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Histological changes in fish after psychoactive pollutants exposure

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Projektpartner:
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27.02.2020

_____
Datum der Abgabe

1. Statutory declaration

Statutory declaration

I declare under penalty of perjury that I have authored the present thesis independently and without use of any other than the declared sources and aids. All material which has been quoted either literally or by content from the used sources is explicitly market as such.

Yspertal, 26.02.2020 Author: Francesca Kastner

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Yspertal, 26.02.2020 Author: Lea Klatzl

2. Abstract

Water pollution is becoming a growing environmental problem, which can cause damage on fish. Psychoactive substances enter domestic watercourses in various ways, where they cause damage to aquatic life. This thesis deals with the effects of psychoactive substances on the liver and heart of fish. The work is the result of a cooperation with the South Bohemian University of Ceské Budjovice, within the framework of the ISS 2019 (International summer school 2019), in the Faculty of Fisheries and Water Protection. The project is carried out under the supervision of Msc. Maria Eugenia Sancho Santos.

The fish, whose organs are being examined, were previously tested in a trial by Msc. Maria Eugenia Sancho Santos. They were divided into a control group and an exposed group. The control group was not exposed to any substances. The exposed group was exposed to 1µg/l of a drug cocktail consisting of tramadol, citalopram, sertraline, oxazepam, venlafaxine and meth amphetamine. There were two sampling days, one 7 days and another one 21 days after exposure.

The tissue samples, not thicker than 3mm, are placed in buffered formalin and transferred to 70% ethanol after 12-24 hours. The heart and liver samples are then transferred to cassettes, but before this happens the liver is separated from the internal organs. The heart can be transferred immediately because it is not connected to the intestines. The cassettes of the respective samples are now placed in the tissue processor, which exchanges water for paraffin. After this procedure the samples are placed in metal tanks, aligned and filled with hot paraffin. A cover plate is placed on the hot paraffin, which is then labelled. After the samples have cooled down, they are carefully removed from the tank. The paraffin blocks obtained are cut with a microtome into 4. 5µm thick slices. These slices are now transferred to a water bath and carefully removed with a microscope slide. Two different stains are made for each organ. The heart is stained with haematoxylin and eosin, as well as Masson's trichrome. The liver is also stained with haematoxylin and eosin as well as with PAS (Periodic-acid Schiff reaction). The samples are examined under the microscope for various damages, these are evaluated with the semi-quantitative classification and the significance is calculated with the Kruskal-Wallis test.

In addition to the practical research activities, a handout is prepared. This can be divided into 4 parts, the description of the fish anatomy, the pollution of the water, the resulting damage to the heart and liver of the fish and a crossword puzzle.

After evaluating the damage in the tissue, changes in both the heart and the liver are detectable. These are degeneration, fibrosis, oedema and pigmented macrophages in the heart. In addition, infiltration has occurred in the myocardium and pericardium. In the liver, vacuole as well as macrovacuole are clearly visible.

3. Zusammenfassung

Wasserverschmutzung wird zu einem immer größeren Umweltproblem, worunter insbesondere Fische leiden. Psychoaktive Substanzen kommen auf verschiedene Wege in die heimischen Fließgewässer, dort führen sie zu Schädigungen der Wasserlebewesen. Diese Diplomarbeit befasst sich mit der Auswirkung von psychoaktiven Substanzen auf die Leber und das Herz von Fischen. Die Arbeit ist durch eine Kooperation mit der Südböhmischen Universität Budweis, im Rahmen der ISS 2019 (International summer school 2019), in der Fakultät für Fischerei und Wasserschutz entstanden. Das Projekt steht unter der Leitung von Msc. Maria Eugenia Sancho Santos.

Die Fische, deren Organe untersucht werden, wurden zuvor in einem Versuch von Msc. Maria Eugenia Sancho Santos in eine Kontrollgruppe und eine Testgruppe eingeteilt. Die Kontrollgruppe war keinen psychoaktiven Substanzen ausgesetzt. Die Testgruppe wurde 1µg/l eines Drogencocktails, bestehend aus Tramadol, Citalopram, Sertralin, Oxazepam, Venlafaxine und Meth Amphetamin, ausgesetzt. Es gab zwei Tage, an welchen die Proben genommen wurden, einer 7 Tage nach der Verabreichung des Cocktails und einer nach 21 Tage.

