



# Longitudinal study of northern fur seal (*Callorhinus ursinus*) hematology

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**ABSTRACT.** Although the causes have not been specified yet, wild populations of northern fur seals (*Callorhinus ursinus*) have been decreasing, which is why conservation techniques such as captive breeding and health maintenance should be established. Hematological parameters can be used to maintain the health status of northern fur seals kept in captivity for artificial reproduction and public education. Year-round fluctuations of blood parameters have not been examined for northern fur seals due to the difficulties in obtaining serial blood samples from wild animals during the oceanic migration period from late autumn to spring. In this study, blood samples were collected from four captive northern fur seals and analyzed monthly for more than three years to clarify the seasonal fluctuation patterns in 14 hematological parameters. Many hematological parameters seemed to be seasonal patterns: summer–autumn and winter–spring; leukocyte-related parameters were higher in summer and autumn than in winter and spring; erythrocyte-related parameters were lower in summer and autumn than in winter and spring. Significant seasonal differences in nine of the 14 parameters were observed using a generalized linear mixed model (GLMM) analysis. These results have improved our understanding of the seasonal patterns of hematological characteristics in the northern fur seal and can contribute to the health care of protected or captive northern fur seals.

**KEY WORDS:** erythrocyte, hematology, leukocyte, northern fur seal, seasonal change

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The northern fur seal (*Callorhinus ursinus*) is a subarctic otariid endemic to the North Pacific Ocean [31]. This species lives in two different environments, land and open ocean, depending on the season. They breed annually on several islands in the North Pacific Ocean, the Bering Sea, and the Okhotsk Sea for approximately five months from June to October (breeding seasons), and then migrate offshore to forage for approximately seven months, from November to May (non-breeding seasons). Adult males stay ashore to guard specific territories on the breeding grounds (called “rookeries”) during the mating season; during the migration period, males continually stay at sea and forage once they leave the breeding islands [9, 13, 14, 19]. Adult females deliver and nurse pups, are inseminated, and forage at sea near the rookeries during summer and early autumn but migrate south to oceanic areas from late autumn to spring. Since northern fur seals have distinct annual reproductive cycles, as with other pinnipeds [1, 5], they are likely to switch their physiological state such as blood and endocrine parameters according to their life stages in terrestrial or oceanic habitats. However, the seasonal physiological changes are difficult to assess in free-ranging wild individuals throughout the year due to the difficulties in collecting serial blood samples during the oceanic migration period.

Recently, the population of wild northern fur seals has been declining, particularly in the Alaskan subpopulation, and their conservation is an international concern [11, 12, 39, 40]. Several factors are believed to contribute to the population decline, such as changes in prey availability and species composition related to commercial fisheries and climate change, incidental mortality in commercial fishing, entanglement in marine debris, and environmental contaminants, but the specific causes of this decline remain unknown [25, 39, 40]. Hematological parameters are effective at monitoring the health condition of seals and can be used to detect disease and provide crucial information regarding their metabolism, nourishment states, or infectious diseases [3, 18, 27, 32]. Monitoring the normal ranges and seasonal fluctuation patterns of blood parameter values of northern fur seals across the breeding and non-breeding seasons enables us to better understand their reproduction and health conditions. Considering this, samples

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from captive individuals provide useful reference values for us to compare them with free-ranging wild animals. Previously, we examined the blood and serum biochemistry of captive northern fur seals and found some differences in the values from the levels reported in free-ranging animals, such as those of erythrocytes, hemoglobin, and hematocrit [21]; however, seasonal patterns were not examined. Therefore, the aim of this study was to determine seasonal variations in blood parameters obtained from four captive northern fur seals across more than three years.

## MATERIALS AND METHODS

### *Animals and breeding environment*

Four northern fur seals were examined (Table 1). Three (Seals 1, 3, and 4) were born in an aquarium and one (Seal 2) was live-stranded and rescued in Taro Town, Iwate, Japan with the permission of the Ministry of Agriculture, Forestry and Fisheries of the Japanese Government. The age of the stranded seal (Seal 2) at rescue was estimated to be less than 1 year based on its morphometrics (body length 73.0 cm and body weight 7.65 kg). Blood was sampled from Seal 1 from March 2009 to June 2012, and from Seals 2, 3, and 4 from April 2013 to June 2016 (Table 1). Judging from the age of each animal and from the information in the literature about northern fur seal maturation [14], Seals 2 and 3 were sexually mature at the beginning of the experiment, while Seals 1 and 4 matured during the course of the study. In addition, Seal 3 was confirmed to be pregnant for the first time in the final year of this experiment and delivered normally after the blood samples were collected. Thus, the experimental periods corresponded to the pre-maturation and post-maturation stages. These seals were also used in a previous hematological study [21]. All procedures were approved by the Gifu University Animal Care and Use Committee (approval nos. 14094 and 17186).

Seal 1 was maintained with two adult females (not used in this study) in an indoor facility with an area of approximately 46.8 m<sup>2</sup> and a pool of 40 m<sup>3</sup> filled with sea water filtered using a pressure-type sand filter (0.5 m<sup>3</sup>/min) (type PFV183; Ebara Corp., Tokyo, Japan). Seals 2, 3, and 4 were maintained together in another indoor facility with an area of 16.8 m<sup>2</sup> and a pool of 4.5 m<sup>3</sup> filled with sea water filtered using a pressure-type sand filter (0.1 m<sup>3</sup>/min) (type S-050-18L10; Nippon Filcon, Tokyo, Japan). Although natural light entered these indoor facilities through windows, overhead fluorescent lights were used during the daytime (generally from 08:30 to 17:00) as the brightness of natural light was insufficient compared with that in outdoor settings. The air temperature of these facilities fluctuated with external atmospheric temperatures but was maintained at 25°C or lower during summer by using an air conditioner. The water temperature of the pool was not controlled and fluctuated in conjunction with the inlet seawater (Fig. 1). All fur seals were fed a diet comprising thawed mackerel (*Scomber australasicus* and *S. japonicus*; 2 to 3 kg/day/animal) for most of the study period from March 2009 to March 2016. The fur seals were healthy throughout the study periods (3 years or more) based on their body conditions from the routine veterinary inspections by on-site veterinarians and the observation of their behavior and appetite (data not shown).

