



ABSTRACT

The white blood cell (WBC) count in peripheral blood of adult South Polar Skua (*Catharacta maccormicki*) was carried out. The blood smears were prepared during the 2001/02 season near Ukrainian Antarctic Station Akademik Vernadsky at Galindez Island (Argentine Islands Archipelago). Blood smears were fixed in 96% ethanol and air-dried. The slides were stained and scored under magnification 100x20. For each individual the number of WBC per 10.000 mature erythrocytes was scored.

The smears were analyzed for the relative total leukocyte number, WBC differential (percentage of heterophils, eosinophils, basophils, lymphocytes and monocytes), and ratio of heterophils to lymphocytes (H:L).

These parameters are reliable and sensitive indicators of bird's general health. We evaluated also the level of genome instability of the same birds by estimation of micronuclei (MN) and other nuclear anomalies (NA) frequency in mature erythrocytes. A mean total leukocyte count was $2,83 \pm 0,8 \%$ per 10000 mature erythrocytes. Mean WBC differential was $54,22 \pm 3,16\%$ heterophils, $1,72 \pm 0,03 \%$ eosinophils, $2,36 \pm 0,66 \%$ basophils, $4,88 \pm 1,27 \%$ monocytes and $36,82 \pm 3,15 \%$ lymphocytes. Mean H:L ratio was $2,34 \pm 0,39$. The average rate of MN was $0,09 \pm 0,04$ per 10 000 erythrocytes. The average rate of total NA was $0,96 \pm 0,17$. The relation of WBC count and the level of genome instability will be discussed.

INTRODUCTION

The white blood cell (WBC) differential is a percentage of different type of white blood cells based on a count of leucocytes on blood smears. Heterophils, basophils, eosinophils, lymphocytes and monocytes are all included. The WBC differential depends on concentration of different white blood cells in the blood. The WBC differential is a good, sensitive indicator of the bird's general health: a change can be noted when no other abnormalities are detected. It is the one of the most important tests to perform with smears of blood collected in the field. Another indicator that may be checked with blood smears is the rate of micronuclei and nuclear anomalies of the cells that reflect the level of chromosome instability.

Changes in the WBC differential are often non-specific, however, reflect response of an organism to anyone stressful impact (various physiological and pathological factors). If all parameters of the WBC differential are normal, this is a good indication that the bird is not currently fighting some type of generalized infection or inflammatory disease process. Another indicator - the ratio of heterophils to lymphocytes (H:L ratio) is an reliable index of avian stress. Thus, analysing the blood smears of birds we may obtain important information on the state of bird's health. That is why it is quite important to know the range of individual variation of WBC differential and distribution of different parameters of WBC in the population.

Here we present data on WBC differential of skua and on correlation between body weight, index of stress (H:L), and chromosome instability (NA).

OBJECTIVE

To figure out the WBC differential and H:L ratio for *Catharacta maccormicki* and to evaluate possible correlation between studied indices of WBC differential, chromosome instability and body weight.

METHOD

Blood samples from 45 adults of South Polar Skua (*Catharacta maccormicki*) were analysed. The blood samples of birds were collected near Ukrainian Antarctic Station Akademik Vernadsky at Galindez Island (Argentine Islands Archipelago) during austral summer 2001-2002.

Slides preparation. Blood was obtained by incision of pounce of the leg 5-th rudimentary finger. The smears were fixed in 96% ethanol for 30 min, and stained with a combination of May-Grunwald and Geimsa stains.

Cytogenetic and statistical processing. The smears were scored at magnification 100x20 under oil immersion. White blood cells (WBC) count was performed. The total leucocyte number, WBC differential (percentage of heterophils, eosinophils, basophils, lymphocytes and monocytes), and ratio of H:L were estimated. The birds' chromosome instability was tested using micronucleus and nuclear anomalies tests. Micronuclei (MN) and other nuclear anomalies (NA) were counted in mature erythrocytes of the same birds. Studied parameters were counted and estimated per 10.000 mature erythrocytes for each individual.

RESULTS

A. WBC count

There are five types of WBC's: granulocytes - heterophils, eosinophils, basophils and mononuclear cells - lymphocytes and monocytes. Some of them are shown on Fig.1. Mean, standard error, limits, confidence interval, standard deviation and variance of numbers of different types of leucocytes and H:L ratio for *Catharacta maccormicki* are presented in Table 1.

