Załącznik 3

Autoreferat

1. First name, last name: **Elżbieta Anna Kondera**

2. Diplomas, academic degrees:

2012 – Doctor of Biological Sciences in Biology (Ph.D.)

University of Natural Sciences and Humanities Siedlce, Faculty of Natural Sciences Ph.D. dissertation supervisor: prof. dr hab. Małgorzata Witeska

Doctoral dissertation: „Changes in hematopoietic tissue and peripheral blood of carp (*Cyprinus carpio* L.) under the influence of cadmium and copper"

2005 – Master in Biology (M.Sc.)

University of Podlasie, Siedlce, Faculty of Agriculture

M.Sc. thesis supervisor: prof. dr hab. Barbara Jezierska

Master's thesis: „Minimum concentrations of copper toxic to embryonic development of carp (*Cyprinus carpio* L.)"

2003 – Bachelor in Biology (B.Sc.)

University of Podlasie, Siedlce, Faculty of Agriculture

B.Sc. thesis supervisor: prof. dr hab. J. Uchmański

Bachelor's thesis: „The state of the environment in the municipality of Chorzów"

3. Employment in academic institutions:

a) from **10.2013 to present:** Assistant professor, Department of Animal Physiology, Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities,

b) **10. 2007 – 09.2013**: Assistant, Department of Animal Physiology, Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities,

c) **10. 2006 – 02. 2007:** Contract work (teaching Animal Physiology and Hematology lab), Department of Animal Physiology, Faculty of Agriculture, University of Podlasie, Siedlce,

d) **04. 2006 – 08. 2006:** Contract work (research project 2 P04G 011 29 "Physiological and developmental after-effects of short-therm heavy metal exposures in common carp juveniles", Department of Animal Physiology, Faculty of Agriculture, University of Podlasie, Siedlce,

e) **09. 2005 – 02. 2006:** internship, Department of Animal Physiology, Faculty of Agriculture, University of Podlasie, Siedlce,

In 2008, the name of Faculty of Agriculture was changed to Faculty of Natural Sciences, whereas in 2010 and the name of the University of Podlasie was changed to Siedlce University of Natural Sciences and Humanities.

4. Indication of the scientific achievement in accordance with Art. 16 paragraph. 2 of the Act of 14 March 2003. On Academic Degrees and Titles (Journal of Laws. No. 65, item. 595, as amended.)

a) Title of scientific achievement

„The effect of selected environmental factors on cellular composition and activity of head kidney hematopoietic tissue in fish"

b) Author/ authors, title/ titles, name and year of publication

Scientific achievement comprises the series of seven publications listed below. Value of impact factor (IF) and the number of points for journals, according to the scoring system of Ministry of Science and Higher Education, was presented in accordance with the publication years.

1. Sikorska J., **Kondera E**., Kamiński R., Ługowska K., Witeska M., Wolnicki J. 2018 Effects of four rearing temperatures on the performance of larval and juvenile crucian carp, *Carassius carassius*, under controlled conditions. Aquaculture Research 49(12): 3874–3880. DOI: 10.1111/are.13855

(IF_{2017}=1,475; 30 pts. MNiSW)

(my contribution – 35%), number of citations: 0

2. **Kondera E.**, Teodorczuk B., Ługowska K., Witeska M. 2018: Effect of glyphosate-based herbicide on hematological and hemopoietic parameters in common carp (*Cyprinus carpio* L). Fish Physiology and Biochemistry 44(3): 1011-1018. DOI:10.1007/s10695-018-0489-x .

(IF_{2017}=1,735; 20 pts. MNiSW)

(my contribution – 80%), number of citations: 1

3. **Kondera E.**, Kościuszko A., Dmowska A., Witeska M. 2017: Hematological and hematopoietic effects of feeding different diets and starvation in common carp *Cyprinus carpio* L. Journal of Applied Animal Research 45(1): 623–628 DOI: 10.1080/09712119.2016.1251926

(IF_{2018}=0,826; 15 pts. MNiSW)

(my contribution – 80%), number of citations: 3

4. **Kondera E.** 2014: Cell composition of the head kidney of European chub (*Squalius cephalus* L.). Archives of Polish Fisheries 22(4): 271-280. DOI: 10.2478/aopf-2014-0029

(IF_{2014}=0; 8 pts. MNiSW)

(my contribution – 100%), number of citations: 1

5. Witeska M., **Kondera E.**, Belniak N. 2013: Hematological and hematopoietic changes induced by formaldehyde and malachite green in common carp (*Cyprinus carpio* L.). Zoology and Ecology (23)3: 245-251. DOI: 10.1080/21658005.2013.821790

(IF_{2013}=0; 5 pts. MNiSW)

(my contribution – 45%), number of citations: 3

My contribution to each of five papers included in the scientific achievement consisted in proposing the hypotheses and designing the concept of the study, preparing the research scheme, conducting the laboratory experiments, interpreting the results, and writing the text of the manuscript. My average contribution to each of five articles presented as the achievement is 68%.