### *Hematological analyses*

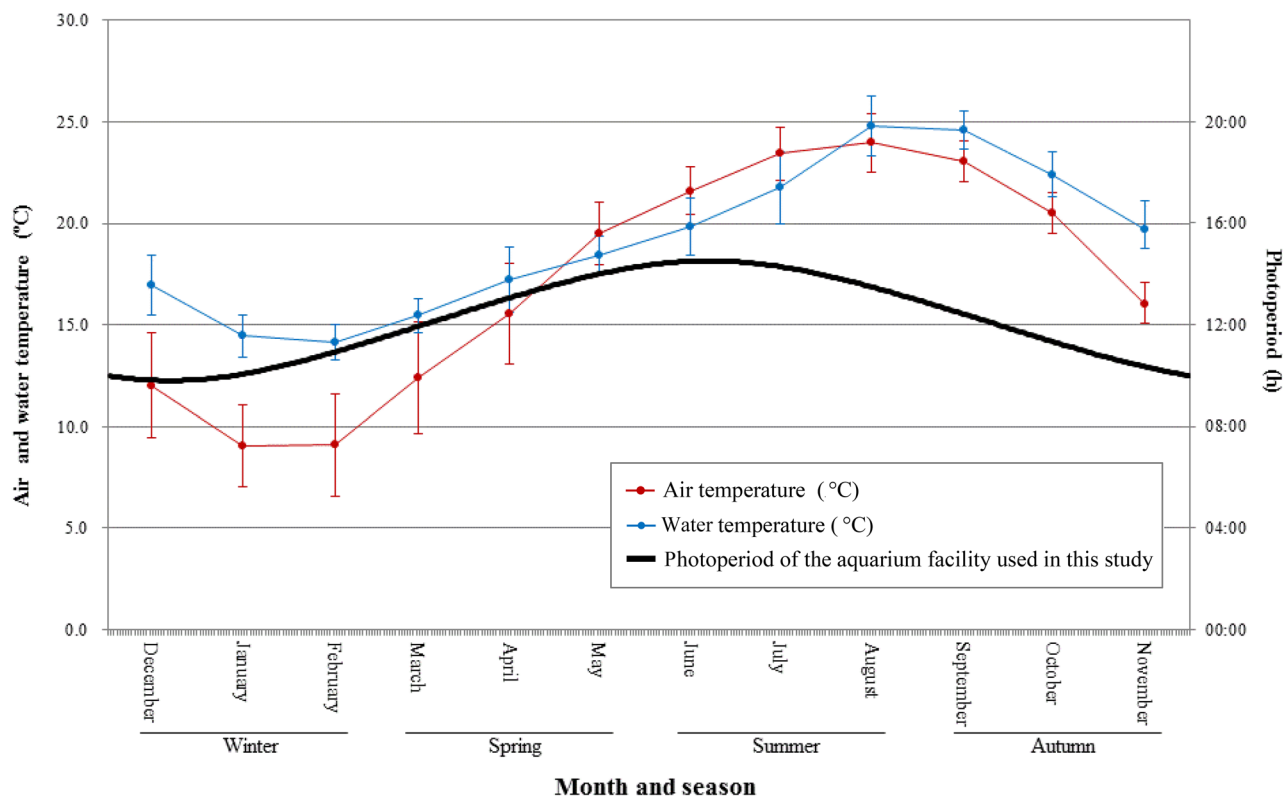
Blood samples (approximately 10 ml) were collected from the hind flippers of four seals monthly during the study period using disposable syringes (TERUMO syringe 10 ml, TERUMO, Tokyo, Japan) and needles (TERUMO NEOLUS 22G: φ 0.70 × 32 mm, TERUMO). The seals were trained to enter a metal cage by themselves, and their hind flippers were pulled out from the cage and fixed without chemical restraint during the blood sampling. The collected blood samples were dispensed into EDTA-containing tubes (TERUMO VENOJECT II VP-DK052K, TERUMO). In total, 157 blood samples were collected from the animals during the study period.

Fourteen hematological parameters—leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, reticulocytes (immature red blood cells), segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils—were measured. The hematologic analyses were conducted by a clinical laboratory testing company, SRL (Tokyo, Japan), using an XN-9100 automatic hematology analyzer (Sysmex, Kobe, Japan) (Table 2) within 12 hr of blood collection. Conventionally, the values of segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils are given in relative percentages. In this study, these values were converted to cell counts to eliminate the influence of super-abundant cell types on other cell types. The measurement with the automatic hematology analyzer at SRL was performed using the settings optimized for human blood cells. To verify the

**Table 1.** Northern fur seals used in this study

| Seal ID | Sex    | Date of birth                   | Collection period       |                   | Sample number          |                        |                        |                        |       |
|---------|--------|---------------------------------|-------------------------|-------------------|------------------------|------------------------|------------------------|------------------------|-------|
|         |        |                                 | Date                    | Age <sup>b)</sup> | 1st year <sup>c)</sup> | 2nd year <sup>d)</sup> | 3rd year <sup>e)</sup> | 4th year <sup>f)</sup> | Total |
| 1       | Male   | 25-June-2007                    | March 2009 to June 2012 | 1Y9M to 4Y9M      | 13                     | 12                     | 12                     | 3                      | 40    |
| 2       | Male   | Unknown July 2009 <sup>a)</sup> | April 2013 to June 2016 | 3Y9M to 6Y11M     | 12                     | 12                     | 12                     | 3                      | 39    |
| 3       | Female | 26-June-2009                    | April 2013 to June 2016 | 3Y10M to 7Y0M     | 12                     | 12                     | 12                     | 3                      | 39    |
| 4       | Female | 30-June-2010                    | April 2013 to June 2016 | 2Y10M to 6Y0M     | 12                     | 12                     | 12                     | 3                      | 39    |

a) Rescued in Taro town, Iwate, Japan, estimated to be less than one year old based on morphometrics. b) Year (Y) and Month (M)-old. c) Seal 1, March 2009–March 2010; Seals 2–4, April 2013–March 2014. d) Seal 1, April 2010–March 2011; Seals 2–4, April 2014–March 2015. e) Seal 1, April 2011–March 2012; Seals 2–4, April 2015–March 2016. f) Seal 1, April 2012–June 2012; Seals 2–4, April 2016–June 2016.



