

UNIVERSITY OF PLOVDIV PAISII HILENDARSKI FACULTY OF BIOLOGY



Abstract of the materials under Art. 65 of PRASPU of chief assistant professor Stela Georgieva Stoyanova, PhD

regarding my participation in a competition for the occupation of the academic position "associate professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological sciences, scientific specialty Morphology announced in the State Gazette No. 98 of 19.11.2024

Compliance with the conditions for the occupation of the academic position "associate professor" under Art. 65 of the Regulations for the Development of the Academic Staff of University of Plovdiv "Paisii Hilendarski"

Since 2015, I have been an assistant in the Department of Developmental Biology at the Faculty of Biology of Plovdiv University. I obtained my PhD degree in June 2015, and from 2016 until now I have been working in the same department.

In regards with my participation in the contest for the academic position of "associate professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences (Morphology), announced in the State Gazette No. 98 of 19.11.2024, I present a total of 33 scientific works that have not been submitted in the procedures for acquiring my PhD degree and the academic position "chief assistant professor". In accordance with the minimum national requirements of the ZRASRB and the Regulations for the implementation of the ZRASRB, the attached scientific works are classified as follows:

- \triangleright Scientific papers for indicator B4 10 (Q2 1 paper; Q3 6 papers; Q4 3 papers).
- ➤ Scientific papers for indicator G7 23 (Q1– 2 papers; Q2– 3 papers; Q3 2 papers; Q4 16 papers).
- \triangleright Study guides 2.

The scientific works are published in English, with the exception of 2 study guides, which are published in Bulgarian. All scientific works are published in co-authorship, and in 8 of them I am the first author – in 2 papers according to indicator B4 and in 6 papers according to indicator G7. Total number of citations in world databases (Scopus) – 534.

Abstract annotation of scientific papers of

of chief assistant professor Stela Georgieva Stoyanova, PhD

to indicator B4 in accordance with the minimum national requirements of the ZRASRB and the Regulations for the implementation of the ZRASRB

B4_1. Yancheva V., Georgieva E., Velcheva I., Iliev I., Vasileva T., Petrova S., Stoyanova S. 2014. Biomarkers in European perch (*Perca fluviatilis*) liver from a metal-contaminated dam lake. Biologia, 69(11): 1615-1624. eISSN: 1336-9563

ABSTRACT: The present study was carried out in three seasons – spring, summer and autumn in Topolnitsa Dam Lake (Bulgaria) which has been subjected to continuous contamination with trace metals due to copper extraction in the area. The trace metal levels in surface water and liver samples of European perch (*Perca fluviatilis* L.) were investigated. We also linked the metal levels we determined with the various histological and biochemical changes which we observed. Lesions in the liver parenchyma were found to be degenerative and necrotic, as well as, they were presented as hyperemia which consequently leads to disturbances in the hepatic blood circulation. Activities of the hepatic enzymes lactate dehydrogenase (LDH), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were found to be significantly elevated, particularly in summer. Therefore, we could recommend that the investigated tissue and cell alterations may be successfully applied as reliable biomarkers for monitoring polluted with a mixture of trace metals freshwater ecosystems.

B4_2. Todorova K., Velcheva I., Petrova S., Yancheva V., Stoyanova S., Georgieva E. 2014. Effects of heavy metals on survival and oxygen consumption in common carp. Oxidation Communications, 37(2): 563-571. eISSN 0209-4541

ABSTRACT: Effects of brief toxicity test with Cd, Cd+Zn, Cd+Pb and Cd+Ni on behaviour, survival and oxygen consumption of carp (*Cyprinus carpio* L.) was studied ex *situ*. Cadmium intoxication was associated with oxidative stress leading to significant negative changes in all parameters investigated. Antagonistic relationships between Cd–Pb, Cd–Zn and Cd–Ni were found. Descending row of the studied heavy metals on the basis of their toxic effect on ish revealed as follows: Cd > Cd+Pb > Cd+Ni > Cd+Zn. Fish biotest, as a fast, non-expensive, easily applied and reliable method, could be included in an integrated environmental monitoring.

B4_3. Yancheva V., Stoyanova S., Velcheva I., Petrova S., Georgieva E. 2014. Metal bioaccumulation in common carp and rudd from the Topolnitsa reservoir, Bulgaria. Archives of Industrial Hygiene and Toxicology, 65(1): 57-66. eISSN: 18486312

ABSTRACT: Concentrations of arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) were determined in water samples and five fish organs (gills, liver, kidney, spleen, and muscle) of common carp (*Cyprinus carpio* L.) and common rudd (*Scardinius erythrophthalmus* L.) from the Topolnitsa reservoir (Bulgaria) in three seasons (spring, summer, and autumn). This water ecosystem is located in a copper mining and metallurgical region. Water metal concentrations were significantly higher in the summer than in the spring (p<0.05).

Moreover, As, Cd, Cu, and Zn concentrations were higher than the national limits. Qualitative factors "element" and "fish organ" had a stronger influence on metal bioaccumulation than the factors "season" and "fish species". In fish, the highest metal levels were detected in the liver, spleen, kidney and gills, and the lowest in the dorsal muscle. Tissue levels were higher in the summer, but in general they were similar between the two Cyprinid fish. Fish muscles had the lowest metal levels at all times, but As and Pb exceeded the national and international standards. Therefore, we would not recommend fish consumption from Topolnitsa, as continuous metal contamination of the reservoir may seem to present human health risk.

B4_4. Yancheva V., Velcheva I., Stoyanova S., Georgieva E. 2016. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: a review. Applied Ecology and Environmental Research, 14(1): 47-75. eISSN:1785-0037

ABSTRACT: Water contamination, both in freshwater and marine ecosystems, has been a serious environmental problem all over the world in the last few decades. One of the most common anthropogenic pollutants, which enter the water bodies are metals and metalloids with no biological functions (As, Cd, Hg, Ni and Pb), pesticides and other persistent organic pollutants such as PAHs, PCBs, tributil tins, dioxins, etc. Most of these pollutants tend to accumulate in biota, biomagnify in the food chains and they are also difficult to break down to less harmful substances. In order to better understand the negative effects on living organisms, and particularly fish, biomarkers at different levels (cell, tissue, organism and population) are applied. In addition, the biomarkers at tissue level such as histological alterations in different fish organs give valuable information about the xenobiotic impact. Thus, they are recommended as useful biomarkers in eco-toxicological research, risk assessment and monitoring programs. In the present paper we aimed to review the use of histological alterations in fish organs such as gills, liver and kidney in ecotoxicological studies, based on collected scientific data from the late 1960's until today.

