 2***Immune response and body weight:*

The voles kept solitarily on a 10L:14D cycle at 5°C (SOL,  $n = 9$ ,  $3.15 \pm 0.62$  [mean  $\pm$  SE]) showed superior immune response in comparison with voles kept solitarily on a 12L:12D cycle (WLT,  $n = 12$ ,  $1.56 \pm 0.37$ ) at 5°C (Table 5, ANOVA;  $F = 5.34$ ,  $P = 0.03$ ).

Table 5. Comparison of titres measured in Hemagglutination test between voles kept on a 12L:12D cycle and voles kept on a 10L:14D cycle.

Factor	<i>df</i>	<i>F</i> -value	Prob.	adjusted R <sup>2</sup>
Full Model	2	3.225	0.064	0.182
Group	1	6.000	0.025	
Body weight	1	1.082	0.312	

The following numbers of voles were examined: 12 voles on 12L:12D and 9 voles on 10L:14D.

Body weights of SOL were significantly lighter than those of WLT throughout the experiment (T-test;  $t = 3.85$ ,  $P = 0.001$  at the 1st day;  $t = 4.13$ ,  $P < 0.001$  at the immunization;  $t = 3.59$ ,  $P = 0.002$  at the end of the experiment, mean  $\pm$  SE [g];  $29.8 \pm 0.90$  for WLT and  $24.9 \pm 0.83$  for SOL at the 1st day;  $32.5 \pm 1.18$  for WLT and  $26.0 \pm 0.87$  for SOL at the immunization;  $32.7 \pm 1.23$  for WLT and  $27.2 \pm 0.69$  for SOL at the end of experiments). Change in body weights of WLT and SOL were as mentioned above.

*Food intake:*

Food intake of SOL showed significant high value in comparison with WLT for both 8th-9th days of the experiment (mean  $\pm$  SE [g];  $9.80 \pm 0.29$  for WLT,  $9.96 \pm 0.33$  for SOL, T-test;  $t = 0.38$ ,  $P = 0.708$ ) and 14th-15th days of the experiment ( $9.16 \pm 0.26$  for WLT,  $10.2 \pm 0.43$  for SOL, T-test;  $t = 2.12$ ,  $P = 0.048$ ). Food intakes of WLT decreased from 8th-9th days to 14th-15th days, although food intakes of SOL did not differ between 8th-9th days and 14th-15th days (Paired T-test;  $t = 4.05$ ,  $P = 0.002$  for WLT;  $t = 1.13$ ,  $P = 0.291$  for SOL).

*Riney's KFI, internal and lymphatic organs:*

There were no significant differences in Riney's KFI, weights of internal organs (liver, kidney, and heart), and weights of lymphatic organs (spleen and thymus) between WLT and SOL (ANCOVA;  $F = 1.336$ ,  $P = 0.263$  for Riney's KFI;  $F = 0.002$ ,  $P = 0.963$  for liver;  $F = 0.0001$ ,  $P = 0.991$  for kidney;  $F = 0.901$ ,  $P = 0.355$  for heart;  $F = 0.0005$ ,  $P = 0.983$  for spleen;  $F = 3.19$ ,  $P = 0.09$  for thymus).



## Discussion

### *Cold stress on immune function*

The gray red-backed voles (*Myodes rufocanus*) exposed to low temperature (5°C) displayed inferior immune responses to the voles at room temperature (23°C) in experiment 1 (Fig. 1, Table 1), although it is thought that this species has been adapted to cool environments. The voles (*M. rufocanus*) exposed to low temperature (5°C) also showed higher food intakes and reductions of fat reserves in comparison with the room temperature (23°C) voles in experiment 1 (Table 2). Previous studies have reported that energy demands increase under low temperature conditions on a 12L:12D cycle in the laboratory mice<sup>2,13,30</sup>. The energy demands of gray red-backed voles (*M. rufocanus*) also increase under low temperature conditions. This means that the gray red-backed voles (*M. rufocanus*) exposed to a low temperature would require more energy for thermoregulation than the voles at room temperature. Furthermore, internal organs such as heart, liver, kidney and small intestine are hypertrophied under low temperature<sup>2,13,14,21</sup>. In this study, the internal organs (heart and kidney) of gray red-backed voles (*M. rufocanus*) exposed to low temperature were significantly heavier than those of the voles at room temperature (Table 2). Low temperature conditions would induce stress against organs related to metabolism (especially thermoregulation). Therefore, gray red-backed voles (*M. rufocanus*) exposed to low temperature could not allocate enough amount of energy for immunity. The results in this study are consistent with those of the previous studies mentioned above and interpretable as a trade-off between thermoregulation and immunity<sup>2</sup>.

Immunity is one of the important functions to maintain life, and is a major countermeasure against pathogens. Although the role of pathogens in the rodent cycle has noticed traditionally and theoretically<sup>7</sup>, it has largely neglected owing to a lack of direct evidence of the field in gray red-backed voles (*M. rufocanus*). As mentioned above, immunity competes with thermoregulation for energy allocation. The effects of cold stress on immune function could be one of the possible mechanisms of the density-dependent population decline during winter in the gray red-backed vole populations.

### *Short photoperiod and aggregation on immune function*

Humoral immune responses of gray red-backed voles (*Myodes rufocanus*) were enhanced by a short photoperiod (10L:14D cycle). The humoral immune responses of the gray red-backed voles (*M. rufocanus*) exposed to low temperature (5°C) on a 10L:14D cycle were similar to those of the voles kept on a 12L:12D cycle at room temperature (23°C). These results were similar to humoral immune responses enhanced by short photoperiods under low temperature in previous studies (e.g. deer mice (*Peromyscus maniculatus*)<sup>4,22</sup>). Deer mice (*P. maniculatus*) kept on an 8L:16D cycle at low temperature (8°C) displayed superior immune response (IgG) to those kept on a 16L:8D cycle at 8°C. Furthermore, serum IgG levels on an 8L:16D cycle at 8°C were comparable to those on a 16L:8D cycle at 20°C<sup>4</sup>. Therefore, reductions of immune function caused by cold stress could be

compensated by short photoperiods<sup>4,22</sup>).