**Fig. 1.** Air temperature, water temperature and photoperiod in the breeding facility. Air and water temperatures are presented as the average and standard deviation of each month from 2013 through 2016. Photoperiod is shown for 2014 as a representative example. Red line, air temperature (°C), blue line, water temperature (°C), and black line, photoperiod of the aquarium facility used in this study.

**Table 2.** Measuring methods for hematology in clinical laboratory

| Parameter             | Measuring method <sup>a)</sup> |
|-----------------------|--------------------------------|
| Leukocytes            | FCM by semiconductor laser     |
| Erythrocytes          | sheath flow DC                 |
| Hemoglobin            | SLS-hemoglobin                 |
| Hematocrit            | sheath flow DC                 |
| MCV                   | calculation                    |
| MCH                   | calculation                    |
| MCHC                  | calculation                    |
| Platelets             | sheath flow DC                 |
| Reticulocyte          | FCM by semiconductor laser     |
| Segmented neutrophils | FCM by semiconductor laser     |
| Lymphocytes           | FCM by semiconductor laser     |
| Monocytes             | FCM by semiconductor laser     |
| Eosinophils           | FCM by semiconductor laser     |
| Basophils             | FCM by semiconductor laser     |

a) Measured by Sysmex XN-9100 Hematology analyzer. FCM, Flowcytometry; DC, Direct current; SLS, Sodium lauryl sulfate; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

validity of hematologic measurement in this study, we compared the measurement by SRL with that by Fujifilm VET Systems (Tokyo, Japan), who use a flow cytometry system (ADVIA2120i multispecies hematology analyzer, Siemens Healthineers, Erlangen, Germany) that is not affected by blood cell size. Table 3 shows the results of blood analysis for five northern fur seals including two individuals used in this study. The average measurement by SRL was approximately 5% higher for erythrocytes and approximately 13% lower for leukocytes. Although there is a possibility of minor errors in the absolute counts of fur seal blood cells using the automatic hematology analyzer with the setting for human blood samples, it is considered that the relative values reflect seasonal trends correctly since all measurements were made under the same settings in this study.

#### Data analysis

To visualize the monthly fluctuations of each hematological parameter, a seasonal decomposition analysis (SDA) was implemented. A locally weighted regression (loess) filter procedure was used to segregate the observed time series of each individual into trend, seasonal, and residual components [6]. The stl function of R 3.6.3 [30] was used for the seasonal decomposition analysis with an additive decomposition. The smoothing parameter for the seasonal component was set at 7 but had little influence on the resultant seasonal decomposition.

Since many hematological parameters exhibited seasonal fluctuation patterns, the blood parameter data were summarized by season and then analyzed statistically. According to the classification of the Japan Meteorological Agency ([https://www.jma.go.jp/jma/kishou/now/yougo\\_hp/toki.html](https://www.jma.go.jp/jma/kishou/now/yougo_hp/toki.html)), which fits the monthly changes in air temperature, water temperature, and photoperiod (Fig. 1), the following seasons were defined: winter, from December to February; spring, from March to May; summer, from June

**Table 3.** Comparison of measurements by an automatic blood cell counter and a flow cytometry system for five northern fur seal samples

| Fur seal ID | Sex | Erythrocyte × 10,000 |       |            | Leukocyte × 100 |      |            |
|-------------|-----|----------------------|-------|------------|-----------------|------|------------|
|             |     | BCC                  | FC    | BCC/FC (%) | BCC             | FC   | BCC/FC (%) |
| 3           | F   | 567                  | 577   | 98.3       | 53              | 49   | 108.2      |
| 4           | F   | 639                  | 671   | 95.2       | 49              | 44   | 111.4      |
| 16          | F   | 519                  | 554   | 93.7       | 83              | 74   | 112.2      |
| 18          | M   | 572                  | 579   | 98.8       | 82              | 71   | 115.5      |
| 19          | M   | 554                  | 571   | 97.0       | 72              | 60   | 120.0      |
| Average     | -   | 570.2                | 590.4 | 96.6       | 67.8            | 59.6 | 113.4      |

BCC, Blood cell counts using XN-9100 automatic hematology analyzer; FC, Flow cytometry using ADVIA2120i multispecies hematology analyzer.

to August; autumn, from September to November. The hematological parameter values were aggregated into these seasons, and averages and standard deviations were calculated for each individual. To examine the seasonal differences in each hematological parameter, generalized linear mixed models (GLMMs) were applied to the monthly data. Since the number of experimental animals was limited, fur seal individuals were treated as random intercepts, and season was treated as a categorical variable. Means and standard errors (SE) were compared among the four seasons, and the seasonal differences were confirmed using Tukey's multiple comparisons test. These analyses were conducted using R 3.6.3 [30] with the packages lme4 and multicomp.

