# „Factors affecting the body condition and immuno-physiological parameters of the High Arctic seabird, Little Auk (Alle alle)"

**Izabela Kulaszewicz**

In the bird's annual cycle, there are several phases that require increased energy expenditure of the organism, like migration, moulting and breeding period. Breeding is considered as one of the most time and energy demanding phase of the birds annual cycle (Drent & Daan 1980; Walsberg 1983). During breeding, adult birds need more energy than in any other part of the year to meet the requirements associated with reproductive activities (hormonal changes, egg formation). The energy-consuming activities associated with reproduction include: territory and mate guarding, finding a partner, nest building, egg incubation, brooding and chick feeding. These high energy requirements may be meet by increased foraging intensity. However, if breeders fail to modify their foraging behavior and time budget in a way that allows all of these extra energy expenditures to be met they may limit the available energy resources of the organism, used and invested in immune defense (Sheldon & Verhulst 1996) or egg incubation (Moreno & Carlson 1989; Bryan & Bryant 1999). Since the proper and effective functioning of the immune system plays a significant role as a defending the organism against infection with pathogens and parasites (Roitt et al. 1998), the reduction of immunocompetence usually results in a reduction in the individuals fitness. The fact that the maintenance of immune system is costly for organism has been proven many times (Råberg et al. 1998). Also short-term decrease in the food availability, which results in a reduction in the amount of acquired food may result in decrease in immunocompetence (Klasing 1988). In addition, decreased food availability may increase the foraging time of an adults, which may result in prolonged hatchling exposure to adverse environmental conditions (low temperature) (Kidawa et al. 2011). Nesting in polar regions also means energy challenges for the avian organism resulting from low temperatures, strong winds and short breeding season. Meteorological conditions can directly affect offspring, especially in the case of species developing full thermoregulation a few days after hatching (Krijgsveld et al. 2003, Tortosa & Castro 2003). Growth and development of birds are strongly dependent on temperature (Gillooly et al. 2002). It is believed that a proper egg temperature maintained by an adult during incubation is crucial for the appropriate development of birds embryos (Webb 1987). It has been proven that in several species prolonged exposure to low temperatures reduces the development rate (Tazawa et al. 1989), extends the incubation period (Lyon & Montgomerie 1985) and may affect negatively chick growth after hatching (Sockman & Schwabl 1998).

Parental investments are expenditures (in the form of time, energy, etc.) which increase the chance of survival of their offspring while at the same time reducing the condition and survival of the parent (Trivers 1972). In a breeding system where both birds from a pair participate in the care of the offspring (incubation and feeding of the chicks), the reproductive efforts of both partners may be different in terms of the nature of the performed duties. Seabirds, due to the high costs of foraging, have a monogamous reproductive system, with similar participation of both sexes in breeding care (Gowaty 1996). They are characterized by longevity, long period of individual development, a small number of eggs in brood (1-3) and extended parental care (Ricklefs 1990). Therefore, despite the participation of both parents, the annual reproductive success is small. However, such a strategy allows both parents to maintain good condition and energy reserves for the next breeding season. In addition, seabirds are characterized by a low degree of extra-pair paternity (Quillfeldt et al. 2001; Wojczulanis-Jakubas et al. 2009). Given all these features, seabirds are a very interesting group in the context of research on the mechanisms controlling hematological and biochemical parameters during the breeding season in both sexes.

Choosing the little auk (*Alle alle*) as a research species is associated with its life strategy and a specific breeding site (nest burrows in scree in climate with low air temperatures) and the ability to collect sufficient sample size for research (nesting in large breeding colonies with relatively easy access to the nests). The little auk breeds exclusively in the High Arctic. It is a monogamous and long-lived seabird with biparental care. Both partners are involved in egg incubation, brooding and feeding of the chick. Little auks lay only one egg annually (Wojczulanis-Jakubas et al. 2009). Therefore the quality of offspring is an important element influencing the demographic parameters of the population. Adults should maximize parental investment for effective chick rearing and having offspring of the best quality. The fact of nesting the Arctic regions also allows to analyze the impact of the environmental conditions (low air temperature) on the development of the chicks.