Die höchstens 3mm dicken Gewebeproben werden in gepuffertes Formalien geben und nach 12-24 Stunden in 70% Ethanol überführt. Danach werden die Herz- und Leberproben in Kassetten expandiert, bevor dies passiert wird jedoch die Leber von den inneren Organen getrennt. Das Herz kann gleich überführt werden da es nicht mit den inneren Organen verbunden ist. Die Kassetten der jeweiligen Proben werden nun in den Gewebeeinbettungsautomaten geben, dieser tauscht das im Gewebe enthaltene Wasser gegen Paraffine aus. Nach dieser Prozedur werden die Proben in Metalltanks gegeben, ausgerichtet und mit heißem Paraffin aufgefüllt. Auf das heiße Paraffin wird eine Deckplatte geben, welche beschriftet wird. Nachdem die Proben abgekühlt sind, werden sie vorsichtig aus dem Tank gelöst. Die erhaltenen Paraffinblocks werden mit einem Mikrotom in 4,5µm dicker Scheiben geschnitten. Diese Scheiben werden nun in ein Wasserbecken überführt und vorsichtig mit einem Objektträger entnommen. Es werden 2 Färbungen für jedes Organ angefertigt. Das Herz wird mit Hämatoxylin und Eosin sowie Masson’s trichrome gefärbt. Die Leber

wird ebenfalls mit Hämatoxylin und Eosin wie auch mit PAS (Periodic-acid Schiff reaction) gefärbt. Die Proben werden unter dem Mikroskop auf verschiedene Schädigungen untersucht, diese werden mit Hilfe der semi-quantitative Klassifizierung bewertet und mit dem Kruskal-Wallis Test wird die Signifikanz berechnet.

Neben der praktischen Forschungstätigkeit wird ein Handout erstellt. Dieses kann in 4 Teile eingeteilt werden, die Beschreibung der Fisch Anatomie, die Verschmutzung des Wassers, die dadurch entstehenden Schäden an Herz und Leber der Fische und ein Kreuzworträtsel.

Nach der Bewertung der Schäden im Gewebe sind Veränderungen sowohl im Herz als auch in der Leber nachweisbar. Diese sind im Herz, Degeneration, Fibrose, Ödeme und pigmentierte Makrophagen. Außerdem ist Infiltration im Myokard und im Herzbeutel aufgetaucht. In der Leber sind Vakuole als auch Makrovakuole deutlich erkennen bar.

4. Acknowledgements

We would like to thank all those who supported us with this diploma thesis.

In particular, we would like to thank our thesis supervisors, Ms. Mag. Isabel Urban and Mr. OStR Mag. Gunter Gortan, who have always actively supported us.

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We would also like to thank the University of South Bohemia in Ceske Budejovice, as well as Dl Peter Böhm and PaedDr. Jiří Koleček, because they made the cooperation and thus this diploma thesis possible.

Further thanks go to Michaela Dirnová for the great organisation of the Summer School 2019.

Last but not least, we would like to thank our families, because they made our education and this project possible.

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6. Introduction

Aquatic organisms are exposed to many threats. The pollution of waters with psychoactive substances is an increasing problem. Traces of psychopharmaceuticals and illegal drugs have been reported in effluents, influents and surface waters. This pollution poses a threat to aquatic ecosystems and is mainly absorbed by fish. The psychoactive substances damage their health, which can be detected in the tissue of their organs. Psychoactive substances, such as methamphetamine and tramadol, are released by humans into wastewater. However, they cannot be completely filtered from water by sewage treatment plants, which leads to a residual content of psychoactive substances in water.

On that account, the effects of this pollution on the tissues of the heart and the liver of fish were analyzed. The practical research work took place in the framework of a cooperation with the *University of South Bohemia in Ceske Budejovice*. The work took place at the *Faculty of Fisheries and Protection of Waters* under the supervision of Maria Eugenia Sancho Santos, a PhD student. Our study is mainly based on literature provided by the faculty. Furthermore, reference is made to unreleased papers by Eugenia Sancho Santos.

The aim of this work is to detect and determine changes in the tissues of the liver and heart of fish. Other organs are not discussed, as they are not relevant to this study, or would go beyond the constraints of this diploma thesis. The results of the research work will be used to create learning materials for science teaching in schools. These will also contain general information on the histology of fish and the effects and functioning of psychoactive substances. These learning materials are intended to raise awareness concerning drug abuse and environmental protection.

In order to be able to see the effects of psychoactive substances in the tissue of the organs under the microscope, the samples have to be prepared correctly. The

samples are collected by Squalius cephalus (chub). After collecting, the samples need to be fixated in formalin. As soon as the fixation is complete the tissue needs to be dehydrated as well as infiltrated. Next the samples get embedded in paraffin blocks. Once the paraffin blocks have hardened, they get cut by the microtome, which allows the transfer of the tissue onto microscope slides. Finally, the tissue needs to be deparaffinized, rehydrated and stained.

7. Foundation

7.1. Psychoactive substances

In general, the term psychoactive substance defines drugs and other substances which affect brain functions and cause changes in the mood, awareness, thoughts, feelings or behaviour. They can cause hallucinations and permanent changes in the mood, behaviour, cognition and perception of the consumer. Examples for these kinds of drugs are on the one hand illicit and licit drugs, such as alcohol, caffeine, cocaine, heroin, marijuana and methamphetamines. On the other hand, they are psychiatric drugs and painkillers such as Citalopram, Sertraline, Oxazepam, Tramadol and Venlafaxine.