## RESULTS

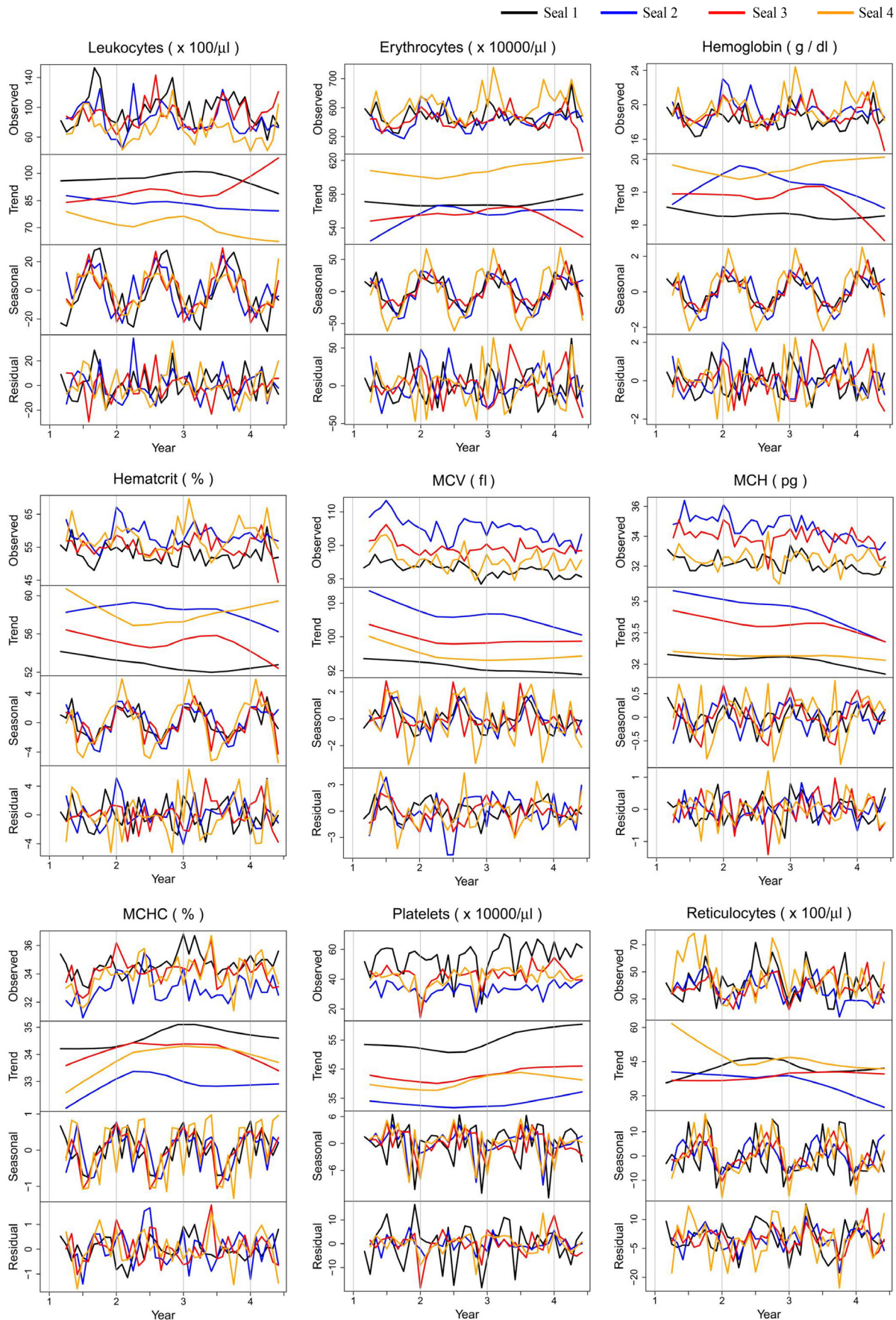
The observed time series, as well as the trend, seasonal, and residual components by the SDA are shown in Fig. 2. The seasonal components extracted by the SDA indicated that there were two distinct seasonal cycles: leukocyte, reticulocyte, segmented neutrophil, and monocyte counts were higher in summer and autumn and lower in winter and spring. In contrast, erythrocyte counts, hemoglobin levels, and hematocrit levels were higher in autumn and winter and lower in spring and summer. Additionally, MCHC was lower in summer than in other seasons. However, MCV, MCH, platelet, lymphocyte, eosinophil, and basophil counts showed no clear seasonal patterns.

The seasonal averages and standard deviations of each parameter for the four individuals are summarized in Table 4. The results of the GLMM analyses and Tukey's multiple comparisons test revealed no significant differences ( $P > 0.05$ ) between winter and spring or summer and autumn, but showed significant differences across winter and spring vs. summer and autumn for many hematological parameters (Fig. 3). For example, leukocytes, segmented neutrophils, and monocytes were significantly lower in winter and spring than in summer and autumn. Reticulocytes showed a similar seasonal pattern to that of leukocytes, segmented neutrophils, and monocytes, but the spring mean was not significantly different from the summer mean. Contrastingly, erythrocytes, hemoglobin, and hematocrit were significantly higher in winter and spring than in summer and autumn. In addition, MCHC showed a similar seasonal pattern to that of erythrocytes, hemoglobin, and hematocrit, but the autumn mean was not significantly different from the spring mean. However, MCH, platelets, lymphocytes, eosinophils, and basophils did not show significant seasonal differences.

## DISCUSSION

In the present study, the blood characteristics of four captive northern fur seals were monitored continuously. Consequently, many hematological parameters seemed to have seasonal patterns: leukocyte-related parameters were higher in summer and autumn than in winter and spring; erythrocyte-related parameters were lower in summer and autumn than in winter and spring. Although several studies have examined the blood characteristics of northern fur seals, such seasonal changes in blood parameters have not been reported till date [2, 23, 28]. Seasonal hematological changes have been described for terrestrial mammals such as brown bears (*Ursus arctos*) [17] and Angora rabbits (*Oryctolagus cuniculus domesticus*) [7]; however, limited information is available on the seasonal changes in the blood characteristics of marine mammals. To the best of our knowledge, only one study has shown annual changes in the hematological parameters of marine mammals, i.e., bottlenose dolphins (*Tursiops truncatus*) [38]. There are a few reports on marine mammals that indicate the differences in the hematological parameters between breeding and non-breeding seasons, or differences based on age [24, 34, 42]. We, therefore, compared the results of this study with information in the literature on the changes in the blood characteristics of California sea lions (*Zalophus californianus*) [3, 4], Galapagos sea lions (*Zalophus wollebaeki*) [29], and Steller's sea lions (*Eumetopias jubatus*) based on season or age [15]; however, these previous studies did not provide data that could refer to the results of this study. Gerlinsky *et al.* (2018) reported that in Steller's sea lions, erythrocytes tended to increase with growth, whereas leukocytes tended to decrease with growth and suggested that the changes in blood cell counts were related to the physiological state of the animals [15]. In our study, the northern fur seals showed seasonal changes in erythrocytes and leukocytes both pre-maturity and post-maturity. This suggests the possibility that erythrocytes and leukocytes fluctuate seasonally throughout the growth period of northern fur seals. The difference by sex, age, or sexual maturation could not be assessed in this study.