B4_5. Yancheva V., Stoyanova S., Velcheva I., Georgieva E. 2016. Histological response of fish gills to metal pollution: Common Carp, *Cyprinus carpio* L., and Common Rudd, *Scardinius erythrophthalmus* L., from Topolnitsa Reservoir, Bulgaria. Acta Zoologica Bulgarica, 68(1): 103-109. eISSN: 2603-3798

ABSTRACT: Concentrations of six metals (As, Cd, Cu, Ni, Pb and Zn) were measured in surface waters of Topolnitsa Reservoir, Bulgaria, located in a region with intensive copper mining. The process of metal bioaccumulation in gills of two cyprinid fish species: common carp (*Cyprinus carpio* L.) and common rudd (*Scardinius erythrophthalmus* L.), was studied in spring, summer and autumn. Higher metal concentrations in both water and fish gill samples in summer were detected. Histological alterations in the gill surface tissues of both fish species and the degree of expression of lesions were determined in the three seasons. Mostly proliferative and degenerative changes in gill epithelium were observed. Changes in the blood circulatory system were presented as vasodilatation in the central venous sinus and secondary lamellae, as well as aneurysms. Overall, the extent and severity of lesions were more expressed in gills of the common carp gills than of in the common rudd. This is likely because the carp is a benthic feeder and more often has contacted with the sediments where the metal concentrations were times higher than in water. In addition,

the histological alterations were more severe in summer in both fish species, which could be linked to the higher concentrations of metals in water and gills in this season.

B4_6. Yancheva V., Mollov I., Velcheva I., Georgieva E., Stoyanova S. 2016. Heavy metal effects on the lysosomal membrane stability and respiratory rate in Chinese Pond mussel (*Sinanodonta woodiana*) under ex *situ* exposure: Preliminary data. Biharean Biologist, 10(1): 55-57. eISSN: 2065-1155

ABSTRACT: The Chinese pond mussel (*Sinanodonta woodiana*) is a unionid mussel, which is known to accumulate heavy metals, making it useful for biomonitoring. The current preliminary research aimed to study the lysosomal membrane stability in heamocytes of Sinanodonta woodiana by applying the neutral red retention assay (NRR), as well as changes in the respiratory rate under acute metal exposure. The mussels were treated with different concentrations of Ni and Pb in laboratory conditions for 72 h. After the 72nd h exposure to Ni and Pb the lysosomes retained the dye between 30 to 60 minutes in the mussels exposed to the higher concentrations. The respiratory rate was measured at the 24th and 72nd hour and it increased in a dose-dependent manner. The acute metal exposure, including all metal concentrations below the allowable concentrations, lead to destabilization of the lysosomal membrane stability and changes in the respiratory rate.

B4_7. Stoyanova S., Yancheva V., Iliev I., Vasileva T., Bivolarski V., Velcheva I., Georgieva E. 2016. Biochemical, histological and histochemical changes in *Aristichthys nobilis* Rich. liver exposed to thiamethoxam. Periodicum Biologorum, 118(1): 29-36.

ABSTRACT: The aim of the present study was to investigate the effects of the insecticide thiamethoxam on some biochemical, histological and histochemical parameters of bighead carp liver (Aristichthys nobilis), which is an economically important fish species for aquaculture. Different increasing concentrations of 6.6 mg l-1, 10 mg l-1 and 20 mg l-1 of the test chemical under laboratory conditions were tested for their toxicity on fish for 96 h. The hepatic activity of the enzymes lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were measured. In addition, the hepatic histological structure was observed for alterations, as well as the lipid content was observed by histochemical staining. It was determined that the enzymatic activity of the exposed fish was increased compared to the control group (p<0.05). Furthermore, the enzymatic activity was increased proportionally to the increasing thiamethoxam concentrations. The histological lesions, which were observed in the liver parenchyma, were degenerative and necrotic. The degenerative alterations were as follows: granular, balloon and fatty degeneration. The necrotic alterations in the fish liver were associated with presence of karyopyknosis, karyorrehsis and karyolysis, respectively. The histological alterations in the liver blood vessels were hyperemia and lymphocyte proliferation. Along with the established histological changes in the liver parenchyma, we found presence of fatty degeneration in the hepatocytes using Sudan III staining on cryostat sections. Our study shows that there is a relation between the concentration of the insecticide and biochemical changes, as well the severity of expression of the histological and histochemical alterations in the bighead carp liver. Overall, such experiments could be successfully applied in research and monitoring programs to study the effects of pesticides on fish.

B4_8. Georgieva E., Yancheva V., Velcheva I., Iliev I., Vasileva T., Bivolarski V., Becheva M., Stoyanova S. 2016. Histological and biochemical changes in common carp (*Cyprinus carpio* L.) liver under metal exposure. North-Western Journal of Zoology, 12(2): 261-270. eISSN: 1843-5629

ABSTRACT: Topolnitsa reservoir is located in a region with intensive copper mining, which has been constantly contaminated with metals in the last few decades. Thus, we measured As, Cd, Cu, Ni, Pb, and Zn concentrations in surface water samples, as well as, in liver of common carp in three different seasons – spring, summer, and autumn 2013. Furthermore, we also studied the carp response to the water quality by observing its hepatic morphological structure and measuring some biochemical parameters (LDH, ALAT, and ASAT activity). In general, metal concentrations in the water varied, but As, Cu and Ni were present in all the three seasons. Cu concentrations were above the maximum permissible levels set by law. Metal concentrations in the liver were significantly higher than in the water (p<0.05). In addition, severe hepatic histological alterations, which included degenerative changes – granular degeneration, vacuolar degeneration, hydropic degeneration, and fatty degeneration; necrotic changes (necrobiosis) – karyopyknosis, karyorrexis and karyolysis, and necrosis; and changes in the blood vessels – hyperemia in sinusoids and major blood vessels were observed. Moreover, the LDH activity was inhibited in the common carp liver from the Topolnitsa reservoir compared to the reference fish, but ALAT and ASAT activity was significantly increased compared to the reference. Based on our findings we consider that the metal-contaminated waters of the Topolnitsa reservoir lead to negative changes at tissue and cellular level in the fish organism, which include altered structure and impaired functions of the liver.