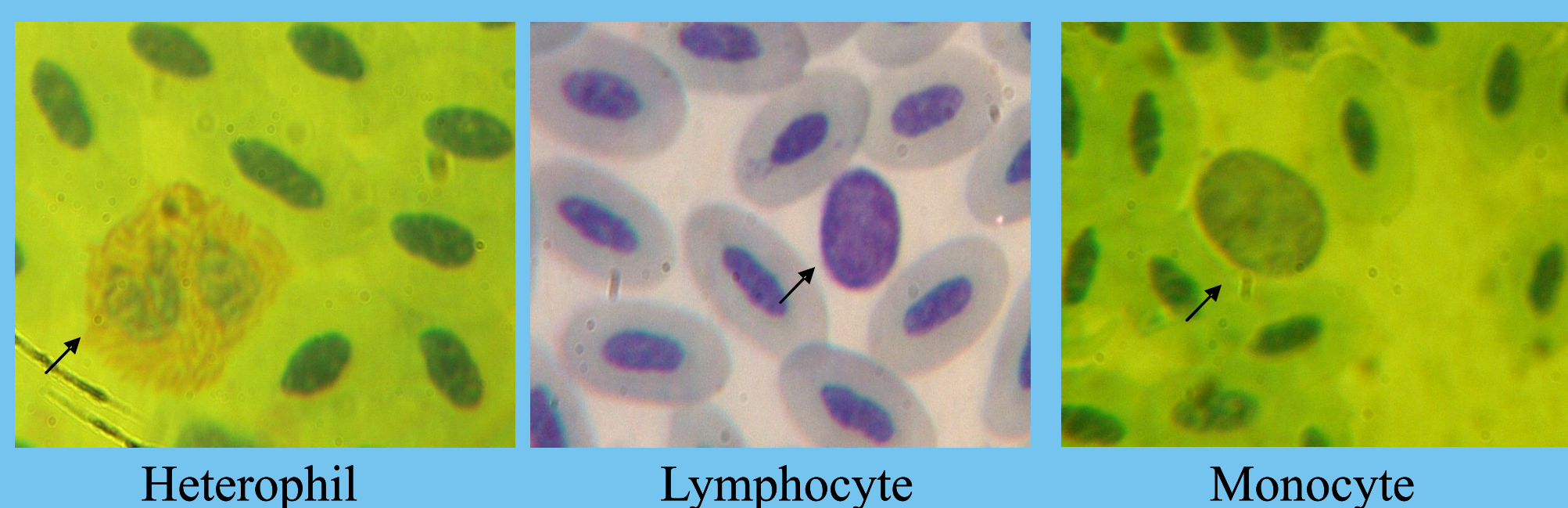


Fig. 1. Heterophil, lymphocyte and monocyte of *Catharacta maccormicki*.

Table 1. WBCs and H:L ratio (n = 45).

Number per 10000 RBC	Mean	± SE	Limits (min-max)	Confidence interval (P=0,005)	SD	Variance
Heterophils	16,71	± 2,25	1,0 - 80,0	14,18-21,24	15,08	227,48
Lymphocytes	9,42	± 1,10	0 - 34,0	7,21-11,63	7,35	54,07
Monocytes	1,20	± 0,28	0 - 8,0	0,63-1,77	1,9	3,62
Basophils	0,58	± 0,17	0 - 6,0	0,24-0,92	1,14	1,29
Eosinophils	0,42	± 0,12	0 - 3,0	0,18-0,66	0,81	0,66
H:L	2,34	± 0,39	0 - 13	1,55-3,13	2,62	6,87
WBC	28,33	± 2,84	6 - 109	22,61-34,05	1,90	67,2