**Fig. 2.** Seasonal decomposition analysis. The observed time series and trend, seasonal, and residual components extracted by seasonal decomposition analysis of blood parameters for the four fur seals. Black line, Seal 1; blue line, Seal 2; red line, Seal 3; yellow line, Seal 4.

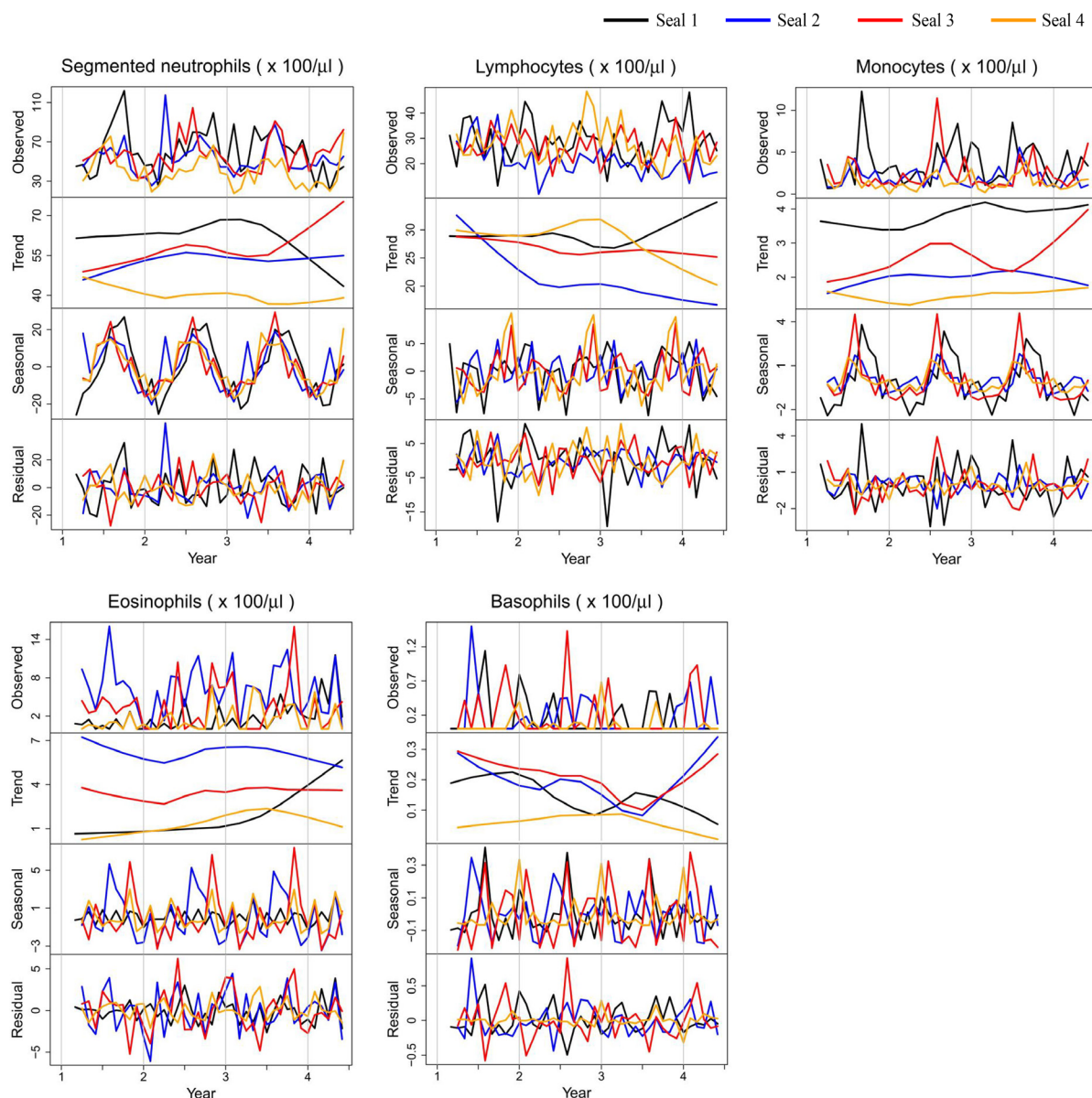


Fig. 2. Continued.

Riley and Rupert (2015) suggested that the increased leukocyte counts could be related to microbial infection, since leukocyte counts are known to increase in animals in normal environments compared with those in germ-free animals [16, 35]. The number of microorganisms encountered by northern fur seals foraging in oceanic areas is likely to be much lower than that in coastal zones and terrestrial rookeries [37]. One possible evolutionary explanation for the increased leukocyte levels observed during the summer–autumn period in northern fur seals is that this species evolved this trait as an adaptation to resist bacterial exposure in terrestrial and near-shore environments during the breeding season.

A causal explanation for the seasonal changes in hematological parameters may be related to reproductive physiology, *inter alia*, sex-steroid hormones. Reportedly, in adult male northern elephant seals (*Mirounga angustirostris*), leukocyte and neutrophil counts significantly increased during the reproductive period than in the molting period [42]. In addition, it is known that the leukocyte count is affected by steroids [8, 10, 26]. Moreover, elevated seal leukocyte counts may be affected by testosterone or corticosteroids. A similar possibility can be considered for male fur seals. However, seasonal variations in leukocyte counts were also observed in Seal 1, which was considered immature based on his age in the first year of sampling [14]. In addition, the female northern fur seal in this study showed seasonal leukocyte fluctuations similar to those shown by males, although fluctuations in steroid hormones occurred at different timepoints between the sexes [20, 22, 41]. Therefore, the effects of testosterone or other steroid hormones alone cannot explain seasonal variations in leukocyte counts in fur seals. Differences in erythrocyte counts, hemoglobin concentrations, and hematocrit levels between the breeding and molting seasons have been reported in male northern elephant seals [42]; however, the study did not consider their relation to breeding and non-breeding seasons.