B4_9. Yancheva V., Mollov I., Georgieva E., Stoyanova S., Tsvetanova V., Velcheva I. 2017. Ex situ effects of chlorpyrifos on the lysosomal membrane stability and respiration rate in Zebra mussel (*Dreissena polymorpha* Pallas, 1771). Acta Zoologica Bulgarica, Supplement 8: 85-90. eISSN: 2603-3798

ABSTRACT: The present study examines the lysosomal membrane stability in haemocytes of Zebra mussel (*Dreissena polymorpha*) by applying the neutral red retention assay (NRRA) as well as the respiration rate and survival under acute pesticide exposure. The mussels were treated with different concentrations of chlorpyrifos in laboratory conditions for a total acute exposure period of 72 hours. The pesticide concentrations were prepared as 50 and 30% of the maximum permissible level (100%) set by the national and EU legislation. Destabilization of the lysosomal membrane stability occurred at all tested concentrations and the respiration rate was time- and dose-dependent were found. Overall, we consider that the results from such experiments can be successfully applied in risk assessment, monitoring programs and water policy, and the use of pesticides such as chlorpyrifos should be controlled very cautiously in plant protection and agriculture.

B4_10. Stoyanova S., Velcheva I., Yancheva V., Mollov I., Georgieva E. 2017. Biomarkers for ex situ Ni and Pb exposure in common carp (*Cyprinus carpio* L.). Acta Zoologica Bulgarica, Supplement 8: 163-168. eISSN: 2603-3798

ABSTRACT: This work aimed to study the effects of heavy metal exposure on the respiration rate and histological structure of common carp (*Cyprinus carpio* L.) gills. Fish were treated with different soluble concentrations of Ni and Pb in laboratory conditions for a total acute period of 72 hours. The metal concentrations were prepared as 75, 50 and 25% of the maximum permissible levels (100%) set by law. The results showed a higher index of respiration rate in the fish from all experimental tanks for both metals, compared to the control in the beginning of the experiment, but there was no pattern of increase or decrease in relation to the metal concentrations. After 72 hours of exposure we observed the same pattern, but in addition the respiration rate of the fish in the tanks treated with Pb showed an increase in a dose-dependent manner. We also observed different histological changes in the gill epithelium, which included proliferative and degenerative changes, as well as changes in the circulatory system. In addition, the degenerative changes were more pronounced in the fish, treated with Pb concentrations, and the blood circulatory system showed mainly vasodilatation, which caused pathological changes in the gills. In sum, we can conclude that Ni and Pb have severe effects on the respiration rate and gill histology of common carp, even at concentrations, which were lower than the allowable ones.

Abstract annotation of scientific papers of

of chief assistant professor Stela Georgieva Stoyanova, PhD

to indicator G7 in accordance with the minimum national requirements of the ZRASRB and the Regulations for the implementation of the ZRASRB

Γ7_1. Yancheva V., Mollov I., Velcheva I., Stoyanova S., Todorova K., Georgieva E. 2017. Lysosomal membrane stability and respiration rate in zebra mussel (*Dreissena polymorpha* Pallas, 1771) as biomarkers for *ex situ* heavy metal exposure. Periodicum Biologorum, 119(4): 229-237. eISSN: 1849-0964

ABSTRACT: The scientific publication includes a study that addresses to the lysosomal membrane stability in haemocytes of the invasive mollusk zebra mussel (*Dreissena polymorpha* Pallas, 1771) by applying the neutral red retention assay (NRRA), as well as changes in the respiration rate and survival under acute heavy metal exposure. The mussels were treated with different decreasing concentrations of nickel (Ni) and lead (Pb) in laboratory conditions for a total acute period of 72 hours. These metals are considered as priority substances in surface waters according to Directive 2013/39/EU of the European parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. The metal concentrations were prepared as 75, 50 and 25 % of the maximum allowable concentrations (100% MAC) set by law. In sum, after 24 and 72 h was found that the lysosomes in the mussels exposed to all Ni and Pb concentrations, including the ones below the MAC (75 and 50 % from MAC) retained the dye shorter than the accepted minimum of 90 min. In addition,

the respiration rate increased in a dose-dependent manner. Findings of such changes indicate that the acute metal exposure lead to destabilization of the lysosomal membrane stability and changes in the respiration rate of zebra mussel, therefore altered physiological functions. It is suggested that such experiments could be successfully applied in risk assessment and monitoring programs on metal-contaminated aquatic systems, and the obtained results could be used in the field of water policy, respectively.

Γ7_2. Stoyanova S., Yancheva V., Velcheva I., Mollov I., Todorova K., Tomov S., Tsvetanova V., Georgieva E. 2018. Glyphosate-based herbicide alters the histological structure of gills of two economically important cyprinid species (common carp, *Cyprinus carpio* and bighead carp, *Aristichthys nobilis*). Applied Ecology and Environmental Research, 16(3): 2295-2305. eISSN: 1785-0037 Q4(SJR 0.224) IF 0.34

ABSTRACT: The scientific publication investigates the histopathological effects which a glyphosate based herbicide could cause on the gills of two economically important Cyprinid fish (common carp and bighead carp), and to determine which species is more sensitive in terms of glyphosate contamination. The pesticide concentrations tested in laboratory conditions were decreasing (72 mg/l, 40 mg/l and 20 mg/l) and prepared by dilution of the stock solution of the commercial product used in plant protection. The experiment was short-term of 96 h. In general, the herbicide caused different pathological alterations in the fish gills, such as lamellar lifting, edema, proliferation of the glandular cells and epithelium, covering the gill filament, fusion, vasodilatation of the secondary lamellae and aneurysms. In addition, a tendency towards the enhancement of the gill histological changes which degree of expression was proportional to the increasing pesticide concentrations was observed. However, bighead carp was more sensitive compared to common carp when it comes to the tested chemical and the alterations in the gill histological structure were more pronounced.