(National Cancer Institute. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/796898 , 13.12.19)

7.1.1. Licit drugs

Licit drugs are often used to treat mental disorders. The number of people that suffer from these diseases has increased drastically over the last years, as a consequence, also the amount of psychiatric drugs that is being consumed has risen significantly.

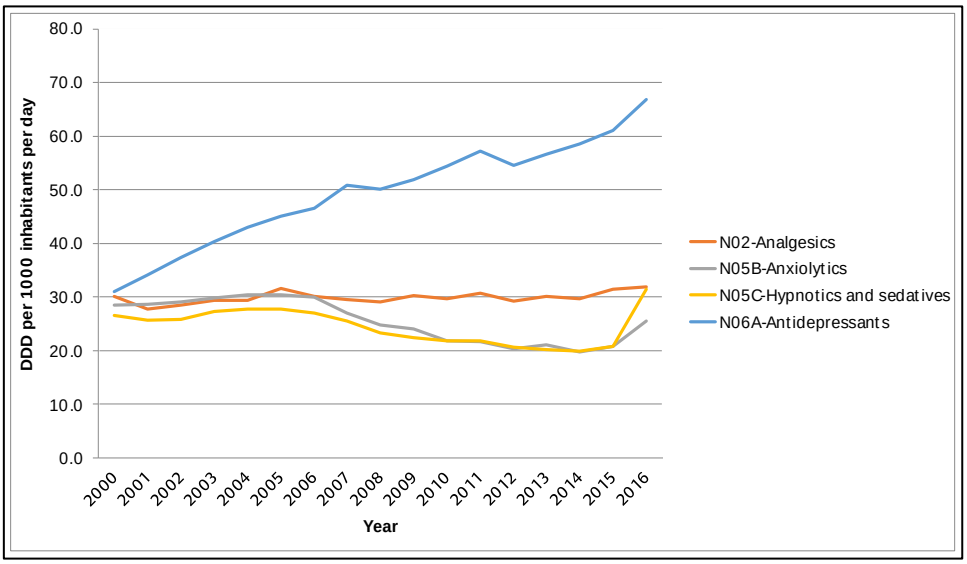


Figure 1: Consumption of antidepressants

The line graph from the OECD Statistics 2017 (figure 1) gives an overview over the rise of the defined daily dose per 1000 inhabitants per day per year. In general, it can be seen that the blue line, which shows the antidepressants, rises constantly since 2000. According to the graph the consumption at the beginning of the measurement is similar to them from the other medications. In the year 2002 the bar chart shows a big rise to the point of 40. The highest point of the blue line is in the year 2016 at nearly 70. If the line continues rising, one possible outcome could be that the consumption as well as the pollution rises, this can lead to devastating problems for the environment.

(self-interpretation by Francesca)

“These medications are almost the most prescribed globally (Calisto & Esteves, 2009), and the actual trends indicate that their use is still rising among the years (Indicators, 2017). Even so, psychotropic medication suffers great variations among the European countries due to the cultural variability, being the culture a great influencer in the identification, management and treatment of mental diseases (Hoebert, MantelTeeuwisse, Leufkens, & Van Dijk, 2017), so each country has its own patterns of usage although always in rising (Sempere Verdú, Salazar Fraile, Palop Larrea, & Vicens Caldentey, 2014)”

(Sancho Santos, Maria Eugenia: MSc. Psychoactive compounds in aquatic environment and their effects on fish. not yet published)

Currently society is facing a big problem with the consumption of antidepressants. Many people are already get antidepressions prescribed, when there is only a suspicion or higher risk for depressions or burn out. This leads to an increase in consumption. In addition, many people fall into depression or burnout more quickly. Reasons for this development are stress, pressure at work and loneliness in large cities.

(J. Goetz, etal ARD documentary „Gefährliche Glückspille – Milliardenprofite mit Antidepressiva, Name Autor 2013)

7.1.2. Illicit drugs

These substances have different effects, which can be roughly divided into the following categories:

- Stimulating effects
- Inhibitive effects on the central nervous system
- Hallucinogenic effects

(A. Uutela. International Encyclopaedia of the social & Behavioural Sciences, Pages 3877-3881, https://www.sciencedirect.com/science/article/pii/B0080430767038869, 13.12.19, 2001)

7.1.2.1. Stimulating effects

These effects are caused by substances like cocaine or amphetamines. The stimulation appears immediately after ingestion in the form of increased blood pressure, pulse acceleration, bronchodilatation, enlargement of the pupils and increase in body temperature. This increase is due to the stimulation of the synapses. A release of dopamine and norepinephrine follows, which leads to an increased availability of monoamine transmitters in the synaptic cleft. This leads to stimulation. A side effect of stimulation is the reduction of gastrointestinal activity, which leads to a diminishing feeling of hunger.