RBC = red blood cells; SE = Standard error; SD = Standard deviation

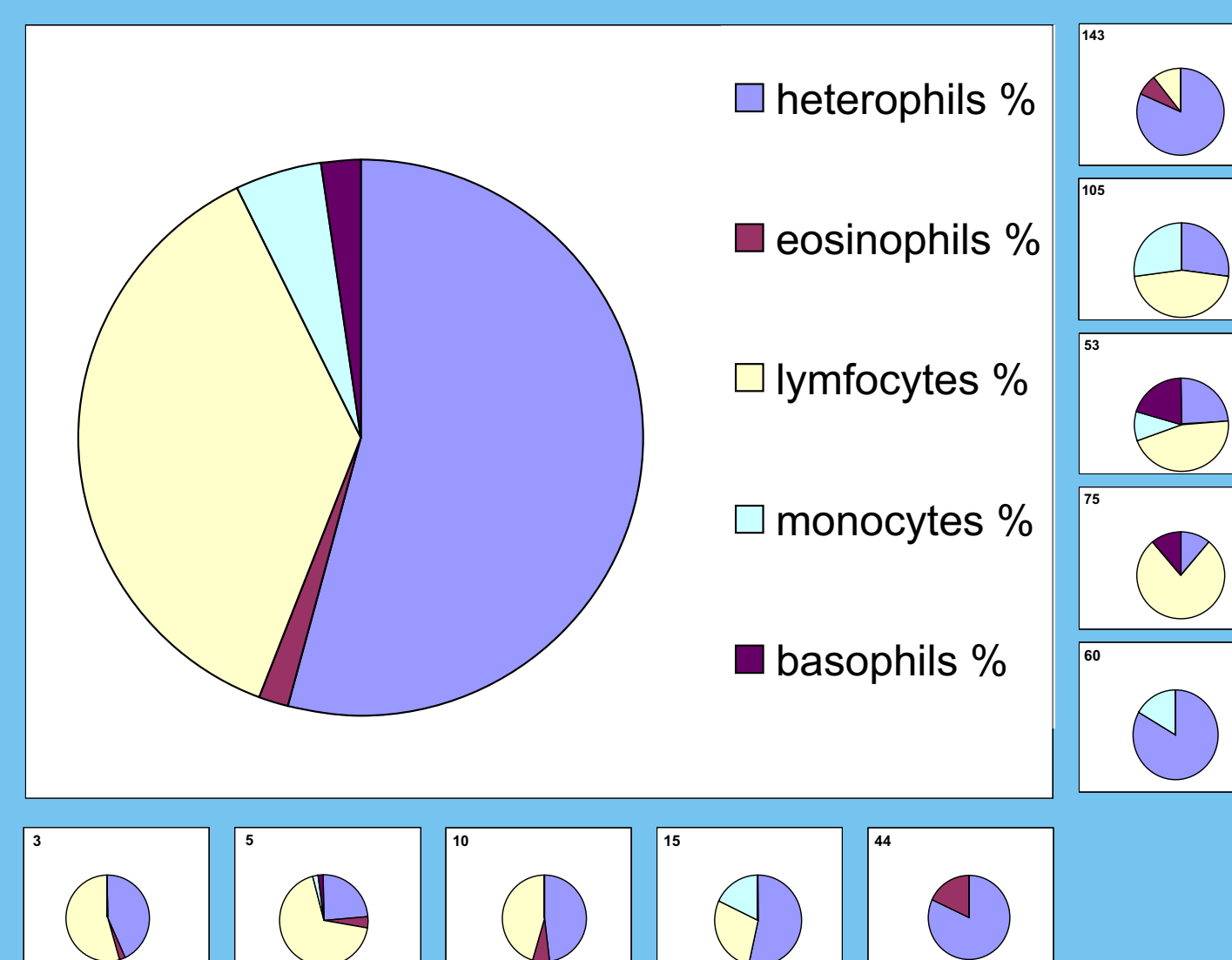


Fig. 2. White blood cell differential.

For *Catharacta maccormicki* the total leucocytes number is $28,3 \pm 2,84$ per 10000 erythrocytes (Table 1.) that is 0,28 % of the erythrocytes number. The normal ranges of total leucocytes proportion in blood are approximately 0,5-1,0 % of erythrocytes number for birds (in particular, for psittacine birds are 0,1-0,6 %), and 0,1-0,2 % for mammals (for human they are 0,07-0,27 %.) [Nozdrachev, 1991; McDonald, 1996].

The WBC differential, found in the present study (Fig. 2.), is within the normal ranges for birds, which may vary significantly for different species [McDonald, 1996]. The individual variability of this trait for *Catharacta maccormicki* seems enough high (Fig.2) The most variable parameter of WBC differential was heterophils number. The less variable were eosinophils, basophils and monocytes numbers (Table 1).

It is known the H:L ratio is reliable physiological index of avian stress. The H:L average rate of South Polar Skua equals $2,34 \pm 0,39$ and is relatively high for birds. For instance, the H:L ratio for white leghorn chickens is 1,65 [Gross, 1992], for penguin *Pygoscelis papua* is $1,35 \pm 0,08$ [Afanasieva et al., 2004].

B. Micronucleus and nucleus anomalies tests

Micronucleus test consists in calculation of micronucleus frequency in interphase cells of tissues with certain level of mitotic activity. Micronuclei appear during mitosis and are derivatives of acentric chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division. Our previous data showed that micronuclei in bird's erythrocytes appear quite rarely. Therefore, we used also other nuclear anomalies as additional parameters of genome instability. We classified more frequently registered NA into "budding nucleus" (BN), "two-lobe nucleus" (TLN) and "tailed nucleus" (TN) and described (see Fig. 3).