**Table 4.** Seasonal averages and standard deviations in hematological parameters of four captive northern fur seals used in this study

| Parameter             | Unit                 | Seal 1                      |                              |                              |                             | Seal 2        |                |                |               |
|-----------------------|----------------------|-----------------------------|------------------------------|------------------------------|-----------------------------|---------------|----------------|----------------|---------------|
|                       |                      | Winter <sup>a)</sup><br>n=9 | Spring <sup>b)</sup><br>n=12 | Summer <sup>c)</sup><br>n=10 | Autumn <sup>d)</sup><br>n=9 | Winter<br>n=9 | Spring<br>n=11 | Summer<br>n=10 | Autumn<br>n=9 |
| Leukocytes            | ×10 <sup>2</sup> /μl | 90.3 ± 12.5                 | 78.5 ± 13.4                  | 99.8 ± 13.8                  | 120.6 ± 19.1                | 65.0 ± 9.8    | 81.1 ± 17.5    | 93.7 ± 15.2    | 91.4 ± 18.2   |
| Erythrocytes          | ×10 <sup>4</sup> /μl | 584.7 ± 21.7                | 587.5 ± 37.1                 | 551.2 ± 15.5                 | 550.2 ± 29.5                | 575.3 ± 35.1  | 571.7 ± 19.2   | 551.7 ± 35.3   | 530.1 ± 27.0  |
| Hemoglobin            | g/dl                 | 18.8 ± 1.0                  | 19.0 ± 1.2                   | 17.7 ± 0.6                   | 17.7 ± 1.0                  | 20.0 ± 1.5    | 19.6 ± 0.8     | 19.1 ± 1.2     | 18.4 ± 0.9    |
| Hematocrit            | %                    | 53.8 ± 2.2                  | 54.4 ± 3.7                   | 51.6 ± 0.9                   | 51.2 ± 2.7                  | 59.9 ± 3.6    | 59.7 ± 2.0     | 58.3 ± 2.0     | 56.2 ± 2.2    |
| MCV                   | fl                   | 91.9 ± 2.2                  | 92.6 ± 2.2                   | 93.6 ± 1.7                   | 93.1 ± 0.7                  | 104.1 ± 2.4   | 104.4 ± 3.6    | 106.0 ± 4.6    | 106.0 ± 2.2   |
| MCH                   | pg                   | 32.2 ± 0.6                  | 32.3 ± 0.6                   | 32.0 ± 0.4                   | 32.1 ± 0.3                  | 34.7 ± 1.0    | 34.3 ± 0.8     | 34.7 ± 0.8     | 34.7 ± 0.6    |
| MCHC                  | %                    | 35.0 ± 0.9                  | 34.8 ± 0.8                   | 34.2 ± 0.7                   | 34.5 ± 0.2                  | 33.4 ± 0.8    | 32.8 ± 0.7     | 32.8 ± 1.3     | 32.7 ± 0.6    |
| Platelets             | ×10 <sup>4</sup> /μl | 51.8 ± 11.1                 | 56.2 ± 9.3                   | 54.7 ± 11.0                  | 56.3 ± 7.1                  | 32.6 ± 4.7    | 34.1 ± 2.7     | 35 ± 3.6       | 31.3 ± 5.9    |
| Reticulocyte          | ‰                    | 6.1 ± 1.4                   | 7.0 ± 1.6                    | 7.7 ± 2.5                    | 9.0 ± 2.3                   | 5.4 ± 1.0     | 6.1 ± 1.4      | 7.2 ± 1.2      | 7.7 ± 2.4     |
| Segmented neutrophils | ×10 <sup>2</sup> /μl | 54.0 ± 17.0                 | 45.1 ± 16.1                  | 68.2 ± 16.4                  | 80.7 ± 21.6                 | 39.1 ± 7.0    | 53.0 ± 21.2    | 61.9 ± 10.9    | 59.1 ± 13.1   |
| Lymphocytes           | ×10 <sup>2</sup> /μl | 31.6 ± 10.4                 | 28.2 ± 6.7                   | 26.5 ± 5.7                   | 30.9 ± 8.8                  | 19.8 ± 3.5    | 20.0 ± 5.9     | 22.5 ± 7.5     | 21.6 ± 7.5    |
| Monocytes             | ×10 <sup>2</sup> /μl | 3.2 ± 1.6                   | 2.3 ± 1.5                    | 3.4 ± 2.3                    | 6.1 ± 2.6                   | 1.2 ± 0.4     | 1.9 ± 0.9      | 2.2 ± 1.5      | 2.6 ± 0.8     |
| Eosinophils           | ×10 <sup>2</sup> /μl | 1.4 ± 1.1                   | 2.5 ± 3.5                    | 1.1 ± 1.3                    | 1.8 ± 1.9                   | 4.5 ± 3.6     | 5.6 ± 3.4      | 6.6 ± 4.4      | 7.8 ± 2.6     |
| Basophils             | ×10 <sup>2</sup> /μl | 0.1 ± 0.3                   | 0.1 ± 0.1                    | 0.3 ± 0.4                    | 0.1 ± 0.2                   | 0.2 ± 0.2     | 0.1 ± 0.2      | 0.3 ± 0.5      | 0.1 ± 0.2     |