Tracing immunophysiological parameters in consecutive phases of the reproductive period gives an opportunity to study trade-offs between reproduction investments and maintaining own oxidative or immunological balance in the parent birds. There are studies reporting reduced immunocompetence in individuals in condition of low food availability; they had to increase their energy expenses in order to feed chicks (Norris & Evans 1999). According to the 'oxidative cost of reproduction' hypothesis (Alonso-Alvarez et al. 2004) resources allocated to reproduction are no longer available to protect the animal against oxidative stress (Wiersma et al. 2004; Alonso-Alvarez et al. 2004; Costantini et al. 2016). For example, zebra finches (*Taeniopygia guttata*) responded to artificially increased breeding effort (brood size enlargement) by increase in antioxidants level in blood (Alonso-Alvarez et al. 2004). Studies on resources allocation also indicate reduction of immunocompetence and growth rate in embryos or chicks exposed to prolonged low ambient temperatures both during incubation and period before developing full thermoregulation (Mujahid & Mitsuhiro 2009; Durant et al. 2012).

The aim of this doctoral dissertation is to analyze the factors affecting the condition and immunophysiological parameters of the little auk. I focused on three issues:

1) analysis of the relationship between reproduction and self-maintenance of the organism (the immune response to bacterial infection and body mass);
2) measuring of the oxidative stress level in relation to the phase of the breeding season and sex, and its influence on leukocyte profiles;
3) comparison of the ambient air temperature and nest burrow air temperature and their influence on the chick growth.

Respectively, I hypothesized that:
1) immunocompetence of the organism, measured using the level of bacteria killing capacity, H/L ratio and the number of leukocytes per 10,000 red blood cells, will decrease with the progress of the breeding season (growing parental investment); individuals with a higher body mass will have a higher immunocompetence (a higher value of the bacteria killing capacity, a lower H/L ratio); females may have lower bacteria killing capacity and body mass than males due to earlier brood desertion;
2) due to the increase in parental investments along with the progress of breeding season, the level of oxidative stress will be the lowest during incubation and the highest during chick rearing; one of the reasons for the female's earlier brood desertion may be her worse condition, manifested by a higher level of oxidative stress; the level of oxidative stress will be positively correlated with the H/L ratio, the number of heterophils per 10,000 red blood cells (rising during stress or chronic bacterial infection) and the number of lymphocytes per 10,000 red blood cells (mobilization of the immune system);
3) different location of nest burrows (altitude, meters above sea level) will affect the variability of inner nest temperature; nest burrows will have more stable air temperature compared to the ambient air temperature, the chicks from colder nests will grow slower due to higher costs of thermoregulation and they will be hatching later.

## Methods

• Fieldwork

The fieldwork was carried out in the colony of the little auks at Hornsund (south-west Spitsbergen), in three research seasons: 2013, 2014, 2015 (June-August). This colony is one of the largest on Spitsbergen (Isaksen 1995), which ensures the representativeness of the studied population as well as guarantees the possibility of obtaining the appropriate sample size (number of birds) for statistical analyses.

The following morphological and immunophysiological parameters of adult birds were investigated:

• Immunophysiological/biochemical parameters

**Immune response to bacterial infection.** The bacteria killing capacity of the birds blood includes many components of the innate immune system, including antibodies, proteins and lysozyme. It is considered as an indicator of innate immunity (Merchant et al. 2005). The presence of soluble proteins in blood plasma also plays a key role in reducing infection. Antibodies form molecules with abilities of non-specific detection of infection and have the potential to reduce microbial infection (Ochsenbein et al. 1999). For the purpose of this study, strains ATCC8739 of *Escherichia coli* bacteria representing a gram-negative group were used. This type of microorganisms was chosen mainly because of their prevalence and to minimize the possibility of misinterpreting the immune response to the occurrence of less common antigens. It is believed that for the elimination of *E. coli*, organism activates, mainly in the plasma, the complement cascade (Millet et al. 2007).