(Köhler, Thomas: Prof. Dr. med. Dr. phil. Dipl.- Psych. Dipl.-Math. Rauschdrogen: Geschichte, Substanzen, Wirkung. München: C.H.Beck Wissen, 2008.)

7.1.2.2. Inhibitive effects on the central nervous system

Psychoactive substances, such as heroin or sedative-hypnotics cause these effects. One of the most important effects of these substances is their analgesic effect. This is achieved by binding to receptors in the brain area. These receptors are responsible for the processing and transmission of pain trajectory impulses, as well as the limbic system. It also inhibits synaptic transmission from the first to the second neuron in the pain pathway of the spinal cord, one of the most important pain transmitters to the brain. This creates the pain-inhibiting effect which is located in the central nervous system.

(Köhler, Thomas: Prof. Dr. med. Dr. phil. Dipl.- Psych. Dipl.-Math. Rauschdrogen: Geschichte, Substanzen, Wirkung. München: C.H.Beck Wissen, 2008.)

7.1.2.3. Hallucinogenic effects

Marijuana and LSD are the best-known substances which cause these kinds of effects. There is no hallucination immediately after consumption of the substances. In this first phase, the symptoms are similar to those of the above-mentioned stimulation. In contrast, side effects such as drowsiness, dizziness and nausea occur. However, the consumer rarely notice these symptoms. Only in the second phase of intoxication, the sensory phase, do the characteristic changes in perception occur. In this phase:

- colours and tones are felt more intensely
- odours or tones become visible
- objects change their size

In addition, it feels as if the time passes slower than normally. Optical hallucinations appear in the form of non-existent shadows and objects. Most consumers are aware that this is an intoxication and not reality.

(Köhler, Thomas: Prof. Dr. med. Dr. phil. Dipl.- Psych. Dipl.-Math. Rauschdrogen: Geschichte, Substanzen, Wirkung. München: C.H.Beck Wissen, 2008.)

7.1.3. Prevention

Based on European surveys, the use of illicit drugs has increased. To prevent this, there are different programmes. These programmes take place in schools, at workplaces and in public places. On the one hand people are informed about the dangers of drug abuse, on the other hand addicts are able to get help.

(Quelle: A. Uutela, International Encyclopedia of the social & Behavioral Sciences, 2001, Pages 3877-3881, https://www.sciencedirect.com/science/article/pii/B0080430767038869, 13.12.19)

There are different methods of drug prevention used in central Europe. Germany has one of the most effective ways. Their strategy is to prevent drug abuse through social learning. Even in kindergarden the children learn, and train how to live a healthy and active life in a playful way. They also learn how to cope with stressful situations without harming someone. Such games teach the children knowledge which they would not gain in their daily lives. Often children from poorer families do not learn these skills from their parents, some of them see their parents fighting in state of discussing a problem. With this pre-school teaching teachers try to protect the children from an early start of drug abuse and help them deal with an instable environment. In primary school teachers use this crucial phase of life to talk to the children about drug abuse. The children participate in activities that inform on the damage of drugs. For teenagers they have peer groups, where other young adults talk with them. They influence each other and prevent the children from trying drugs because of group pressure. Germany basically tries to prevent drug abuse by creating strong social structures.

(Reisner, Philipp. www.drogen.net/drogenpraevention.php 12.01.2020)

7.1.4. Psychiatric drugs

7.1.4.1. Citalopram

Citalopram is an antidepressant agent used to treat depression, panic and obsessive-compulsive disorders. The drug inhibits the reuptake of serotonin in the presynaptic nerve cells. Normally, it is taken once a day in the form of a film-coated tablet, but there is the possibility of the active substance as an infusion to be

administered, but this happens very rarely. The medication shows many side effects, too, these are among others:

- dry mouth
- excitement
- increased perspiration
- nausea
- reduced appetite
- sleeplessness
- fatigue
- libido loss
- sexual disorders

(https://www.pharmawiki.ch/wiki/index.php?wiki=Citalopram , 14.12.2019)

Structure:

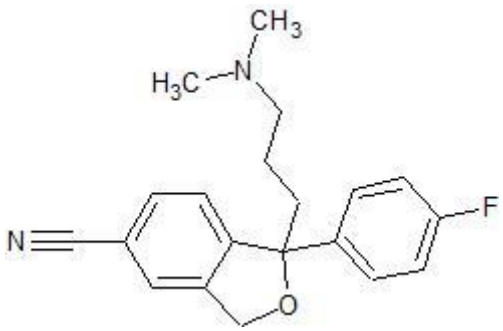


Figure 2: Structure Citalopram

This compound is not soluble in water due to its apolar structure. It is apolar due to the aromatic compounds and the carbon chains.

(P. Pfestrof, H.Kadner, et.al Chemie: Ein Lehrbuch für Fachhochschulen. Seite 480 bis 485, Auflage 2000, Thun und Frankfurt am Main: Harri Deutsch, 2000.)