Total *impact factor* of five publications included in the scientific achievement = 4,036
Total MNiSW score for the publications included in the scientific achievement = 78

c) Discussion of the aims, results, conclusions and possible application of the works included in the scientific achievement

In fish, as in other vertebrates, lifetime of blood cells is limited (depending on the type of cells, it ranges from several days to several months), so these cells undergo repeated exchange during the life of an individual. Despite significant differences in the longevity of blood cells, the body maintains their number at a more or less constant level thanks to efficient hematopoiesis - the process of continuous proliferation and differentiation of blood cells occurring in the hematopoietic organs.

In most species of teleost fish (Teleostei), the dominant hematopoietic organ and the reservoir of blood cells is the head kidney - *pronephros*. It combines blood-forming, immune and endocrine function (production of blood cells, antibodies, cortisol and catecholamines). It is known that all hematopoietic cell lines, together with the hematopoietic stem cell, may be present in the fish head kidney. The available works show that the basic structures of fish hematopoietic tissue and hematopoietic mechanisms are very similar to those of other vertebrates. Data on histology and ultrastructure of hematopoietic tissue of fish head kidneys are scarce. There is also little information on the percentage of cells belonging to the particular lines in hematopoietic organs of bony fishes.

Peripheral blood parameters and blood cell morphology are often used to assess the health and general state of fish. Blood changes are also one of the early signs of the influence of environmental factors and a fairly sensitive indicator of physiological disorders (caused eg. by changes in physicochemical parameters of water, the presence of toxic substances, pathogens or other stressors). Changes in the values are often non-linear, which may indicate the effect of a particular factor on various physiological processes. However, it is not clear whether hematological changes observed in fish result from the action of environmental factors on circulating blood cells or from disorders of hematopoietic processes, because information concerning impact of environmental factors on the functions of the hematopoietic system in fish is missing in the literature.

There is no doubt that hematopoietic tissue of the head kidney is very active. The processes of cell proliferation, differentiation and maturation take place constantly. Proliferation and apoptosis are the basic processes that determine the effectiveness of blood formation. It can therefore be assumed that due to the high rate of cell exchange in the hematopoietic tissue of the fish head kidney, its sensitivity to various factors disturbing homeostasis is high. However, in the literature there is no information on the hematopoietic

activity of *pronephros*, although this knowledge is very helpful to interprete the changes in peripheral blood and immune system of fish.

The aim of the studies included the following detailed:

1. Presentation of detailed characteristics of head kidney hematopoietic tissue in cyprinid fish and indication of morphological features of hematopoietic cells common for the investigated species, making detailed photographic documentation facilitating identification of various cell types and immunocytochemical evaluation of the tissue to determine the blood-forming activity.
2. Determination of changes in cellular composition and activity of head kidney hematopoietic tissue in cyprinid fish in relation to the changes in peripheral blood caused by:
   - natural factors: (temperature, different diet and starvation),
   - anthropogenic factors: therapeutics (malachite green and formaldehyde) and glyphosate-based herbicide.
3. Creating a methodology for evaluation of the influence of various factors on the fish hematopoetic system and assessment of their bioindicative value.

The following is a summary of the results of the research published in a series of five papers submitted for evaluation as a scientific achievement.

The aim of study by **Kondera (2014)** was a detailed description of blood precursor cells in the head kidney of juvenile chub (*Squalius cephalus*) reared under optimal conditions, and comparison of the obtained results with available literature data. The head kidneys were isolated from fish organism, tissue smears were made and stained using Pappenheim method. From each fish, 500 blood cells were identified based on cell morphology (size, shape and color, presence or absence of granularity in the cytoplasm, size and location of cell nucleus), and their relative abundance was calculated. The study resulted in identification of 21 types of hematopoietic precursors and mature cells in the chub head kidney belonging to 8 main groups: unidentified blast cells (the youngest stages of various lineages were morphologically very similar, so they were included in one common group to avoid identification bias), erythroid, granuloid (neutrophilic and basophilic), lymphoid, monocytoid, and thrombocytoid. The lymphoid lineage was the most abundant, followed by granuloid, erythroid, thrombocytoid, blast, and monocytoid cells. Lymphocytes were the most frequently occurring

cells in the kidney and the most numerous of the lymphoid cells. Neutrophilic lineage was the most numerous among the granuloid cells. The erythropoietic series comprised five stages (erythroblasts: basophilic, polychromatic, and orthochromatic, accompanied by young and mature erythrocytes), more than the numbers of developmental stages of any other lineage. Only mature eosinophils and thrombocytes were found in chub hematopoietic tissue and no precursor cells of these lineages were observed. In summary, cellular composition of chub hematopoietic tissue was similar as in other fish species, although there were quantitative differences. It is obvious that the composition of hematopoietic tissue and the frequency of different cell lineages also change even in the same fish species and that this reflects the adaptation of organisms to changing environmental conditions.