**Oxidative stress.** The level of oxidative stress is defined as the ratio of reactive oxygen metabolites (ROM) to antioxidants (OXY) (x 1,000) in blood plasma (Barja 2004). Oxidative stress occurs when the production of reactive oxygen species (ROM) exceeds the organism antioxidant capacity (Cadenas & Davies 2000). ROMs are a byproduct of respiratory chains and make up 0.1-4.0% of the oxygen molecules consumed during breathing (Cadenas & Davies 2000). They are unstable compounds that can damage: DNA, proteins, lipids, decrease gene expression, functioning of tissues and affect negatively the reproductive success and vitality of the organism. They can directly damage the reproductive system or reduce the quality of sperm and oocytes. Alternatively, ROMs can weaken the parents' somatic system and, consequently, reduce their physiological or physical ability to care for offspring and endurance for environmental conditions. In the long term, the accumulation of ROM can contribute to a gradual deterioration in the functioning of all organs and death (Barja 2004). Therefore, differences in susceptibility to oxidative stress may differentiate survival and fertility of an individual. Because reproduction is an energetically demanding period that increases the metabolic load (Nilsson et al. 2007) and, consequently, the production of ROM, individuals with a high level of resistance to oxidative stress can tolerate better reproductive costs for the organism.

**Leukocyte profiles**. The relative number of different types of leukocytes in peripheral blood is a measure frequently used to evaluation of the immune system (Davis 2005; Salvante 2006). The increased total number of leukocytes indicates the response to inflammatory processes caused both by microbiological reasons and infection with internal parasites (e.g,. Dein 1986). Lymphocytes and heterophils constitute the majority (almost 80%) of leukocytes in the peripheral avian blood (Campbell 1995). Lymphocytes are involved in the immune response in the fight against pathogens and their increase can also be observed during parasitic infections (Ots & Horak 1998). Heterophils are cells with the ability of phagocytosis; they are especially sensitive to microbial infections (Campbell 1995). Their number increases during stress, trauma and chronic bacterial infection. The ratio of heterophils to lymphocytes (H/L) is often used as an indicator of stress in birds (Bonier et al. 2007; Davis 2005). The remaining 20% of leukocytes represent three groups of white blood cells. Changes in the number of leukocytes in the blood were examined basing on the count of number of white blood cell per 10,000 erythrocytes. It dives a chance to note the changes in the absolute number of leukocytes (not just the proportions) allowing to indicate immunosuppression (Moreno et al. 2002).

• Chicks growth

Prolonged cold exposure may reduce the development rate (Tazawa et al. 1989), extend the duration of incubation (Lyon & Montgomerie 1985) and may negatively affect the growth of chicks after hatching (Sockman & Schwabl 1998). Changes in ambient temperature were previously associated with variation in breeding success of seabirds (Konarzewski & Taylor 1989; Skinner et al. 1998). To maintain the temperature in the optimal range for the embryo/chick development, birds must supplement the heat lost by incubating eggs / brooding hatchlings. Therefore, the quality of the nest and its location is considered as an important factor reducing the energy costs of nestlings development (Furness & Bryant 1996).

**Results**

• Relationship between reproduction and self-maintenance of the organism (immune system function and body mass) in adult little auks (article No. 1)

The level of bacteria killing capacity of adult little auks plasma was affected by the phase of the breeding season. Birds had a higher plasma bacteria killing capacity at the initial stages of the breeding season (during pre-laying period and at the beginning of incubation) compared to the later stages (end of incubation and chick-rearing period). The body mass of birds also influenced the level of bacteria killing capacity of the plasma. Individuals with a higher body mass index had a higher bacteria killing capacity.