$\Gamma7_3$. Yancheva V., Stoyanova S., Georgieva E., Velcheva I. 2018. Mussels in ecotoxicological studies – are they better than fish? Ecologia Balkanica, 10(1): 57-84. Q4(SJR 0.103)

ABSTRACT: EU Member states are required to apply the EU Water Framework and its Daughter Directives in order to achieve Good Environmental Status (GES) for all 11 qualitative descriptors by 2015 in all water bodies for a list of priority and specific pollutants. Therefore, environmental indicators and biological-effect techniques have to be carefully selected for the management of chemicals in the aquatic environment and for developing an integrated framework. The most commonly applied biological-effect tools are measures of the biochemical and physiological state of selected organisms, such as mussels or fish. This study was designed to provides basic information on the EU Water Directive, the essence of biomarkers, and outlines why mussels may be the better choice of indicators in toxicological research and monitoring programs in order to study the impact of contaminants in water ecosystems.

Γ7_4. Yancheva V., Georgieva E., Stoyanova S., Tsvetanova V., Todorova K., Mollov I., Velcheva I. 2018. Short and long-term toxicity of cadmium (Cd) and polyaromatic hydrocarbons (PAHs) on zebra mussel (*Dreissena polymorpha* Pallas, 1771). Acta Zoologica Bulgarica, 70(4): 557-564. eISSN: 2603-3798 Q4(SJR 0.190)

ABSTRACT: This study was designed to examine the possible negative effects, which cadmium (Cd) and polyaromatic hydrocarbons (PAHs) could have on the lysosomal membrane stability in haemocytes of the invasive mollusk zebra mussel (*Dreissena polymorpha*) by applying the neutral red retention assay (NRRA). The mussels were exposed to different concentrations of Cd and PAHs in laboratory conditions for 96 hours (acute exposure) and 31 days (chronic exposure). These are considered as priority substances in surface waters according to Directive 2008/105/EC. Lysosomal membrane destabilisation in all mussels treated with Cd and PAHs, including concentrations was found, which were lower than the allowable ones. In addition, a trend of lower retention time in the mussels treated with Cd as compared to the ones treated with PAHs, although these differences were not significant (p>0.05) was determined. The results confirmed that the neutral red retention assay could be used as a cheap, fast and reliable biomarker for Cd and PAHs effects on freshwater mollusks and that zebra mussel could be suggested as a freshwater bioindicator for water contamination. However, further studies are required in order to better understand the negative effects of Cd and PAHs on this bivalve species.

Γ7_5. Todorova K., Velcheva I., Yancheva V., Stoyanova S., Dimitrova P., Tomov S., Georgieva E. 2018. Interactions of Pb with other heavy metals (Cd, Ni and Zn) and toxic effects on gills histological structure of common carp (*Cyprinus carpio* Linnaeus, 1775). Acta Zoologica Bulgarica, 71(1): 95-102. eISSN: 2603-3798 Q4(SJR 0.119) IF 0.53

ABSTRACT: A laboratory experiment was performed in order to study the toxic effects of five different concentrations of single lead (Pb) and its interactions with other heavy metals, cadmium (Cd), nickel (Ni) and zinc (Zn), on the gills of common carp (*Cyprinus carpio* Linnaeus, 1775). Cadmium, Ni and Pb are considered as priority polluting substances in surface waters. A short-term experiment (96 h) in laboratory conditions was performed. The fish were treated with decreasing concentrations of single Pb as well as with its combination with Cd, Ni and Zn. Then we examined the fish gill histological changes and the degree of expression of each histological alteration. Several alterations were observed, i.e. lamellar epithelium lifting, edema, proliferation of the stratified epithelium and cartilage tissue, fusion and degeneration in the gill epithelium as well as vasodilatation and aneurysms in the gill blood circulatory system. The alterations were more pronounced for the single Pb-exposed groups than for its combination with the other tested heavy metals. Overall, the study has contributed to clarify the toxicity of single Pb as well as its mixtures (Pb2++Cd2+, Pb2++Ni2+ and Ni2++Zn2+) and the interactions between the metals on the commercially important freshwater common carp.

Γ7_6. Yancheva V., Velcheva I., Georgieva E., Mollov I., Stoyanova S. 2019. Chlorpyrifos induced changes on the physiology of common carp (*Cyprinus carpio* Linnaeus, 1785): a laboratory exposure study. Applied Ecology and Environmental Research, 17(2): 5139-5157. eISSN: 1785-0037 Q4(SJR 0.229) IF 0.34

ABSTRACT: The scientific publication includes a study that addresses the effects of the pesticide chlorpyrifos (CPF) on the physiology of the economically important fish species common carp (*Cyprinus carpio* Linnaeus, 1785) by applying a biomarker approach. The pesticide is considered as a priority substance in surface waters according to Directive 2013/39/EU of the European Parliament and of the Council. The fish were treated with decreasing concentrations of CPF for 72

hours (acute exposure) and the histological structure of gills and respiration rate were examined. In sum, we found pronounced alterations in the gill structure and changes in the respiration rate index, regardless of the applied pesticide concentrations which indicates its negative effects on non-target aquatic species such as common carp. The results from such studies could be incorporated in the legislation to prevent water contamination in areas with intensive agricultural practices by applying biomarkers, and an update could also be initiated on the maximum permissible concentrations of CPF in surface waters.

Γ7_7. Stoyanova R., Tomov S., Georgieva E., Atanassova P., Dechev I., Yancheva V., Petrova S., Stoyanova S. 2019. Influence of exogenous factors on the maturation levels of spermatozoa chromatin in sub/infertile men treated with nutritional supplement PAPA®. Ecologia Balkanica, 11(1): 179-189. eISSN: 1314-0213 Q4(SJR 0.134) IF 0.53

ABSTRACT: Changes in the hereditary information during the nucleus maturation of the sperm cells can occur under the influence of exogenous or endogenous factors. Exogenous factors could be linked with unfavorable working environment or bad habits. In the recent years nutritional supplements have been used for male infertility treatment. In addition, they can improve the morpho-physiological status of the sperm. The present study aims to investigate the effect of nutritional supplement PAPA on the spermatozoa chromatin maturity level by using an Aniline Blue staining test (aniline blue staining - AB) in sub/infertile men exposed to exogenous factors.. A group of men (n = 88) was examined after their written agreement and a completed questionnaire. The tested group with established diagnoses of asthenoteratozoospermia, oligoasthenoteratozoospermia, astenoteratozoospermia with oligoasthenoteratozoospermia with hypovolemia took orally PAPA® supplement for three months. All patients were tested for DNA damage by an AB test twice before and after the treatment. The patients in the tested group were divided based on the type of harmful work environment (I-IV) and harmful habits (smoking I-III). The results showed that after the treatment with a nutritional supplement PAPA®, the levels of condensation or maturity of sperm chromatin increased by 16.11%. In addition, connections between the effect of treatment and the conditions of the harmful working environment, as well as the amount of received tobacco by the smokers were found. A better effect of the treatment in the tested group with the absence of harmful working environment and non-smokers was found. Therefore, the lowest treatment efficacy was found in the patients who are chronically exposed to occupationally harmful environment, associated with increased body and scrotal temperature (seated workers in factories, ect., drivers, welders, bakers, founders, heavy physical labour workers). On the other hand, a negative connection between the smoked cigarettes per day and the effect of the food supplement in the tested group of men with harmful habits, such as smoking was established.