| Parameters            | Unit                 | Seal 3        |                |                |               | Seal 4        |                |                |               |
|-----------------------|----------------------|---------------|----------------|----------------|---------------|---------------|----------------|----------------|---------------|
|                       |                      | Winter<br>n=9 | Spring<br>n=11 | Summer<br>n=10 | Autumn<br>n=9 | Winter<br>n=9 | Spring<br>n=11 | Summer<br>n=10 | Autumn<br>n=9 |
| Leukocytes            | ×10 <sup>2</sup> /μl | 79.7 ± 14.5   | 82.4 ± 10.8    | 104.0 ± 19.9   | 95.0 ± 7.5    | 62.9 ± 13.8   | 58.9 ± 10.3    | 84.5 ± 14.5    | 78.3 ± 20.1   |
| Erythrocytes          | ×10 <sup>4</sup> /μl | 567.9 ± 22.5  | 574.5 ± 33.5   | 533.6 ± 32.5   | 543.3 ± 10.6  | 645.8 ± 44.0  | 626.2 ± 34.3   | 561.2 ± 12.2   | 599.9 ± 41.7  |
| Hemoglobin            | g/dl                 | 19.4 ± 0.9    | 19.5 ± 1.4     | 18.0 ± 1.3     | 18.4 ± 0.6    | 21.0 ± 1.6    | 20.5 ± 1.2     | 18.1 ± 0.5     | 19.3 ± 1.4    |
| Hematocrit            | %                    | 56.0 ± 2.0    | 56.9 ± 3.1     | 53.6 ± 3.5     | 53.6 ± 1.3    | 61.3 ± 3.8    | 59.7 ± 3.7     | 54.2 ± 2.4     | 57.1 ± 3.5    |
| MCV                   | fl                   | 98.6 ± 0.9    | 99.1 ± 1.4     | 100.5 ± 3.3    | 98.7 ± 1.4    | 94.9 ± 1.9    | 95.4 ± 2.3     | 96.5 ± 4.3     | 95.3 ± 3.3    |
| MCH                   | pg                   | 34.2 ± 0.5    | 33.9 ± 0.7     | 33.7 ± 0.6     | 33.8 ± 0.7    | 32.5 ± 0.4    | 32.7 ± 0.4     | 32.3 ± 0.4     | 32.2 ± 1.0    |
| MCHC                  | %                    | 34.7 ± 0.6    | 34.2 ± 0.7     | 33.6 ± 1.2     | 34.2 ± 0.4    | 34.3 ± 0.8    | 34.3 ± 0.9     | 33.5 ± 1.6     | 33.8 ± 1.0    |
| Platelets             | ×10 <sup>4</sup> /μl | 40.6 ± 11.7   | 42.5 ± 2.6     | 43.2 ± 3.3     | 45.8 ± 3.3    | 39.8 ± 7.8    | 41.4 ± 4.1     | 42.9 ± 2.8     | 39.3 ± 8.3    |
| Reticulocyte          | ‰                    | 5.8 ± 0.9     | 6.2 ± 1.3      | 7.6 ± 0.9      | 8.3 ± 1.2     | 6.2 ± 1.7     | 6.5 ± 1.8      | 9.3 ± 2.8      | 8.8 ± 2.8     |
| Segmented neutrophils | ×10 <sup>2</sup> /μl | 46.2 ± 11.2   | 50.9 ± 10.9    | 71.9 ± 19.5    | 59.6 ± 10.4   | 29.4 ± 9.1    | 30.3 ± 6.7     | 55.3 ± 13.7    | 44.9 ± 13.2   |
| Lymphocytes           | ×10 <sup>2</sup> /μl | 27.3 ± 8.0    | 28.0 ± 4.2     | 24.2 ± 5.1     | 27.4 ± 4.9    | 30.8 ± 8.1    | 26.1 ± 6.2     | 25.9 ± 5.2     | 29.5 ± 9.2    |
| Monocytes             | ×10 <sup>2</sup> /μl | 1.9 ± 1.1     | 1.7 ± 0.9      | 4.0 ± 3.0      | 2.4 ± 1.6     | 1.1 ± 0.8     | 0.9 ± 0.5      | 2.2 ± 0.9      | 1.5 ± 0.9     |
| Eosinophils           | ×10 <sup>2</sup> /μl | 3.8 ± 3.1     | 1.4 ± 1.8      | 3.5 ± 2.9      | 5.1 ± 4.8     | 1.3 ± 2.0     | 1.4 ± 2.1      | 1.0 ± 1.6      | 2.2 ± 2.2     |
| Basophils             | ×10 <sup>2</sup> /μl | 0.3 ± 0.3     | 0.1 ± 0.3      | 0.2 ± 0.4      | 0.2 ± 0.3     | 0.1 ± 0.2     | 0.0 ± 0.0      | 0.0 ± 0.0      | 0.1 ± 0.1     |

a) From December to February; b) from March to May; c) from June to August; d) from September to November. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