The characteristics of head kidney hematopoietic tissue were used in subsequent works as an indicator of the influence of selected factors (natural and anthropogenic) on hematopoetic system in cyprinid fish. As a novelty, immunocytochemical methods were used in order to identify hematopoietic precursor cells in mitosis and apoptosis stages (standardized methods by Dako Cytomation). Cells in stage of proliferation showing the presence of proliferating cell nuclear antigen (PCNA) protein were treated with mouse monoclonal anti-PCNA antibodies (Dako) and visualized using the Dako mouse PCNA Envision System. In apoptotic cells, active caspase 3 (an enzyme participating in degradation of cytoplasmic and nuclear proteins) was detected using rabbit anti-caspase 3 active antibodies (Sigma) and visualized using the Dako rabbit anti-caspase Envision System. The brown-stained PCNA-positive and caspase 3-positive cells (other cells remained unstained) were counted in smears in at least 6 fields of view per 500 hematopoietic cells, and their percentage was calculated. The rate of cell exchange (hematopoietic activity) was also calculated as the ratio of PCNA-positive cells to caspase 3-positive fraction. In all experiments, quantitative and qualitative identification of blood cells in head kidney was made according to the uniform (described above) criteria.

Determination of thermal sensitivity and optimal temperature range for head kidney hematopoiesis in juvenile crucian carp reared at four different temperatures (22, 25, 28 and 31°C) are presented in work Sikorska J., **Kondera E.**, Kamiński R., Ługowska K., Witeska M., Wolnicki J. (2018). In this study, leucocyte count (WBC) decreased with temperature: at 28 and 31°C it was significantly lower than at 22°C. Differential leucocyte count also differed among the groups – the percentage of lymphocytes decreased, and that of neutrophils and monocytes increased with a temperature increase. Frequencies of lymphocytes and neutrophils in fish from the highest temperature significantly differed from the values in all other groups.

Differential leucocyte counts were also much more variable (higher standard deviations) at 28 and 31°C than at 22 and 25°C. It is noteworthy that the highest percentage of proliferating cells in head kidney was observed at 28°C, while the lowest at 31°C. However, the highest frequency of apoptotic cells occurred also at 28°C, while the lowest at 25°C. The maximum hematopoietic activity of head kidney (measured as the ratio of proliferating to apoptotic precursor cells) occurred at 25°C, and the lowest at 31°C. The frequency of hematopoietic early blast cells, erythroid cells and erythroblasts was highest in fish from 31°C. On the contrary, the abundance of lymphoid cells in this group was significantly lower compared to the other groups, while neutrophilic, monocytoid and basophilic cells were the most abundant. The highest level of thrombocytoid lineage in head kidney tissue was observed at 28°C. These results indicate changes in immunological status of fish at 31°C. Hematopoietic activity and leucocyte count were significantly disturbed at temperatures of 28 and 31°C. These temperatures caused stress to the juveniles, as shown by lymphopaenia and neutrophilia found in their peripheral blood and hematopoietic kidney. These results indicate that lower temperatures (25°C) are more favourable and safer for juvenile crucian carp.

Effects of starvation and malnutrition on composition and activity of hematopoietic tissue in common carp, and oxygen consumption rate were evaluated by **Kondera E.**, Kościuszko A., Dmowska A., Witeska M. (2017). Fish were fed Aller Aqua Classic (control diet), ground barley (diet of low nutritional value) or starved. The values of most peripheral blood parameters did not significantly differ among experimental groups but erythroblast frequency was reduced in starved fish. Much more differences were noted in hematopoietic tissue. In control fish significantly lower percentage of early blast cells and erythroid cells, was observed, compared to the other groups. Neutrophilic lineage was the least frequent in starved fish, and the most abundant in the control (statistically significant differences). Basophilic cells were most frequently represented in Aller Aqua Classic-fed carp, and the least in fish from the group fed with barley. Frequency of lymphoid, monocytoid and thrombocytoid cells did not significantly differ among experimental groups. The control fish showed significantly higher hematopoietic activity and highest oxygen consumption rate compared to the other groups, and starved fish - the lowest. Starvation or low-quality feeding did not cause anaemia or significant immunodeficiency in carp but reduced haematopoietic activity, which was directly related to the reduction of metabolic rate.