Γ7_8. Stoyanova S., Georgieva E., Velcheva I., Yancheva V. 2019. Histochemical alterations in bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) liver under two pesticides exposure: A comparative study. Ecologia Balkanica, 11(2): 63-71. eISSN: 1314-0213 Q4(SJR 0.134)

ABSTRACT: The scientific publication includes a study that addresses the toxicological effects of a fosetyl-Al and fenamidone based fungicide and a glyphosate based herbicide on the liver lipid

accumulation in bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) in a short-term laboratory conditions (96 hours). A histochemical method with Sudan III staining was applied. Concentrations of 30 mg/L, 38 mg/L and 50 mg/L concentrations fungicide, representing 50, 40, 30 times dilution and 20 mg/L, 40 mg/L and 72 mg/L representing 70, 40, 20 times dilution of the fungicide, were used. These concentrations were considered as real applicable pesticide concentrations in plant protection practices. Overall, a different degree of lipid accumulation in the fish liver. In terms to the histochemical alterations, we found that the fungicide had a more sever effect compared to the herbicide was established.

Γ7_9. Yancheva V., Velcheva I., Georgieva E., Stoyanova S. 2019. Bioaccumulation of polyaromatic hydrocarbons (PAHs) and cadmium (Cd) and its toxic effects on zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Bivalvia: Dreissenidae). Acta Zoologica Bulgarica, 71(4): 567-574. eISSN: 2603-3798 Q4(SJR 0.211) IF 0.53

ABSTRACT: The possible negative effects of polyaromatic hydrocarbons (PAHs) and cadmium (Cd) on the lysosomal membrane stability (LMS) in haemocytes of the invasive mollusc zebra mussel (*Dreissena polymorpha*) were studied by applying the neutral red retention time assay (NRRT). In addition, the process of bioaccumulation of PAHs and Cd in the gills of zebra mussel was examined and the bioaccumulation factor (BFA) was calculated. The mussels were exposed to different concentrations of Cd and PAHs in laboratory conditions for 96 hours (acute exposure) and 31 days (chronic exposure). Higher toxicant concentrations at the 24th h as well as on the 31st day compared to the other tested time periods was found. These results were linked with the faster lysosomal membrane destabilisation in all mussels treated with Cd and PAHs in the beginning of the experiment.

Γ7_10. Stoyanova S., Nyeste K., Georgieva E., Uchikov P., Velcheva I., Yancheva V. 2020. Toxicological impact of a neonicotinoid insecticide and an organophosphorus fungicide on bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) gills: a comparative study. North-Western Journal of Zoology, 16(1): 64-73. eISSN: 1843-5629 Q3(SJR 0.28) IF 0.69

ABSTRACT: The present study was designed to compare the toxicological effects of a fosetyl-Al and fenamidone based fungicide and a thiamethoxam based insecticide on the gill histological structure of bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) in a short-term laboratory conditions (96 hours). Different concentrations were used – for the insecticide – 6.6 mg/l, 10 mg/l and 20 mg/l representing 30, 20, 10 times dilution, for the fungicide – 30 mg/l, 38 mg/l and 50 mg/l representing 50, 40, 30 times dilution, respectively. These concentrations were considered as real applicable pesticide concentrations in plant protection practices. Overall, pronounced alterations in the gill histological structure such as proliferative and regressive, as well as changes in the circulatory system in the fish treated with both pesticides were found. The histopathological alterations indicated the negative effects of the applied chemicals on non-target aquatic species such as bighead carp. In addition, we found that, in terms of the histological lesions and tested fish species, the fungicide had more severe effects compared to the insecticide.

Γ7_11. Yancheva V., Georgieva E., Stoyanova S., Velcheva I., Somogyi D., Nyeste K., Laszlo A. 2020. A histopathological study on the Caucasian dwarf goby from an anthropogenically

loaded site in Hungary using multiple tissues analyses. Acta Zoologica Sweden, 101(4): 431-446. eISSN: 1463-6395 Q3(SJR 0.414) IF 1.012

ABSTRACT: The scientific publication investigates for the first time the health status of the Caucasian dwarf goby *Knipowitschia caucasica* (Berg, 1916, Fishes of freshwaters of Russian Empire, p. 563, Moscow, Russia: Dep. Zemledeliya) (Teleostei: Gobiidae) from an anthropogenically loaded site in Hungary using histopathological analyses on multiple tissues. For that purpose, fish were collected from the public beach at Tiszafüred near the River Tisza. Gills, liver and kidney were subjected to histopathological analyses, and the results showed different alterations in each organ, which also differed in their extent and severity. In addition, lesions in the reproductive organs of both, male and female fish which, overall, we hypothesized could be due to untreated municipal wastewaters, most likely contaminated with endocrine-disrupting chemicals were also found. The multi-organ histopathological analyses of Caucasian dwarf gobies revealed different lesions, prevalence and severity in each target organ, as follows: liver>gills>kidney>gonad (testes and ovaries). These histopathological lesions can be assessed as good indicators of contamination by endocrine-disrupting chemicals of freshwater ecosystems.