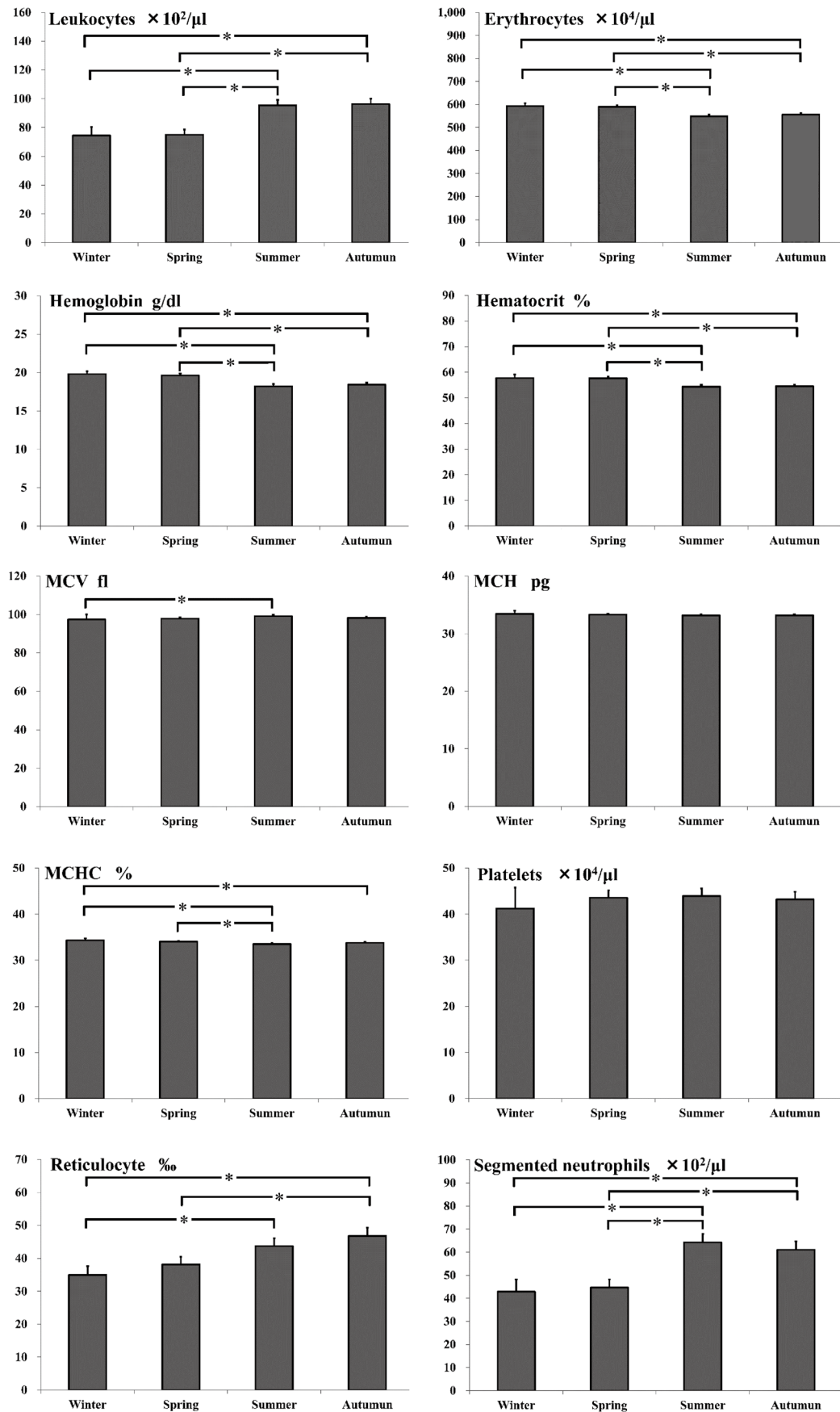
The parameters associated with erythrocytes (such as erythrocyte count, hemoglobin concentration, hematocrit levels, and MCHC) are conventionally used as an index of anemia [36]. In the present study, the erythrocyte count, hemoglobin concentration, and hematocrit levels were lower in summer and autumn than in winter and spring; however, the clinical signs of anemia were not observed during summer and autumn, based on their behavior and external features such as the colors of the mucous membrane. Although conclusive evidence cannot be provided, the higher values of erythrocyte-based parameters in the winter–spring season may be related to the increased swimming and diving activities during the migration period. We observed that only reticulocyte counts expressed in permillage were higher in the summer–autumn season than in the winter–spring season among the erythrocyte-based parameters. When reticulocytes increase, a rise in erythrocytes is observed [33]. It may be possible that the observed increase in reticulocytes in the summer–autumn season is related to the increase in erythrocytes observed in the winter–spring season.

The results of the present study have improved our understanding of the seasonal patterns of northern fur seal blood characteristics and our interpretation of the hematological parameters obtained from opportunistically sampling free-ranging animals. Future studies should find a causal explanation for the seasonal changes in the hematological parameters of the northern fur seals observed in this study. These results would contribute to the accumulation of zoological knowledge and be useful for veterinary medicine when monitoring the health of wild and captive northern fur seals.

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

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**Fig. 3.** Seasonal means of hematological parameters. Estimated by generalized linear mixed model in which northern fur seal individuals were treated as random intercepts. Error bar shows standard errors. \*Significant difference ( $P < 0.05$ ) between two seasons by multiple comparisons with Tukey's test.



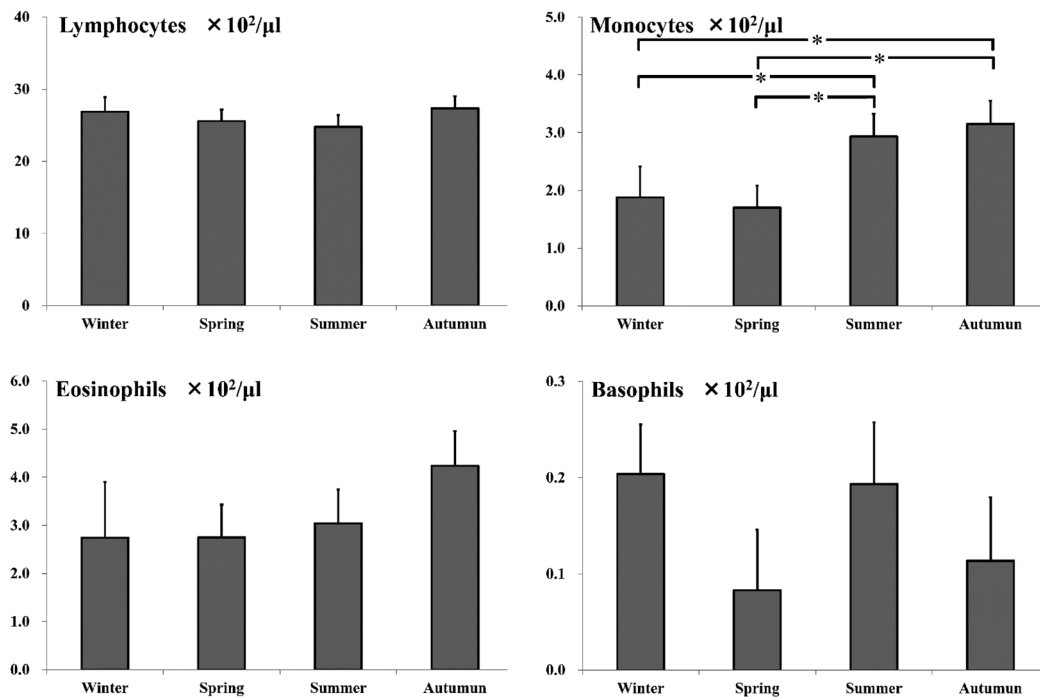


Fig. 3. Continued.

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