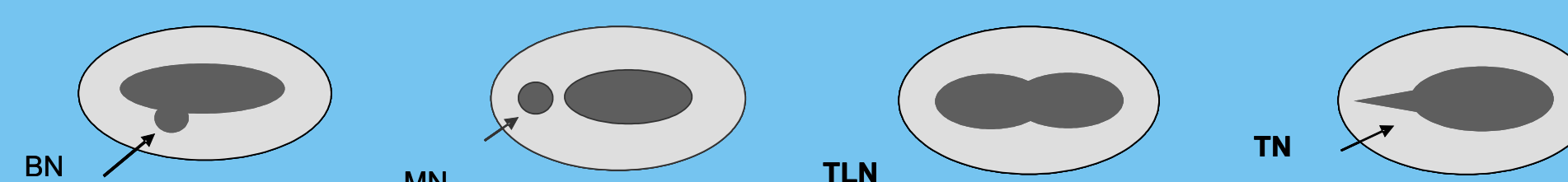


Fig. 3. Nuclear anomalies

As nuclear anomalies had enough low frequency we presented them as a sum marked NA. The average rate of MN and NA of *Catharacta maccormicki* are presented in Table 3.

Table 2. Micronuclei (MN) and nuclear anomalies (NA).

Number per 10000 RBC	Mean	±SE	Limits (min-max)	Confidence interval (P=0,005)	SD	Variance
MN	0,09	±0,04	0-1	0-0,18	0,29	0,08
NA	0,96	±0,17	0-4	0,63-1,39	1,11	1,23

RBC = red blood cells; SE = Standard error; SD = Standard deviation

Usually the level of micronuclei in birds is much less than in mammals. For some species micronuclei weren't find (*Cassidix melanicterus*, *Polyborus plancus*); while owl (*Otus sp.*) have rather high rate (15,8 %). In most cases the frequency of MN is in limits 0,4 - 4,3 % [Zuniga-Gonzalez et al., 2001]. For penguin *Pygoscelis papua* the average rate of MN is $0,05 \pm 0,02 \%$ and NA is $0,22 \pm 0,02 \%$ [Afanasieva et al., 2005]. Thus, South Polar Skua have very low rate of MN (0,01%) and NA (0,096%) that indicate high level of chromosome stability.

C. Correlation of H:L ratio with NA and body weight

Distribution of H:L ratio, NA number and body weight are presented at the Fig. 3a. In most cases the H:L ratio is 0,01-4, NA number is 0-1 per 10000 erythrocytes, body weight is within ranges of 1,011-1,190 kg.

The checked parameters may reflect the stress level and/or the immune state of individual (H:L), the level of genome instability (MN and NA) and the general health condition (body weight). It was supposed that individuals with extremely high or low values of the certain parameter may have different values of other parameters. According to distribution we discriminated the modal group (Fig. 4A, circled values) and the extreme group (rest of the sample), located at the tails of distribution. The modal group (Fig 4B, marked blue) consisted from 35 birds for H:L ratio distribution, 33 birds for NA and 34 birds for body weight distributions respectively (total sample N = 45 birds).