Γ7_12. Stoyanova S., Mollov I., Velcheva I., Georgieva E., Yancheva V. 2020. Cadmium and polyaromatic hydrocarbons exposure changes the condition indices in zebra mussel, *Dreissena polymorpha* (Pallas, 1771): a case study. Acta Zoologica Bulgarica, Supplement 15, 141-146. eISSN: 2603-3798 Q4(SJR 0.237) IF 0.53

ABSTRACT: Cadmium (Cd) and polyaromatic hydrocarbons (PAHs) are priority pollutants in surface waters according to Directive 2013/39/EC. They are toxic, persistent and tend to accumulate in high concentrations in aquatic organisms. In the present scientific publication, zebra mussel *Dreissena polymorpha* was confirmed as a bioindicator for contaminated freshwater ecosystems. The effects of short-term (96 h) and long-term (31 days) exposure to Cd and PAHs on the Condition index (CI) and Soft Tissue Wet Ratio (STWR) in zebra mussel were investigated. Overall, alterations in both CI and STWR (as compared to the control group) at all tested concentrations, including the one below the allowable concentration according to the EU legislation were found. These results demonstrated the toxicity of both contaminants. Furthermore, the study confirmed that the studied condition indices could be successfully applied as biomarkers as they are fast and low-cost in future monitoring and risk assessment of polluted freshwater ecosystems.

Γ7_13. Yancheva V., Velcheva I., Iliev I., Vasileva T., Bivolarski V., Georgieva E., Stoyanova S. 2020. Histochemical and biochemical alterations in zebra mussel, *Dreissena polymoprha* (Pallas, 1771) after cadmium and polyaromatic hydrocarbons exposure. Acta Zoologica Bulgarica, Supplement 15, 155-164. eISSN: 2603-3798 Q4(SJR 0.237) IF 0.53

ABSTRACT: The present publication was developed to examine the possible harmful effects, which cadmium (Cd) and polyaromatic hydrocarbons (PAHs) could cause to the gills and digestive gland of the zebra mussel *Dreissena polymorpha* (Pallas, 1771). For this purpose, for the first time their histochemical and biochemical alterations by applying the Periodic-Schiff staining method (PAS) and analysing the catalase (CAT) and cholinesterase (ChE) activity were investigated. The

mussels were exposed to different concentrations of Cd and PAHs in laboratory conditions for 96 hours (acute exposure) and 31 days (chronic exposure). These are considered as priority substances in surface waters according to Directive 2013/39/EU. Moreover, the enzymatic measurements are included as biomarkers for biota in the EU Water Framework Directive, the Marine Strategy Framework Directive and in the mussel component of the International Council for the Exploration of the Sea/Oslo-Paris convention for the Protection of the Marine Environment of the North-East Atlantic (ICES/OSPAR) integrated monitoring framework. Based on the results, the PAS reaction as an easy, fast, low-cost and trustworthy biological tool, which could be used for biota in monitoring programs was aslo proposed. Overall, we found alterations both in the gill structure and enzymatic activity in the digestive gland at all tested concentrations, including the one below the allowable concentration according to the EU legislation. These results confirmed the toxicity of Cd and PAHs. Furthermore, Cd was more toxic compared to PAHs in terms of the studied parameters.

Γ7_14. Stoyanova S., Georgieva E., Velcheva I., Iliev I., Vasileva T., Bivolarski V., Tomov S., Nyeste K., Antal L., Yancheva V. 2020. Multi-biomarker assessment in common carp (*Cyprinus carpio*, Linnaeus 1758) liver after acute chlorpyrifos exposure. Water, 12, 1837. ISSN: 2073-4441 Q2(SJR 0.718) IF 2.069

ABSTRACT: The excessive use of pesticides at different stages of crop production can pose a great danger to the aquatic environment, and particularly to fish. The purpose of the present publication was to assess the negative effects of chlorpyrifos (CPF) on the liver histological architecture and the activities of marker enzymes in common carp (Cyprinus carpio Linnaeus, 1758), by applying a multi-biomarker technique. The tested insecticide is categorized as a priority pollutant in surface waters in terms of Directive 2013/39/EU. The carps were exposed to di erent and environmentally relevant CPF concentrations for 72 h (a short-term acute experiment). The results showed that the tested insecticide alters the liver histological structure, causing degenerative lesions, such as granular and vacuolar degeneration; necrobiotic alterations and necrosis, as well as changes in the circulatory system. In addition, CPF induces changes in the enzymatic activity of lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), cholinesterase (ChE), glutathione peroxidase (GPx) and catalase (CAT). The results from such experimental set ups could be successfully used in the legislation related to the protection of water bodies from contamination, in areas with intensive application of plant protection products used in agricultural practices, and also in implementing the Water Frame Directive by using multi-biomarker approaches.

Γ7_15. Gecheva G., Yancheva V., Velcheva I. Georgieva E., Stoyanova S., Arnaudova D., Stefanova V., Georgieva D., Genina V., Todorova B., Mollov I. 2020. Integrated monitoring with moss-bag and mussel transplants in reservoirs. Water, 12, 1800. ISSN: 2073-4441 O2(SJR 0.718) IF 2.069

ABSTRACT: For the first time, transplants with moss-bags and mussels together were applied to study the water quality in standing water bodies. The tested species: *Fontinalis antipyretica* Hedw. and *Sinanodonta woodiana* (Lea, 1834) were collected from unpolluted sites and analyzed to obtain background levels. Then, the moss and mussels were left in cages for a period of 30 days

in three reservoirs where both are not present naturally. Two of the reservoirs suffer from old industrial contamination and one is affected by untreated wastes. Twenty-four compounds were studied, among them trace elements Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn and organic priority substances: six polybrominated diphenyl ethers (PBDEs) congeners and short-chain chlorinated paraffns (SCCPs). The trace element accumulation was significant after the exposition period in all studied stations. PBDEs and SCCPs were also accumulated up to two times more in the moss tissues. PBDEs in the mussels exceeded the environmental quality standard (EQS). The applied combined transplants, and especially the moss-bags, revealed severe contamination with heavy metals not detected by the water samples. The moss and the mussel followed a di erent model of trace element and PBDEs accumulation. The SCCPs levels were alarmingly high in all plant samples. The study confirmed PBDEs and SCCPs as bioaccumulative compounds and suggested that an EQS for SCCPs in biota needs to be established.