7.1.4.2. Sertraline

The antidepressant is used for the treatment of depression, anxiety, obsessive-compulsive disorder and panic disorder. It works similar to Citalopram, which is described in the above paragraph. Sertraline also inhibits the reuptake of the neurotransmitter serotonin in the presynaptic nerve cells. This similarity of the two substances is due to the fact that both are active substances of the SRRI group. As it has a long half-life, it is only taken once a day as a film-coated tablet. The maximum effect is achieved after a two - to four-week intake. This medicine also has several side effects:

- sleeping disorders
- headaches
- dizziness
- overexertion
- diarrhoea
- dry mouth
- impotence
- fatigue

(https://www.pharmawiki.ch/wiki/index.php?wiki=Sertralin , 14.12.2019)

Structure:

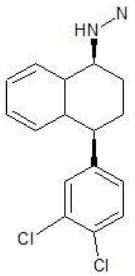


Figure 3: Structure Sertralin

This compound is not soluble in water due to its apolar structure. It is apolar due to the aromatic compounds and the carbon chains.

(P. Pfestrof, H.Kadner, et.al Chemie: Ein Lehrbuch für Fachhochschulen. Seite 480 bis 485, Auflage 2000, Thun und Frankfurt am Main: Harri Deutsch, 2000.)

7.1.4.3. Oxazepam

The active ingredient, administered in tablet form, is used for the symptomatic treatment of anxiety, tension and sleep disorders due to its anxiety-relieving, antispasmodic, calming and sleep-promoting effects. As this drug is only intended for the treatment of symptoms, it should not be taken for a long time under any circumstances. If this is not observed, however, it can lead to a dependency. The following side effects may occur:

- light-headedness
- drowsiness
- depression
- impotence
- nausea

(https://www.pharmawiki.ch/wiki/index.php?wiki=Oxazepam , 14.12.2019)

Structure:

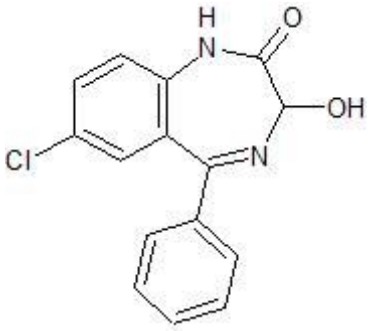


Figure 4: Structure Oxazepam

This compound is not soluble in water due to its apolar structure. It is apolar due to the aromatic compounds and the carbon chains.

(P. Pfestrof, H.Kadner, et.al Chemie: Ein Lehrbuch für Fachhochschulen. Seite 480 bis 485, Auflage 2000, Thun und Frankfurt am Main: Harri Deutsch, 2000)

7.1.4.4. Tramadol

The active substance can be assigned to the group of opioids. It is used to treat moderate as well as severe pain. Although tramadol belongs to the opioids, it has some effects of an antidepressant. Since the substance, which can be taken as tablets, capsules, fused tablets, drops, effervescent tablets and injection solutions, has a high potential for drug interaction, numerous precautionary measures must be observed. The risk of dependency is very low but there is a risk of drug interaction . This medicine may cause the following side effects:

- nausea
- headache
- drowsiness
- vomiting
- constipation
- dry mouth
- sweating
- exhaustion

(https://www.pharmawiki.ch/wiki/index.php?wiki=Tramadol , 14.12.2019)

Structure:

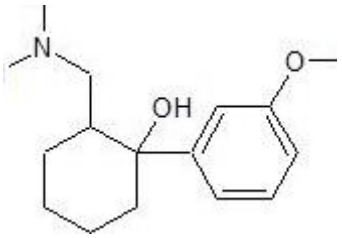


Figure 5: Structure Tramadol

It easily dissolves in water because of the aromatic structures and the carbon chains.

(P. Pfestrof, H.Kadner, et.al Chemie: Ein Lehrbuch für Fachhochschulen. Seite 480 bis 485, Auflage 2000, Thun und Frankfurt am Main: Harri Deutsch, 2000.)

7.1.4.5. Venlafaxine

This antidepressant is used to treat depression, anxiety and panic disorders. It inhibits the reuptake of norepinephrine and serotonin in presynaptic neurons. It is taken once a day in tablet form. The effect starts delayed, only after 1-4 weeks. Since the chemical structure resembles that of tramadol, this substance also has an increased risk of drug interaction. The following side effects may occur:

- nausea
- dry mouth
- headaches
- sleepiness
- insomnia
- sweating

(https://www.pharmawiki.ch/wiki/index.php?wiki=Venlafaxin , 14.12.2019)

Structure:

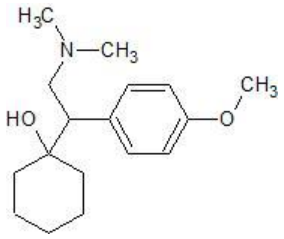


Figure 6: Structure Venlafaxine

It easily dissolves in water because of the aromatic structures and the carbon chains.