The H/L ratio (hematological stress factor) was affected by the phase of the breeding season and the body mass index, but also by sex and the interaction between sex and season phase. Higher values of the H/L ratio occurred at the beginning of the breeding season as compared to the later phases for both sexes. During the pre-laying period, the H/L ratio in males was higher than in females.

The number of white blood cells per 10,000 erythrocytes was another variable affected by the phase of the breeding season was. The numbers of all three groups of blood cells (leucocytes, heterophils, lymphocytes) were higher at the beginning of the breeding season compared to the final phases. During the pre-laying period, males had a significantly higher number of heterophils per 10,000 red blood cells compared to females.

The body mass index of birds was also affected by the phase of the breeding season. Individuals at the early stages of the season had a higher body mass index compared to its final stages.

Bacteria killing capacity of the plasma increased with the increase of the H/L ratio, body mass index, the number of heterophils and leukocytes per 10,000 red blood cells.

- Oxidative balance of the organism depends on the phase of the breeding season in adult little auks (article No. 2)

The level of oxidative stress in adult little auks was affected by the phase of the breeding season. During the incubation the birds had a higher level of oxidative stress compared to the time of feeding the chicks. The body mass index did not affect oxidative stress.

The level of antioxidants (OXY) and reactive oxygen metabolites (ROM) also changed with the progress of breeding season. The level of antioxidants increased with the progress of the breeding season. During the incubation, concentration of OXY was lower compared to the hatching period. The opposite tendency has been found for ROM with significantly higher values recorded during incubation. As in the case of oxidative stress, also here the body mass index did not affect the value of OXY and ROM.

The values of oxidative stress and ROM concentration were positively correlated with the H/L ratio. The reverse pattern has been found for concentration of OXY decreasing with increasing the H/L ratio. The level of oxidative stress and the concentration of ROMs increased with increasing numbers of leukocytes and lymphocytes per 10,000 red blood cells. The concentration of OXY showed a reverse pattern increasing with the decreasing number of leukocytes and lymphocytes per 10,000 red blood cells.

- Influence of nest burrow microclimate on chick growth (article No. 3)

The nest burrows are well insulated from ambient conditions, having a more stable and higher average temperature in the daily cycle. In nests with a higher average temperatures, chicks at aged 15-18 days had higher values of the absolute growth rate basing on total head length and body mass. The same trend was observed in the case of an absolute growth rate calculated on the basis of the total head length in the second measurement cycle (chicks at age 21-24 days). During the first cycle of measurements (15-18 day old chicks), an increase in the average nest temperature by 1°C resulted in increase in the total head length and body mass by 0.81 mm and 0.77 g per day. There was no relationship between the average nest temperature and the date of hatching. Other not studied factors can also affect the growth rate of the chicks, however, given their growth at the limit of thermoneutral zone, influence of nest temperature seems to be considerable.

**Conclusions**

Reproductive effort of parental birds influenced their condition both in the aspect of bacteria killing capacity, leukocyte profiles, body mass index and oxidative stress level. The condition of both males and females decreased with the progress of breeding season (body mass loss, decreased bacteria killing capacity). The beginning of the breeding season was associated with the high values of oxidative stress and the H/L ratio. This result suggests an increase in the mobilization of the breeders organism. Resource allocation aimed at covering reproductive expenditures turned out to affect the condition of parental birds negatively.

In the case of chicks, nest chamber air temperature influenced their growth rate. Chicks from nests with higher temperatures were characterized by higher growth rate suggesting the allocation of larger energy resources for growth than for thermoregulation as in the case of chicks from nests with lower temperatures.

Realization of this project, which is my doctoral dissertation, has so far illustrated the poorly understood mechanisms that control the strength of the immunophysiological response over the energy demanding phase of avian annual cycle, breeding. The relationships between parental investments during the breeding season and the strength of the immune response to bacterial infection have been described so far only in a few seabird species. The project also provided data on the level of oxidative stress that has never been studied in this species before. In addition, project presented the relationship between the nest chamber air temperature and the chick growth, a factor that is so important in the Arctic climate with temperatures close to thermoneutral zone of little auks.