The effects of therapeutic doses of two drugs: formaldehyde (F) at a concentration of 63 mg/dm^3 and malachite green (MG) 3 mg/dm^3 on hematological and hematopoietic parameters in carp is presented in the work by Witeska M., **Kondera E.**, Belniak N. (2013). The results

showed transient macrocytic anemia in fish treated with both therapeutics (decreased RBC erythrocytes, mean hemoglobin concentration in MCHC blood cells, increase in MCV blood volume) followed by regeneration (RBC and MCV returned to the baseline values) and increase in hemoglobin Hb. Exposure of carp to MG caused an increase in the frequency of changed erythrocytes, while formaldehyde caused an increase percentage of erythroblasts. Both groups of fish showed a higher ratio of young to mature erythrocyte cells in head kidney, which indicates a rejuvenation of the erythroid line and indicates accelerated migration of cells into the peripheral blood and / or activation of erythropoiesis. None of the studied therapeutics caused changes in leukocyte differential count or oxidative metabolic activity of phagocytes (NBT). Only MG led to prolonged leukopenia. Both drugs increased the number of mature neutrophils, monocytes and basophils in hematopoietic tissue. Therapeutics also induced an increase in proliferative and apoptotic activity of hematopoietic cells, however formaldehyde activated more strongly proliferation, whereas malachite green significantly increased apoptosis. This resulted in a significant reduction in hematopoietic activity in fish exposed to MG, and a slight reduction of this process in carp treated with F. Both drugs, used at the recommended concentrations effective against parasites in fish, showed hematotoxic effects. However, malachite green was much more dangerous for fish compared to formaldehyde due to persistent immunosuppressive effect and strong reduction of hematopoietic activity.

In paper by **Kondera E.**, Teodorczuk B., Ługowska K., Witeska M. (2018), the effects of Roundup (commonly used in the world, glyphosate-based herbicide) on the composition and activity of carp hematopoietic tissue was presented. In the Roundup treated fish, hematological changes were observed, but most of them were not directly related to herbicide concentration. Herbicide in concentration 0.1, 0.5 and 5.0 mg/dm^3 of glyphosate caused an increase in hematocrit (Ht) and mean red cell volume (MCV) and a decrease in hemoglobin (Hb, MCH and MCHC) indicating anemia. Although there was no reduction in RBC red blood cells or an increased number of erythrocytes or haemolysis, higher hematopoietic activity (increased hematopoietic cell proliferation rate and frequency of early blasts) associated with a greater number of erythroblasts in the head kidney in fish exposed to Roundup, indicates accelerated erythropoiesis. Fish exposed to herbicide exhibited leukopenia and decreased oxidative metabolic activity of phagocytes (NBT), but without significant changes in the percentage of leukocytes. This means that the toxicant has influenced not only the number, but also the function of these cells. In the presented studies, there were no significant differences in the number of most hematopoietic cell lineages in Roundup-treated fish. However, the number of monocytioid, eosinophilic and basophilic cells significantly

increased compared to the control. The obtained results showed that sublethal concentrations of the Roundup herbicide that may appear in polluted inland waters, caused a slight anemic and significant immunosuppressive effect in juvenile carps, and on the other hand showed significant compensatory potential of the hematopoietic system of fish.

Conclusions

The research allowed to characterize qualitative and quantitative cellular composition of head kidney hematopoietic tissue in 3 species of cyprinid fish in which 21 types of cells were identified, classified into 8 main groups: blast cells, erythroid, neutrophilic, basophilic, monocytioid, lymphoid lineage, eosinophils and thrombocytes. It has been noted that morphology of hematopoietic cells in various fish species was very similar, but contribution of precursors of various cell lineages showed high intraspecific and interspecies variability. Although the composition of head kidney hematopoietic tissue in fish is more variable than peripheral blood, it is very sensitive to environmental factors and thus may be a useful indicator for assessing environmental impact on fish.

Analysis of hematopoietic tissue was helpful in determining hermal sensitivity of fish. Reduced hematopoietic activity, leukopenia and changes in the number of leukocytes in head kidney and peripheral blood in crucian carp showed stress response at unfavorable temperatures. The studies also showed that starvation and malnutrition decreased haemopoietic activity in carp, particularly reduced the rate of erythropoiesis by inhibiting the differentiation of blood cells in hematopoietic tissue. On the other hand, fish were quite resistant to malnutrition, as evidenced by the lack of peripheral anemia and significant immunodeficiency. Commonly used therapeutics caused transient macrocytic anemia in carp, but it was followed by regeneration within one week after exposure. Changes in hematopoietic tissue showed accelerated migration of juvenile erythrocytes to peripheral blood. Both therapeutics were toxic to fish but malachite green turned out to be more dangerous compared to formaldehyde due to persistent immunosuppressive effect and a strong reduction in hematopoietic activity. Low sublethal concentrations of glyphosate-based herbicide caused slight anemia and a significant immunosuppressive response in carp. On the other hand, the obtained results revealed a large compensatory potential of the hematopoietic system, which was activated in fish exposed to the herbicide.