(P. Pfestrof, H.Kadner, et.al Chemie: Ein Lehrbuch für Fachhochschulen. Seite 480 bis 485, Auflage 2000, Thun und Frankfurt am Main: Harri Deutsch, 2000.)

7.1.5. Methamphetamin

7.1.5.1. Methamphetamine as medicine

Methamphetamine is used as a drug for ADHD, obesity and, in the past, narcolepsy. Due to the high incidence of abuse, the narcotic is subject to increased prescription requirements. It has a psychotropic and stimulating effect. It also raises blood pressure, stimulates breathing and inhibits appetite. Usually the white crystalline powder is administered once or twice a day in tablet form. It can cause several side-effects, such as

- high blood pressure
- accelerated pulse
- cardiac arrest
- cardiac arrhythmia
- psychoses
- hallucinations
- aggressiveness
- dizziness
- restlessness
- diarrhoea
- blockages
- dry mouth
- flavour disorders
- hypersensitivity reactions
- impotence
- libido changes

(https://www.pharmawiki.ch/, 23.12.2019)

7.1.5.2. Abuse

There are different ways of consuming the drug. Smoking on the one hand is the less effective one, on the other hand injecting the drug powder which has been dissolved in water or alcohol is the most effective way. Throw the blood the substance gets directly to the brain. There it increases the amount of the natural chemical dopamine. Dopamine is involved in the movement, motivation and reinforcement of rewarding behaviours of the body. Dopamine is reward in areas of the brain which strongly reinforce drugtaking behaviour. A small amount of methamphetamines cause short term effects and can affect patients health. These effects include:

- increased wakefulness and physical activity
- decreased appetite
- faster breathing
- rapid and/or irregular heartbeat
- increased blood pressure and body temperature

Injection of this substance increases the risk of an HIV and AIDS infection. Also, there are several long-term effects from long-time consumption:

- extreme weight loss
- addiction
- severe dental problems
- intense itching, leading to skin sores from scratching
- anxiety
- changes in brain structure and function
- confusion
- memory loss
- sleeping problems
- violent behaviour

- paranoia—extreme and unreasonable distrust of others
- hallucinations—sensations and images that seem real though they aren't

(NIDA. "Methamphetamine." National Institute on Drug Abuse, 16 May. 2019, https://www.drugabuse.gov/publications/drugfacts/methamphetamine. 31.12. 2019.)

7.2. Squalius cephalius

This fish was used for the experiment. The freshwater fish is manly found in Central Europe. He prefers a pH range from 6.0 – 7.8, and a cold-water temperature from 4°C – 20°C. This temperature is typical for the water in Central Europa. His common length is 30.0cm but he can reach a maximal length from 60.0cm. His maximal weight is 8kg and the maximal reported age is 22 years. The fish can be found in a lot of different types of water, for example in small rivers and very small mountain streams. Adults are solitary, the young fish lives occur in groups. They mainly eat plant material. The female fish lays eggs in fast-flowing water. There should be submerged vegetation. Because of the bones, this particular fish is not often used for cocking.

(Binohalm, Crispina: https://www.fishbase.se/summary/Squalius-cephalus.html , 13.1.2020)

Figure 7: Squalius-cephalus

The fish is coloured in a spectrum from silver to olive green. His tummy is withe. The chub has a large mouth without any teeth. Instead of teeth he has a horn edge.

(WESO Software Gmbh, https://www.fischlexikon.eu/fischlexikon/fische-suchen.php?fisch_id=0000000005 ,13.1.2020)

7.3. Fish anatomy

7.3.1. Heart

Typically, the fish heart consists of four heart chambers, which are coupled in series. The four chambers are the sinus venosus, the atrium, the ventriculum and the bulbus, this part is very flexible.

(Franck, Terwinghe, Dangcey: Atlas of fish histology. Page 47. Enfield, NH, USA: Science Publisher, 2009)

“The heart of fishes consists of three layers of tissue, the epicardium, myocardium and endocardium. The external epicardium consists of a single layer of flattened epithelial cells, the mesothelium, on a thin connective tissue layer, that merges with the pericardial cavity lining. The myocardium varies in thickness in different parts of the heart. It is thin in the sinus venosus, but it is contractile. The volume of the sinus venosus […] is equivalent to that of the atrium, which is the next chamber. The muscular layer is somewhat thicker in the cardiac atrium […], where pectinate muscles radiate from the roof of the atrium forming a star-burst muscular net. The ventricle […], as expected, has the thickest layer of cardiomyocytes. Its wall,

which thickness is variable according to sex and age, can contain several layers of muscular fibbers. It is also characterized by an abundant spongy myocardium that leaves in the lumen some lacunae in which blood circulates. In the

ventricle, blood acquires a high pressure and passes, through valves [..], into the last chamber of the heart.