The results of this study can be interpreted in a broader context, e.g. of influence of parental investments on the immunophysiological condition in birds with low annual productivity.

## References


Alonso-Alvarez C., Bertrand S., Devevey G., Prost J., Faivre B. & Sorci G. 2004. Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecology Letters 7: 363–368.

Barja G. 2004. Free radicals and aging. Trends in Neurosciences 27: 595–600.

Bonier F., Martin P. R., Sheldon K. S., Jensen J. P., Foltz S. L. & Wingfield J. C. 2007. Sex–specific consequences of life in the city. Behavioral Ecology 18: 121–129.

Bryan, S. M. & Bryant, D. M. 1999. Heating nest–boxes reveals an energetic constraint on incubation behaviour in great tits, *Parus major*. Proceedings of the Royal Society of London B: Biological Sciences 266: 157–162.

Cadenas E. & Davies K. J. 2000. Mitochondrial free radical generation, oxidative stress, and aging 1. Free Radical Biology and Medicine 29: 222–230.

Campbell T. W. 1995. Avian Hematology and Cytology. Iowa State University Press, Ames, Iowa.

Costantini D., Casasole G., AbdElgawad H., Asard H. & Eens M. 2016. Experimental evidence that oxidative stress influences reproductive decisions. Functional Ecology 30: 1169–1174.

Davis A. K. 2005. Effects of handling time and repeated sampling on avian white blood cell counts. Journal of Field Ornithology 76: 334–338.

Dein, J. 1986. Hematology. Clinical avian medicine. Saunders, London, p. 174–191

Drent R. H. & Daan S. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68: 225–252.

Durant S. E., Hepp G. R., Moore I. T., Hopkins B. C. & Hopkins W. A. 2010 Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. Journal of Experimental Biology 213: 45–51.

Furness R. W. & Bryant D. M. 1996. Effect of wind on field metabolic rates of breeding northern fulmars. Ecology 77: 1181–1188.

Gillooly J. F., Charnov E.L., West G. B., Savage V. M. & Brown J. H. 2002. Effects of size and temperature on developmental time. Nature 417: 70–73.

Gowaty P. A. 1996. Partnerships in Birds: The Study of Monogamy: The Study of Monogamy Oxford University Press, New York.

Isaksen K. 1995. The breeding population of little auk (*Alle alle*) in colonies in Hornsund and northwestern Spitsbergen. Seabird population in the northern Barents Sea. Meddelelser 135: 49–57.

Kidawa D., Jakubas D., Wojczulanis–Jakubas K., Iliszko L. & Stempniewicz L. 2011. The effects of loggers on the foraging effort and chick–rearing ability of parent little auks. Polar Biology 35: 909–917.

Klasing K. C. 1988. Influence of acute feed deprivation or excess food intake on immuno–competence of broiler chicks. Poultry Science 67:626–634.

Konarzewski M. & Taylor J. R. 1989. The influence of weather conditions on growth of Little Auk *Alle alle* chicks. Ornis Scandinavica 20: 112–116.

Krijgsveld K. L., Reneerkens J. W., McNett G. D. & Ricklefs R. E. 2003. Time budgets and body temperatures of American Golden–Plover chicks in relation to ambient temperature. Condor 105: 268–278.

Lyon B. E. & Montgomerie R. D. 1985. Incubation feeding in snow buntings: female manipulation or indirect parental care? Behavioral Ecology and Sociobiology 17, 279–284

Merchant M. E., Pallansch M., Paulman R. L., Wells J. B., Nalca A. & Ptak R. 2005. Antiviral activity of serum from the American alligator (*Alligator mississippiensis*). – Antiviral Research 66: 35–38.