Extension of hematological examination by analysis of head kidney hematopoietic tissue in cyprinid fish subjected to various environmental impacts turned out to be the right choice. Cellular composition of peripheral blood and hematopoietic tissue provided a more

complete information about the changes in fish organism than peripheral blood analysis alone. The obtained results showed very high homeostatic potential of the hematopoietic tissue that is able to compensate damage and loss of blood cells. The hematopoietic tissue of fish was also very sensitive to natural and anthropogenic factors of environment. Therefore, changes in cellular composition and activity of hematopoietic tissue can be used as a sensitive indicator of environmental impacts on fish. However, due to the high variability of the cell structure of the hematopoietic system of fish and the lack of unambiguous reference values (similarly as in the case of peripheral blood of the fish), it is important to provide an appropriate control group. Among the examined parameters, proliferative and apoptotic activity of precursor cells showed the highest bioindicatory potential.

5. Other scientific and research achievements

I graduated in 2005 at the Faculty of Agriculture at the_University of Podlasie in Siedlce obtaining M.Sc. Degree in Biology. My master's thesis: "Minimum concentrations of copper toxic to embryonic development of carp (*Cyprinus carpio* L.)" was supervised by prof. dr hab. Barbara Jezierska.

Then, I have cooperated with the Department of Animal Physiology initially as an intern (09.2005 - 02.2006), and as a contract employee (04 - 07.2006). At that time, I participated in the research project financed by the Committee for Scientific Research "Physiological and developmental after-effects of short-therm heavy metal exposures in common carp juveniles" (project number 2 P04G 011 29).

In the years 2007-2013, I was an assistant at the Department of Animal Physiology at the Faculty of Natural Sciences of the University of Natural Sciences and Humanities in Siedlce, and since 2013 I have been an assistant professor in this unit. My scientific interests are related to the influence of environmental conditions on hematological parameters of fish, but I also continue studies concerning the impact of environmental factors on the early stages of fish development. These studies are carried out in statutory research topics („The effect of toxic substances on fish juveniles” – 20/91/S, „The effect of environmental factors on early developmental stages: embryos, larvae and juveniles of cyprinid fishes” – 387/14/S) and own research projects („Embryonic and postembryonic development of cyprinid fishes” – 899/03/W, „The effect of some environmental factors on common carp blood ” – 532/93/W).

HEMATOLOGICAL STUDIES

When I joined the research team of the Department of Animal Physiology, the experiments concerned mainly the influence of metals on blood parameters. The purpose of hematological examinations was to determine the effects and after-effects of 3-hour exposure to 10 mg/dm^3 Pb on hematological parameters of carp (Witeska M., **Kondera E.**, Szymańska M., Ostrysz M. 2010). Throughout the experiment, fluctuations in red blood cell values were observed. There was also an increase in the frequency of abnormal erythrocytes (mainly nuclear anomalies), elevated to the end of the experiment. In the first days after exposure the percentage of immature neutrophils decreased, which was followed by a decrease in the frequency of mature cells, accompanied by a decrease in oxidative metabolic activity of phagocytes (NBT). Then there was a transient but significant reduction in the number of leukocytes after 8 days from the end of the exposure. Persistent reduction in basophil frequencies was observed up to 8 days after exposure. The changes suggest that toxic effects of lead interfered with nonspecific immune reactions in carp.

Comparison of the long-term impact of short-term exposure of juvenile carps on 2 mg/dm^3 Cu and 6.5 mg/dm^3 Cd on hematological parameters in carp was the subject of works: Witeska M., **Kondera E.**, Lipionoga J., Nienałtowska R. (2009) and Witeska M., **Kondera E.**, Lipionoga J., Jastrzębska A. (2010). During the experiment fluctuations of red blood cell parameters were observed. The level of damaged cells increased in Cu-treated fish shortly after exposure, whereas in groups exposed to Cd later. In the white blood cell system, copper induced significant but only transient leukopenia, whereas after exposure to cadmium the number of leukocytes remained significantly reduced until the end of the experiment. Percentage of neutrophils increased slightly, which was more evident in Cd exposed fish. In both groups there was an increase in phagocyte metabolic activity. The results show that Cu and Cd caused similar changes in white blood cell counts in carp, but cadmium-induced disorders were deeper and more persistent.