Γ7_16. Georgieva E., Yancheva V., Stoyanova S., Velcheva I., Iliev I., Vasileva T., Bivolarski V., Petkova E., László B., Nyeste K., László L. 2021. Which is more toxic? Evaluation of the short-term toxic effects of chlorpyrifos and cypermethrin on selected biomarkers in common carp (*Cyprinus carpio*, Linnaeus 1758). Toxics, 9(6), 124. eISSN: 2305-6304 Q1(SJR 0.8) IF 4.14

ABSTRACT: The general aim of this study was to investigate the negative short-term effects of different concentrations of chlorpyrifos (CPF) and cypermethrin (CYP), based on the EU legislation (MACEQS) in common carp (Cyprinus carpio Linnaeus, 1758) under laboratory conditions and to compare their toxicity. The fish were exposed to the pesticides for 96 h and then different histological and biochemical biomarkers were investigated in the gills and liver, and bioaccumulation analyses were conducted. The chemical studies showed increased pesticide concentrations in the gills as the first site for pollutants compared to the liver at the 96th hour. In addition, the histological analyses showed severe alterations in the gills and liver after exposure to both tested pesticides. In the gills, mainly intense proliferative and, to a lesser extent, degenerative changes and alterations in the circulatory system, such as necrosis and vasodilation were found. In the liver, regressive and progressive lesions, as well as circulatory disturbances and inflammation, were observed. The regressive lesions showed a higher degree of expression compared to the other changes. Furthermore, altered enzymatic activities-catalase, glutathione reductase, and glutathione peroxidase-in the liver, compared to the control were found. Overall, both tested pesticides impacted the studied biomarkers in common carp, even at concentrations lower than those permitted by law. However, the results of the comparative analysis showed a relatively higher toxicity of CYP compared to CPF in the fish. Still, questions persist as to whether the observed changes are adaptive or entirely destructive. To avoid any danger or risk, these pesticides must be applied cautiously, especially near water bodies.

Γ7_17. Georgieva E., Velcheva I., Yancheva V., Stoyanova S., Vasileva T., Bivolarski V., Todorova B., Iliev I. 2021. A review on multi-biomarkers in fish for the assessment on aquatic ecosystem contamination with organic pollutants. Ecologia Balkanica, 13(2): 321-330. eISSN: 1313-9940 Q4(SJR 0.144)

ABSTRACT: The review addresses the most suitable biomarkers for evaluating chemical stress due to organic contamination in aquatic environments, as well as possible ways to reduce or limit this contamination. To safeguard the environmental status, the European Union has implemented the Water Framework Directive (WFD; 2000/60/EC) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC) legislations, which encourage the use of biological tools to detect the quality of aquatic systems. Therefore, monitoring of sentinel fish species is widely used to assess the level of health status. Fishes have been found to be good biomonitors of water pollution because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates, are also more sensitive to different toxicants, including persistent organic pollutants, such as pesticides. Biomarkers are defined as responses to any exposure evidenced in histological, physiological, biochemical, genetic and behavioral modification. The application of multi-biomarker approach is necessary in the development of a protocol for the aquatic assessment of organic pollutants. This protocol can be applied in risk assessment and water monitoring programs in order to provide an adequate legal basis for the presence of organic pollutants in aquatic ecosystems and biological responses under concentrations equal or lower to those permitted under the European and Bulgarian legislation.

Γ7_18. Georgieva E., Antal L., Stoyanova S., Arnaudova D., Velcheva I., Iliev I., Vasileva T., Bivolarski V., Mitkovska V., Chassovnikarova T., Todorova B., Uzochukwu I.E., Nyeste K., Yancheva V. 2022. Biomarkers for pollution in caged mussels from three reservoirs in Bulgaria: a pilot study. Heliyion, 8(3): e09069. eISSN: 2405-8440 Q1(SJR 0.55) IF 3.7

ABSTRACT: The mussel-watch concept was firstly proposed in 1975, which was later adopted by several international monitoring programs worldwide. However, for the very first time, a field experiment with caged mussels was performed in three reservoirs in Bulgaria to follow the harmful effects of sub-chronic pollution (30 days) of metals, trace, and macro-elements, as well as some organic toxicants, such as polybrominated diphenyl ethers and chlorinated paraffins. Therefore, The scientific publication includes a study that addresses the biometric indices, histochemical lesions in the gills, biochemical changes in the digestive glands (antioxidant defense enzymes, such as catalase, glutathione reductase, and glutathione peroxidase; metabolic enzymes, such as lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, and the neurotransmitter cholinesterase), in addition to the DNA damage in the Chinese pond mussel, Sinanodonta woodiana (Lea, 1834) in Kardzhali, Studen Kladenets and Zhrebchevo reservoirs in Bulgaria. Significant correlation trends between the pollution levels, which we reported before, and the biomarker responses were established in the current paper. Overall, based on the obtained results, both tested organs were susceptible to pollution-induced oxidative stress. The different alterations in the selected biomarkers in the caged mussels compared to the reference group were linked to the different kinds and levels of water pollution in the reservoirs, and also to the simultaneously conducted bioaccumulation studies.

Γ7_19. Yancheva V., Georgieva E., Velcheva I., Iliev I., Stoyanova S., Vasileva T., Bivolarski V., Todorova-Bambaldokova D., Zulkipli N., Antal L., Nyeste K. 2022. Assessment of the exposure of two pesticides on common carp (*Cyprinus carpio* Linnaeus, 1758): Are the

prolonged biomarker responses adaptive or destructive? Comparative Biochemistry and Physiology, Part C, 261: 109446. eISSN: 1878-1659 Q2(SJR 0.721) IF 4.2

ABSTRACT: Chlorpyrifos (CPF) and cypermethrin (CYP) are two insecticides that have a proven negative effect on non-target aquatic organisms when they enter the surface waters. However, literature on the comparative effects of these pesticides on important aquaculture fish species, such as common carp (Cyprinus carpio Linnaeus, 1758) is not yet scientifically detailed, especially over the long-term. The idea of conducting a long-term exposure is to find out how the observed biomarkers would change compared to the short-term exposure. In the natural environment, toxicants are not present alone, but in combination. By monitoring the long-term impact of individual substances, the state of aquatic ecosystems exposed to various toxicants could be predicted. Thus, this study aimed to evaluate the toxicity of different concentrations of CYP $(0.0002, 0.0003, \text{ and } 0.0006 \,\mu\text{g/L})$ and CPF $(0.03, 0.05, \text{ and } 0.10 \,\mu\text{g/L})$ in 50-L glass tanks on C. carpio, exposed for 30 days under laboratory conditions. A set of histological and biochemical biomarkers in the gills and liver were applied with the chemical analyses of water and fish organs. Furthermore, the condition and hepatosomatic index were calculated to assess the physiological status of the treated carps. The behavioral responses were also monitored, and the respiration rate was analyzed. The results suggest that CYP had a more prominent effect on the histological structure of fish organs, biochemical responses of anti-oxidant enzymes, behavior, and respiration rate compared to the effect of CPF. In addition, the results also indicate that the liver is more susceptible to chronic and chemically induced cellular stress compared to the gills, with overall destructive changes in the histological biomarkers rather than adaptive. Regardless of the scenario, our results provide novel insights into pesticide exposure and the possible biological impacts on economically important freshwater fish, exposed to lower CYP and CPF concentrations, based on the EU legislation (maximum allowable concentrations, MAC-EQS).