The pear-shaped bulbus arteriosus has no valves and constitutes the thickened base of the ventral aorta, the main vessel leaving the heart and leading the deoxygenated blood into the gills. Its wall contains fibroelastic tissue […] which acts as a “shock absorber” when the blood is pumped by the ventricle. In teleost’s, a very short conus with two valves is present and is immediately anterior to the bulbus.

The conus arteriosus […] of the elasmobranchs has a thick and contractile wall containing numerous cardiomyocytes. It has a series of valvules, the number (from two to seven pairs) and the organization of which are very different according to the groups.

The endocardium, homologous to the tunica intima of blood vessels, consists of a one-cell thick layer (endothelium) that may be highly phagocytic in some species (Atlantic cod, plaice). Unlike the mammalian heart, the teleost myocardium is capable of regeneration.

All the cardiac chambers of the fish heart are enclosed in a pericardium of fibrous tissue variably adhering to surrounding tissues, making a rigid space around the heart. The pericardial space is filled with fluid, an ultrafiltrate from plasma. “

(Franck, Terwinghe, Dangcey: Atlas of fish histology. Page 47. Enfield, NH, USA: Science Publisher, 2009)

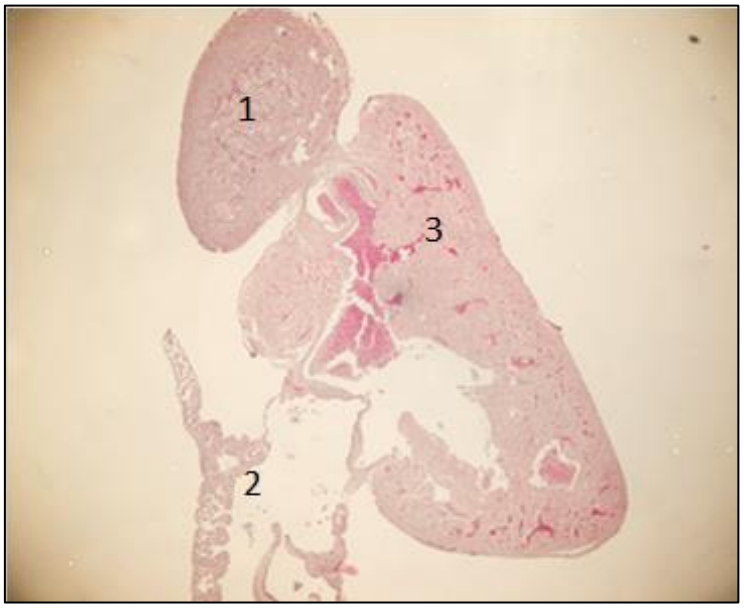


Figure 8: Heart sample

Number 1 is the bulbus arteriosus, the second number shows the atrium and the number three is the ventricle.

7.3.2. Liver

The fish liver and its functions are similar to those of mammals. A few of these important functions are the assimilation of nutrients and the production of bile. But the most important function is detoxification. In addition to that, the liver maintains the body's metabolic homeostasis that includes processing carbohydrates, proteins, lipids and vitamins. Different types of fish show different varieties of liver tissue. The following features are found in the majority of species.

A thin capsule of fibroconnective tissue is found in the parenchyma.

(Franck, Terwinghe, Dangcey: Atlas of fish histology. Page 92. Enfield, NH, USA: Science Publisher, 2009)

"Glycogen deposits […] and fat storage […] often dissolved during the routine histological process, produce considerable histological variability. […]

Bile ducts […] also occur within the parenchyma of the liver. Originating between adjacent hepatocytes, bile canaliculi anastomose to produce ducts of increasing diameter. The ducts merge and almost always end (except some sharks and skates) in the gall bladder […], lined by a pseudostratified epithelium […]. The bile drains into the duodenum by the common bile duct. Smaller ducts within the liver are lined with a single layer of cuboidal epithelial cells. Larger ducts may incorporate connective tissue and a thin muscularis. The hepatic structure normally varies (and considerably) in direct relationship to gender, age, available food (especially with regard to glycogen and fat content), or temperature, and with endocrine influences strongly connected to the environmentally regulated breeding conditions. "

(Franck, Terwinghe, Dangcey: Atlas of fish histology. Page 92. Enfield, NH, USA: Science Publisher, 2009)