I also participated in studies evaluating the impact of short-term and long-term exposures to cadmium on the morphology of carp erythrocytes *in vivo* and *in vitro*. All exposures resulted in a significant increase the number of abnormal erythrocytes, nuclear anomalies being the most common. In fish subjecyed to a long-term cadmium exposure, there was an increase in the frequency of erythroblasts, which indicates a compensatory reaction to erythrocyte damage. It was also noted that cadmium nitrate caused more changes in the

morphology of carp erythrocytes than cadmium chloride or sulphate. The obtained results confirmed the genotoxic and cytotoxic properties of cadmium and showed that fish erythrocytes are good models for cytotoxicity studies. The results were published in the work Witeska M., **Kondera E.**, Szczygielska K. (2011).

The results of hematological studies of fish showed that metals cause different and nonlinear changes in the peripheral blood of fish. This indicates the movement of metals within the body and their effects on various physiological processes. However, it was not clear whether hematological changes are the result of metals only on peripheral blood cells or on hematopoietic system cells. For this reason, I broadened my research and undertook analyses the hematopoietic tissue of the head kidney of the carp. These studies were performed within the research project funded by the Committee for Scientific Research: "The influence of cadmium and copper on the hematopoietic system of fish" (N N304 0021 39). The aim of the study was to compare the effects of cadmium and copper on the cellular composition and activity of the hematopoietic tissue of the carp head kidney in relation to the changes in the peripheral blood and the dynamics of metals in the body, in short-term and long-term exposures. For a month, blood and organs were collected for analysis every week. Smears of hematopoietic tissue were done to assess the percentage composition and morphology of hematopoietic cells (May-Grunwald and Giemsa), and immunocytochemistry was used to identify cells at the stage of mitosis and apoptosis. Concentrations of Cd and Cu were also measured in blood, gills, liver, trunk kidney and head kidney using atomic absorption spectrophotometry (AAS).

The obtained results revealed that both metals: copper and cadmium caused a significant increase in the rate of apoptosis of hematopoietic progenitor cells and a slight increase in the rate of cell proliferation, which led to the reduction of fish hematopoietic potential. However, in all experimental groups, hematopoietic activity (the ratio of proliferating to apoptotic cells) was higher than 1, indicating that regeneration of hematopoietic tissue prevailed over destruction. Fish treated with cadmium (long- term) and copper (short- and long- term) showed an increase in the frequency of changed erythrocytes in the peripheral blood (mainly anomalies in the cell nucleus), effectively compensated by an increase the number of young_erythrocytes in blood, so that the number of red blood cells remained unchanged. In carp treated with Cu, this was accompanied by a slight decrease erythroid precursors in hematopoietic tissue, indicating an accelerated migration of immature cells into the peripheral blood. Short-term exposure in water with cadmium caused fewer anomalies in the structure of erythrocytes, which did not trigger the activation of

erythropoiesis and an increase in the number of erythroblasts released into the blood. In all carp treated with both metals, an increase the percentage of lymphocytes in blood was observed, with a significant decrease in the frequency of these cells in head kidney tissue of carps exposed to cadmium, and to a lesser extent also in fish treated with copper. However, the most serious consequence of intoxication was a decrease in the percentage of phagocytic cells: monocytes and neutrophils in the peripheral blood, which was accompanied by a rapid decrease in the metabolic activity of these cells. This decrease was not compensated by the hematopoietic system despite the observed increase in number of neutrophilic (short-term exposure) and monocytioid (short- and long-term exposure) cell lineage in the head kidney of carp treated with Cu. It was noted that both metals showed high affinity to head kidney, which was significantly higher than binding to other tissues. However, cadmium accumulation factor in trunk kidney, head kidney and liver was higher than for copper. The level of copper in the organs of studied fish increased slightly, and after the end of exposure quickly returned to the control level, while the cadmium content increased significantly and remained elevated for a long time.

The obtained results became the basis of my doctoral dissertation titled: "Changes in hematopoietic tissue and peripheral blood of carp (*Cyprinus carpio* L.) under the influence of cadmium and copper" (supervisor: Prof. Małgorzata Witeska). I obtained the Ph.D. degree in June 2012. The most interesting results of this research were also presented in the publications: **Kondera E.** (2011a)**,** **Kondera E.** (2011b)**, Kondera E.**, Witeska M. (2012), **Kondera E.**, Witeska M. (2013), **Kondera E.**, Ługowska K., Sarnowski P. (2014), and presented at the international scientific conference (The 4th Croatian Congress of Toxicology): **Kondera E.** (2012). In work **Kondera E.** (2019) the literature on haematopoiesis and hematopoietic organs in fish has been reviewed.