Γ7_20. Yancheva V., Velcheva I., Georgieva E., Stoyanova S., Todorova B., Antal L., Nyeste K. 2023. Are Mytilus species suitable bioindicators for assessing aquatic pollution along the Black Sea Coast? A review. Ecologia Balkanica, 15(1): 49-76. eISSN: 1314-0213 Q4(SJR 0.144)

ABSTRACT: This review aims to summarize the possibility of using mussels (Mytilus spp.) as bioindicators to assess aquatic pollution in the Black Sea in Bulgaria. In addition, the main responsive biomarkers that could be applied to study the negative effects of different toxicants on these species in terms of using the Marine Strategy Framework Directive and implementation of environmental quality standards (EQS) in marine biota are also discussed. A specific reference is made to plastic pollution, transplant mussel caging, and mussel watch programs - their application, challenges, and future perspectives in Bulgaria.

Γ7_21. Georgieva E., Kovacheva E., Yancheva V., Velcheva I., Hrischev P., Atanassova P., Tomov S., Stoyanova S. 2023. Pesticides induce fatty degeneration in liver of *Cyprinus carpio* (Linnaeus 1758) after acute exposure. Ecologia Balkanica, 15(2): 77-82. eISSN: 1314-0213 Q4(SJR 0.144)

ABSTRACT: In the present study, results are presented regarding the negative effects of different concentrations of three classes of pesticides - an insecticide, herbicide and fungicide on one relatively uncommon, but reliable histochemical biomarker in the field of fish toxicology – lipid accumulation in the liver of common carp under laboratory conditions.

Γ7_22. Hrischev P., Atanassova P., Georgieva K., Yancheva V., Stoyanova S., Velcheva I., Georgieva E. 2024. Effects of submaximal training on ghrelin in female and male rats with dietary-induced metabolic syndrome. Acta Zoologica Bulgarica, 76(2): 197-205, eISSN: 2603-3798 Q4(SJR 0.237) IF 0.53

ABSTRACT: Ghrelin is a hormone that stimulates appetite and its serum levels are inversely proportional to body weight. Decreased ghrelin levels correlate with obesity, insulin resistance and elevated blood pressure, which are components of the metabolic syndrome (MetS). The effect of physical exercise is studied mainly after an already induced MetS, the results are contradictory and it is not clear what the effect of exercise on ghrelin concentration is. There are no data for gender comparisons of changes in ghrelin due to submaximal training. This study was designed to determine the effect of submaximal training on serum ghrelin concentrations during the dietary induction of MetS in female and male rats. Dietary-manipulated female and male Wistar rats (n = 32) were used. They were divided into four groups: male and female sedentary (MD and FD) and male and female trained on treadmill (MDT and FDT). For 16 weeks, the four groups received high-fat high-carbohydrate (HFHC) food and both MDT and FDT simultaneously with the diet were subjected to submaximal training (70-75% VO_{2max}). At the end of the experiment, mixed blood was collected and ghrelin concentrations were measured. The submaximal training and gender had a significant main effect on ghrelin levels, as trained dietary-manipulated rats had higher serum concentrations compared to the sedentary (p<0.05) and males had higher concentrations compared to females (p < 0.05). Overall, applied for 16 weeks, simultaneously with the combined HFHC diet, the submaximal training had a positive effect, lowering the serum ghrelin concentration. Sexual dimorphism, characterised by higher ghrelin in male rats, was also found.

Γ7_23. Yancheva V., Todorova B., Georgieva E., Stoyanova S., Antal L., Nyeste K. 2024. Seasonal differences on stress on stress responsiveness in the Mediterranean mussel from the Southern Bulgarian Black Sea – an indicator for an ecosystem health decline? Ecologia Balkanica, 16(2): 1-5, eISSN: 1314-0213 Q4(SJR 0.144)

ABSTRACT: The scientific publication includes new results on stress on stress response measurements in both farmed and wild Mediterranean mussels from the town of Sozopol, Bulgaria, collected in the summer of 2024 (August) and compare them with our previous results for the winter and spring season.

Abstract annotation of scientific papers of

of chief assistant professor Stela Georgieva Stoyanova, PhD

to indicator E20 in accordance with the minimum national requirements of the ZRASRB and the Regulations for the implementation of the ZRASRB

1. Georgieva E., Stoyanova S. 2025. Guide on Histology. Plovdiv University Press, pp. 82, ISBN 978-619-281-021-4 (in Bulgarian)

ABSTRACT: Histology is the study of cells, tissues and organs as seen through the microscope. This guide was developed to support the work of students, PhD students and young scientists in the Faculty of Biology how to: organize and conduct their laboratory experiment, to recognize the structure of cells and tissues and understanding how this is determined by their function, shape their thesis. The information presented in the guide is organized in four topics: epithelial, connective, muscle and nervous tissue.

2. Georgieva E., Stoyanova S. 2025. Guide on Histology and pathology. Plovdiv University Press, pp. 116, ISBN 78-619-281-022-1 (in Bulgarian)

ABSTRACT: This guide was developed to support the work of students, PhD students and young scientists in the Faculty of Biology how to: organize and conduct their laboratory experiment, to recognize the structure of cells and tissues and understanding how this is determined by their function, recognize various pathological changes in different organs, shape their thesis. The information presented in the first part "Histology" is related to: epithelial, connective, muscle and nervous tissue. The second is also organized in four topics: histopathological alteration in liver of *Cyprinus carpio* L., histochemical alterations in liver of *Cyprinus carpio* L., histopathological alterations in gill histological structure of *Cyprinus carpio* L. and some human pathological alterations, which are presented in different organs (liver, kidney).

Signature:

(Chief assistant professor Stela Stoyanova, PhD)