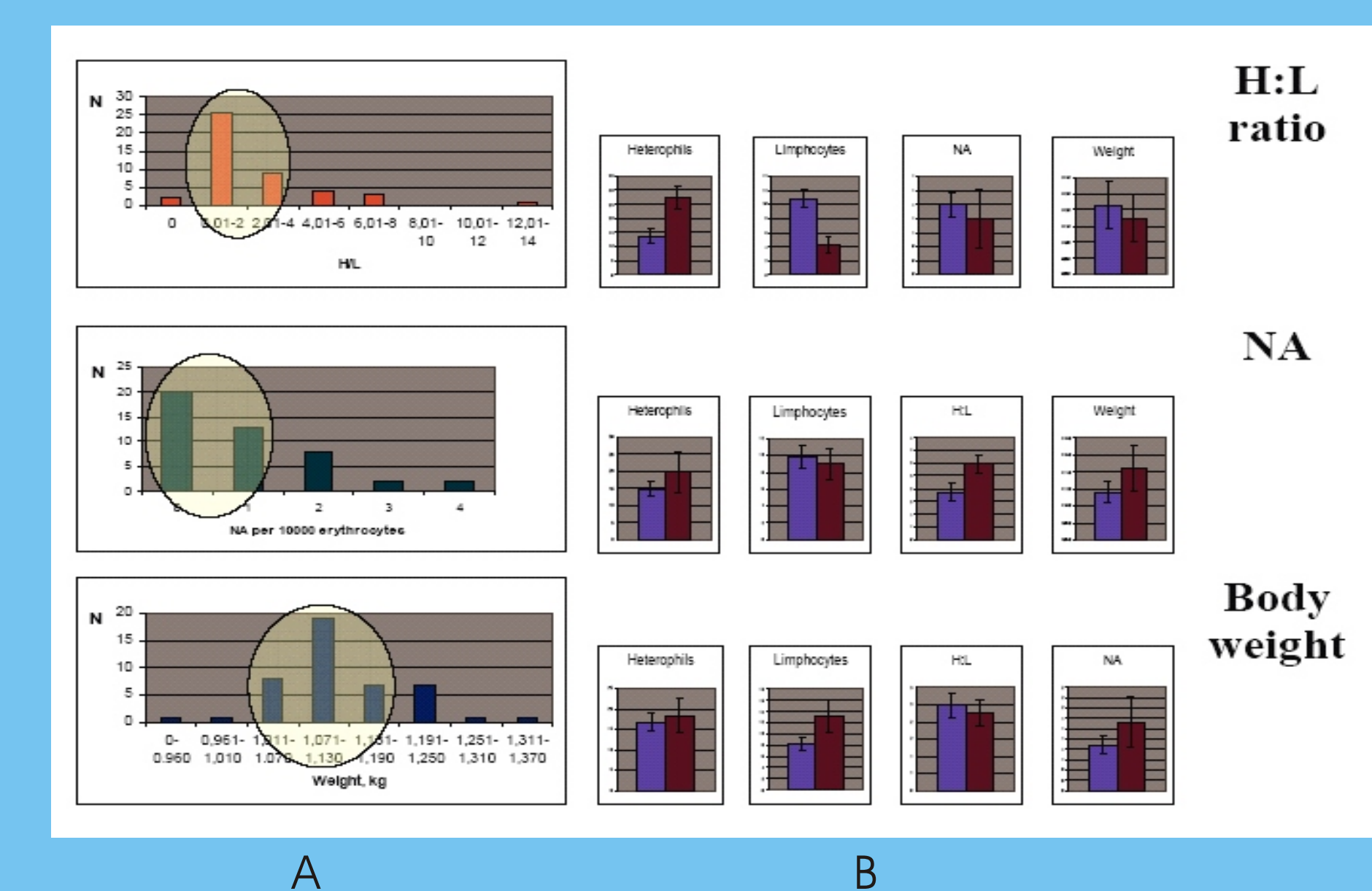


Fig. 4. Distribution of H:L ratio, NA number and body weight.

The modal and extreme groups for H:L ratio were quite different also for heterophils and lymphocytes numbers. At the same time no significant differences were found for such parameters as NA frequency and body weight.

Birds with different level of NA (a parameter of genome instability) – modal and extreme groups – had significant differences for H:L ratio, manifested differences for heterophils number, and insignificant differences for lymphocytes number and body weight.

Similarly, birds from the modal and extreme groups for body weight differed significantly for lymphocytes, non significantly for heterophils and H:L, and had manifested differences for NA.

Thus, the presented data let us to suppose, that the most fundamental features of skua (body weight, index of stress, and genome instability) are correlated by the certain way.

Conclusion

The white blood cell count in peripheral blood of adult *Catharacta maccormicki* was performed. The slides were stained and scored under magnification 100x20. For each individual the number of WBC per 10.000 mature erythrocytes was scored.

The smears were analyzed for the relative total leukocyte number, WBC differential (percentage of heterophils, eosinophils, basophils, lymphocytes and monocytes), and ratio of heterophils to lymphocytes (H:L).

The level of genome instability of the birds by estimation of micronuclei (MN) and other nuclear anomalies (NA) frequency in mature erythrocytes was evaluated. A mean total leukocyte count was $2,83 \pm 0,8 \%$ per 10000 mature erythrocytes. Mean WBC differential was $54,22 \pm 3,16\%$ heterophils, $1,72 \pm 0,03 \%$ eosinophils, $2,36 \pm 0,66 \%$ basophils, $4,88 \pm 1,27 \%$ monocytes and $36,82 \pm 3,15 \%$ lymphocytes. Mean H:L ratio was $2,34 \pm 0,39$.

The average rate of MN was $0,09 \pm 0,04$ per 10 000 erythrocytes. The average rate of total NA was $0,96 \pm 0,17$. There is certain correlation between body weight, index of stress (H:L), and genome instability (NA).

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Acknowledgements. We thank to colleagues of National Taras Shevchenko University of Kyiv and National Antarctic Scientific Center of Ukraine for their constructive comments and helpful suggestions. This work was partly supported by grant INTAS-2001-0517.