Since 2013, after being employed as an assistant professor, my scientific interests mainly concerned the influence of various factors on the cellular composition and the activity of hematopoietic tissue of head kidney in in relation to changes in peripheral blood. The aim of study by **Kondera E.**, Dmowska A., Rosa M., Witeska M. (2012) was a preliminary evaluation of the susceptibility of common carp to anemia and the rate of hematological renewal in fish subjected to experimental blood loss. The results of the study showed that 30% blood loss, due to very fast compensatory response, did not cause severe anemia in studied fish. It has been noted that RBC, Hb and Ht gradually increased, even to the values much higher than before blood loss. This was accompanied by an increase in the frequency of erythroblasts in peripheral blood and hematopoietic tissue. In addition, WBC increased, which was probably related to

lymphocyte release from the spleen or other lymphoid sites, because the percentage of blood lymphocytes and lymphoid lineage in the head kidney remained unchanged. Neutrophil frequency in the blood was also unchanged, however rejuvenation of their population occurred, which was accompanied by a decrease in total neutrophil abundance in hematopoietic tissue, indicating an accelerated migration to the blood. The percentage of circulating monocytes, basophils, and eosinophils decreased, which indicates that their recovery was slow. The obtained results revealed very high hematopoietic capacity in the carp head kidney. This was indicated by a significant increase in the frequency of early blast cells (precursors of various cell lineages) and RBC precursor cells at various stages of development even after 4 weeks of blood loss. This indicates that carp are able to recover from anemia caused by various adverse environmental factors.

The results of the studies on hematopoietic system sensitivity to other environmental factors of various fish species were presented in the works included in the scientific achievement (subsection 4b).

I am also a co-author of methodological studies in the field of fish hematology.

The aim of paper by Witeska M., Ługowska K., **Kondera E.** (2016) was to determine the reference values of peripheral blood parameters of juvenile common carp. It summarized the data obtained in 2006-2014 for groups 41-146 (different number of data for different parameters) of healthy carp, aged 5-8 months, harvested in autumn from ponds and acclimated to laboratory conditions for 3-4 weeks. One of the most stable parameters was lymphocyte count, moderately variable were red blood cell parameters (Hb, RBC, MCV, MCH and MCHC), while the number of thrombocytes and the percentage of phagocytic cells was highly variable.

In work by Lugowska K., **Kondera E.**, Witeska M. (2017), the values obtained during the counting of leukocytes by three different persons (WBC leukocyte differential count) were compared using three diluents and different methods. The obtained results showed that the human factor (the counting person) and the method of counting (directly in diluted blood using a hemocytometer or indirectly on smears, where it number was determined in relation to the number of erythrocytes) was more important than the type of diluent (Hayem, Natt-Herrick or Dacie).

A comparison of the most commonly used methods of blood sampling from fish (from the heart or caudal vein) was presented in Bojarski B, **Kondera E**, Witeska M, Ługowska K, (2018). The results showed that in carp the values of red blood cell parameters (RBC, Ht, Hb) were significantly higher in venous compared to cardiac blood, while other parameters (MCV,

MCH, MCHC, WBC, percentage of erythroblasts, changed erythrocytes, lymphocytes, neutrophils and thrombocytes) did not differ significantly.

The influence of rearing conditions on hematological parameters and carp susceptibility on experimental manipulative stress was the subject of study **Kondera E.**, Witeska M. (2019). In unstressed pond-reared fish higher RBC, WBC, Hb, phagocyte activity and lower MCV were observed, compared to the hatchery-reared fish at the same age and origin The reaction of both fish groups to manipulation was also different. In pond fish swelling of erythrocytes, strong leukopenia (lymphopenia and neutropenia) and decrease in phagocyte activity were observed, whereas in hatchery group there was an increase in RBC and phagocyte activity which indicates that fish from pond showed greater susceptibility to stress compared with fish reared in the indoor tanks.

I also participated in studies concerning hematological changes in juvenile carp at various stages of *Ichthyophthirius multifiliis* infection (showing slight or severe symptoms, and after a 3-week recovery period). In fish infected with the parasite no significant decrease in Ht and Hb values occurred, however, a decrease in RBC was observed, compensated by the increase in MCV and MCH. Carp with minor signs of invasion showed a significant increase in WBC, while significant leucopenia and changes in differential leukocyte count were observed in individuals at advanced stage of disease. In all fish, oxidative metabolic activity of phagocytes was significantly reduced. In addition, thrombocytosis and hypercoagulability was reported in the most severe stages of disease. After 3 weeks after the symptoms have resolved (as a result of treatment with a formaldehyde solution) WBC returned to the reference level. The results of these study were published in work Witeska M., **Kondera E.,** Ługowska K. (2010).