Millet S., Bennett J., Lee K. A., Hau M. & Klasing K. C. 2007. Quantifying and comparing constitutive immunity across avian species. – Develop. Comp. Immunol. 31: 188–201.

Moreno, J. & Carlson, A. 1989 Clutch size and the costs of incubation in the pied flycatcher *Ficedula hypoleuca*. Ornis Scandinavica 20: 123–128.

Moreno J., Merino S., Sanz J. & Arriero E. 2002. An indicator of maternal stress is correlated with nestling growth in pied flycatchers *Ficedula hypoleuca*. Avian Science 2: 175–182.

Mujahid A. & Furuse M. 2009. Oxidative damage in different tissues of neonatal chicks exposed to low environmental temperature. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 152: 604–608.

Nilsson J. Å., Granbom M. & Råberg L. 2007. Does the strength of an immune response reflect its energetic cost?. Journal of Avian Biology 38: 488–494.

Norris K. & Evans M. R. 1999. Ecological immunology: life history trade–offs and immune defense in birds. Behavioral Ecology 11: 19–26.

Ochsenbein A. F., Fehr T., Lutz C., Suter M., Brombacher F., Hengartner H. & Zinkernagel R. M. 1999. Control of early viral and bacterial distribution and disease by natural antibodies. – Science 286: 2156–2159.

Ots I. & Hõrak P. 1998. Health impact of blood parasites in breeding great tits. Oecologia 116: 441–448.

Quillfeldt P., Schmoll T., Peter H. U., Epplen J. T. & Lubjuhn T. 2001. Genetic monogamy in Wilson's storm–petrel. The Auk 118: 242–248.

Råberg L., Grahn M., Hasselquist D. & Svensson E. 1998. On the adaptive significance of stress induced immunosuppression. Proceedings of the Royal Society of London. Series B 265: 1637–1641.

Ricklefs R. E. 1990. Seabird life histories and the marine environment: some speculations. Colonial Waterbirds: 1–6.

Roitt I. M., Brostoff J. & Male D. K. 1998.Immunology. London: Mosby.

Salvante K. G. 2006. Techniques for studying integrated immune function in birds. The Auk 123: 575–586.

Sheldon B. C. & Verhulst S. 1996. Ecological immunology: costly parasite defences and trade–offs in evolutionary ecology. Trends in Ecology & Evolution 11: 317–321.

Skinner W. R., Jefferies R. L., Carleton T. J., Rockwell R. F. & Abraham K. F. 1998. Prediction of reproductive success and failure in lesser snow geese based on early season climatic variables. Global Change Biology 4: 3–16.

Sockman K. W. & Schwabl H. 1998. Hypothermic tolerance in an embryonic American kestrel (*Falco sparverius*). Canadian Journal of Zoology 76: 1399–1402.

Tazawa H., Okuda A., Nakazawa S. & Whittow G. C. 1989. Metabolic responses of chicken embryos to graded, prolonged alterations in ambient temperature. Comparative Biochemistry and Physiology 92: 613–617.

Tortosa F. S. & Castro F. 2003. Development of thermoregulatory ability during ontogeny in the white stork *Ciconia ciconia*. Ardeola 50: 39–45.

Trivers R. 1972. Parental investment and sexual selection. Aldine Publishing Company. Chicago.

Walsberg G. E. 1983. Avian ecological energetics. Avian biology. Academic press. London.

Webb D. R. 1987. Thermal tolerance of avian embryos: a review. Condor 89: 874–898.

Wiersma P., Selman C., Speakman J. R. & Verhulst S. 2004. Birds sacrifice oxidative protection for reproduction. Proceedings of the Royal Society of London B: Biological Sciences 271(Suppl 5): S360–S363.

Wojczulanis–Jakubas K., Jakubas D. & Stempniewicz L. 2009. Sex–specific parental care by incubating Little Auks (*Alle alle*). Ornis Fenica 86: 01–08.