Nontropical animals have physiologically and behaviorally adapted to cope with harsh conditions in winter<sup>22</sup>). Generally, small mammals change their reproductive status on the basis of photoperiodic cues and acclimate to changing environmental conditions. In most arvicoline rodents, acclimatization to winter is characterized by a decrease in body weight, a pronounced reduction in the size and activity of gonads and reproductive accessory organs, and an enhanced thermogenic capacity<sup>19</sup>). The decrease in body weight under short photoperiods is accompanied by reduced food intake and is thought to be a strategy for energy conservation in meadow voles (*Microtus pennsylvanicus*), particularly at low ambient temperature<sup>3</sup>). Alteration in photoperiod significantly influenced the thermogenic capacity of Brandt's voles (*Microtus brandtii*)<sup>32</sup>). In this study, food intakes of gray red-backed voles (*M. rufocanus*) on a 10L:14D cycle were higher than those of the voles on a 12L:12D cycle, although body weights of the voles (*M. rufocanus*) on a 10L:14D cycle were lighter than those of the voles on a 12L:12D cycle throughout the experiment. As already mentioned, arvicoline rodents form communal groups associated with environmental stress due to winter conditions and/or high population densities<sup>9,12,31</sup>). The winter aggregation (i.e. communal group) of voles could be regarded as an adaptive strategy to maintain body conditions at low temperature. The ecological significance of communal groups in winter would be regarded as controlling the body temperature to maintain physiological functions. Masaki et al.<sup>20</sup>) suggested that the Korean field mouse (*Apodemus peninsulae*) might aggregate and synchronize torpor and/or body temperature fluctuations to reduce heat loss in winter, although the body temperatures of group housing mice (2-3 individuals per cage) were similar to those of single housing mice at low temperature (4°C). In this study, the humoral immune responses of the aggregation group did not significantly differ from those of the solitary group on a 10L:14D cycle at low temperature (Fig. 3).

#### *Winter ecology of voles*

Winter is often stressful for arvicoline rodents<sup>5</sup>). As mentioned above, immunity is a major countermeasure against pathogens; it is one of the major physiological mechanisms that regulate host survival, and it may compete for available resources with other functions such as growth, reproduction, and thermoregulation<sup>8,18,26,33</sup>). The immune function of gray red-backed voles (*M. rufocanus*) competes with thermoregulation for energy allocation, and a trade-off might exist between thermoregulation and immunity. However, physiological functions are believed to adapt to various environmental conditions. Immune function is enhanced by short photoperiods in the deer mice (*Peromyscus maniculatus*)<sup>4,22</sup>). The humoral immune responses of gray red-backed voles (*M. rufocanus*) were also enhanced by short photoperiods, such as deer mice (*P. maniculatus*)<sup>4,22</sup>). Therefore, the reduction of immune function caused by cold stress could be compensated by short photoperiods<sup>4,22</sup>). Furthermore, winter aggregation would be able to be regarded as an adaptive strategy to maintain body conditions at low temperatures. Arvicoline rodents form communal groups associated with environmental stress due to winter condi-

tions and/or high population densities<sup>9,12,31</sup>). However, in fact, significant effects of aggregation were not observed on the humoral immune function of gray red-backed voles (*M. rufocanus*).

In winter, high amounts of energy are required, however, food resources for arvicoline rodents are limited in winter than in other seasons<sup>22,23</sup>). Resource limitations in winter would cause serious damage to high-density populations of arvicoline rodents. In the gray red-backed vole (*M. rufocanus*), most of the vole populations display the density-dependent population decline in winter<sup>24,25,27,28,29</sup>). In a field experimental study, Huitu et al.<sup>10</sup> demonstrated that *Microtus* vole populations were limited in growth by a lack of food in winter. The laboratory experiment also suggested that food limitation at low ambient temperature would have negative influences on maintenance of body condition, thermoregulation, and immune function under winter conditions (5°C, 10L:14D light cycle) in gray red-backed voles (*M. rufocanus*)<sup>17</sup>). Therefore, resource-limited conditions at low temperature would induce density-dependent population decline of the gray red-backed vole (*M. rufocanus*) in winter.

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## エゾヤチネズミ (*Myodes rufocanus*) の非繁殖個体の 低温下での体液性免疫反応の比較

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### 摘 要

エゾヤチネズミ (*Myodes rufocanus*) の非繁殖個体を実験にもちいて、病原性のない羊赤血球 (SRBCs) に対する体液性免疫反応を低温下で調べた。実験1では、23匹のエゾヤチネズミの非繁殖個体を23℃ (n=11) および5℃ (n=12) の2つのグループに別け、12L:12Dの低温下におけるSRBCsに対する体液性免疫反応を赤血球凝集反応 (HA) 法により調べたところ、5℃にさらされたグループで力価が低かった。また、5℃にさらされたエゾヤチネズミの餌摂取量が有意に多く、内蔵肥大がみられた。さらに実験2では、低温下における集団の効果を調べるために、18匹のエゾヤチネズミの非繁殖個体を単独飼育 (n=9) および集団飼育 (n=9) のグループに別け、冬の条件 (5℃, 10L:14D) 下でSRBCsに対する体液性免疫反応を調べた結果、集団飼育個体の力価の方が単独飼育個体の力価よりも若干高かったが、有意な違いはみられなかった。