Comparisons of changes in hematological parameters after short-term (30 min) crowding of chub and carp were done by Witeska M., **Kondera E**., Ługowska K., Dmowska A. (2015). The hypoglycaemia was observed immediately after stress in both fish species. In chub, it was accompanied by a decrease of oxidative metabolic activity of phagocytes (NBT) and thrombocytopenia. After one week anemia occurred (decrease in Ht and Hb with erythroblastosis). In carp there was a decrease in the number of erythroblasts, and after a week there were also symptoms of leukopenia and thrombocytopenia. The obtained results indicate higher sensitivity of chub to stress caused by crowding compared to carp.

THE EFFECT OF ENVIRONMENTAL FACTORS ON EMBRYOS AND LARVAE

The first studies on the impact of environmental factors on early stages of fish development concerned the effect of copper on carp embryonic development and postembryonic. Carp embryos were exposed from fertilization for 2 h, 24 h or about 80 h (for hatching) in solutions with concentrations of 0.03, 0.05, 0.1 or 0.2 mg/dm^3 of copper. The minimum Cu concentration significantly reduced egg swelling, embryonic survival and the success of hatching was 0.1 mg/dm^3. At exposure throughout the embryonic development period of 0.03 mg/dm^3 Cu was the minimum concentration that caused a significant increase in the proportion of deformed larvae and significantly reduced their food activity compared to the control group. The most frequently observed body deformities included: curvature of the spine, deformation of the yolk sac and head, heart edema and shortening of the body. Development indicators for the most sensitive to copper were: the quality of hatched larvae and activity of their feeding. The results of these studies were published in **Kondera E.** 2016 and presented at international scientific conferences: "29th Annual Larval Fish Conference. Dose and time dependent copper toxicity to carp embryos, Barcelona" in 2005 and "Metals ions and other abiotic factors in the environment. Disturbances in common carp embryonic. Krakow" in 2006.

The study assessing the impact of 0.1 mg/dm^3 Cu or Cd on embryonic development of ide showed that both metals significantly reduced egg swelling, delayed the rate of embryonic development and changed the rate of hatching: Cu extended, and Cd shortened this process compared to the control group. Six types of body deformities were observed in the newly hatched larvae: curvature of the spine, C-shaped larva, deformed skull, deformed yolk sac, cardiac edema and shortened body. Only first two types of deformation were observed in the control group, while more complicated anomalies were more frequent in the groups treated with Cu and Cd. These studies showed that for the early stages of development of ide cadmium was more toxic than copper. The results of the research were presented at the international conference: Ługowska K., **Kondera E.** (2017): „The effect of copper and cadmium on early development of ide (*Leuciscus idus* L).” XLVI Annual meeting of European Society for new methods in agricultural research, Krakow-Wieliczka 2017.

The aim of the subsequent study was to assess the effects temperature on embryonic development of vimba. The embryos were incubated at 14, 18 or 20 °C. The temperature of 20 °C can be indicated as being more suitable for the development of the embryos than 18 °C, which is supported by a higher rate of development, survival and better quality of hatched

larvae. The temperature of 14 °C turned out to be too low for the incubation of embryos of this species. During cleavage, the highest number of morphological anomalies was observed at 14 °C, and during organogenesis it was more frequent at 20 than at 18 °C. The quality of hatched larvae, however, was significantly higher at 20 °C. The results of this research are presented in paper Ługowska K., **Kondera E.** (2018) and at an international conference Ługowska K., Kondera E., Dondzbach M. 2017: XLVI Annual meeting of European Society for new methods in agricultural research, Kraków-Wieliczka 2017, „Early development of vimba (Vimba vimba) at different temperatures".

My further career plans include, among others, studies on the impact of phytotherapeutics and plant protection products on the cellular composition and activity of the hematopoietic tissue of cyprinid fish head kidney and the peripheral blood parameters.

Załącznik 3

Summary of my scientific activity:

Total number of scientific papers: 24

- before Ph.D.: 8

- after Ph.D.: 16

Number of papers published in JCR-listed journals: 20

- before Ph.D.: 7

- after Ph.D.: 13

Number of other papers: 4

- before Ph.D.: 1

- after Ph.D.: 3

Total *impact factor* of publications, according to the year of publication: 15.525

- before Ph.D.: 4.323

- after Ph.D.: 11.202

Total numer of MNiSW (Polish Ministry of Scienceand Higher Education), according to the year of publication: 351

- before Ph.D.: 109

- after Ph.D.: 242

Number of conference presentations: 8

- before Ph.D.: 4

- after Ph.D.: 4

Number of citations (without self-citations), according to the Web of Science: 78

Hirsch index, according to the Web of Science: 6

Elżbieta Gondera

Siedlce, 05 .04. 2019