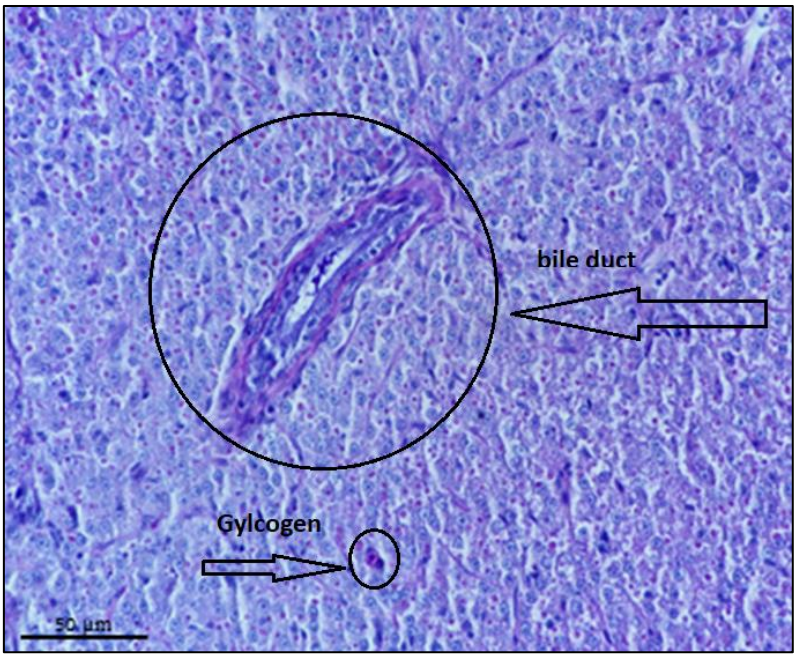


Figure 9: Liver with bile duct

Figure 9 shows a bile duct as well as a cell full of glycogen can be seen.

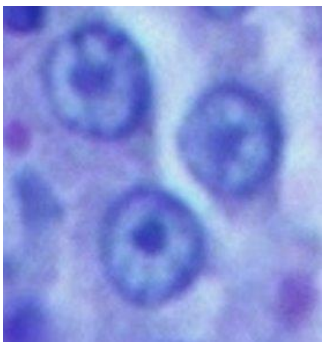


Figure 10: Healthy cells

This picture shows 3 healthy cells with a nucleus in the middle of the cell.

8. Methods

8.1 Used devises

8.1.1 Microtome:

DiaPath Galileo Auto

This rotary microtome can cut histological samples in 0.5 µm thin. It can be used both in automatic and semi-automatic mode.

8.1.2 Water bath:

Leica HI1210

This water bath provides high thermal conductivity rats and outstanding scratch resistance, because of the special plastic coating. It has an overheating protection system and can heat up to 75°C.

8.1.3 Heating plate:

Sakura 1452

It can heat up to 70°C. Also, it has an overheating protection at 90°C.

8.1.4 Slide Stainer:

Tissue-Tek DRS 2000

This stainer handles up to 11 sets of 40 slides at a time. There are 20 different methods stored in the memory. It inculdes27 reservoirs and 1 drying station. The Process Monitor Screen shows the remaining process time for each basket. Up to 20 user defined protocols can be installed which includes up to 50 different steps.

8.1.5 Tissue processor

Bavimed Histomaster 2062/2L

The tissue processor changes water into paraffin. First the water from the tissue get removed by dehydration, which is done by a series of alcohols. As a next step tissue get cleaned with xylene. Now the tissue gets fixed with paraffin.

8.1.6 Microscope

Olympus BX51

Microscopes with an upright-style frame can produce fluorescence illumination either through episcopic or diascopic optical pathways, although the latter is rarely used today. Epi-illuminators usually consist of a mercury or xenon lamphouse coupled to a vertical illuminator that is positioned above the main frame in a separate assembly. The microscope nosepiece and transmitted light components (diascopic illuminator, condenser, field diaphragm, filters, etc.) are built into the main frame, while fluorescence components are housed in the vertical illuminator. These illuminators often contain a revolving or sliding turret that houses four to six "cubes" that contain a mixture of interference filters including a barrier filter, dichroic mirror, and an excitation filter. As illustrated above, light emitted from the lamp positioned in the episcopic lamphouse passes through a collector lens and then the field and aperture diaphragms before entering the first interference filter in the cube set, the emission filter. This light is then directed through the objective and onto the specimen by a special dichroic mirror that reflects certain wavelengths while passing others. Secondary fluorescence, emitted by fluorophores residing in the specimen, travels back through the objective and the dichroic mirror before passing through a barrier filter and into the microscope eyepieces or camera system.

(William K. Fester et.al. - Olympus America, Inc., Two Corporate Center Drive., https://www.olympus-lifescience.com/en/microscope-resource/primer/techniques/fluorescence/bx51fluorescence/, 10.02.2020)

8.1.7 Paraffin embedding station

Leica ER 1150 H

It has a capacity of 3 litres and is high-adjustable. It has removable and heat able paraffin collection trays. The temperature range of whole aperture is adjustable from 55°C to 70°C.

8.1.8 Heat able electric tweezer

Leica EGF

This tweezer can heat up to 68°C, because of the current resistance. A safe sample transfer is enabled. Due to the heat the paraffin does not adhere to the tweezers. This facilitates the embedding process. The tips are not heated, which ensures that the sample is not damaged.

8.2 University of South Bohemia in Ceske Budejovice

The project took place within the framework of the International Summer School 2019.

The practical part of this diploma thesis was completed in the Czech Republic in Vodnany at the Faculty of Fisheries and Protection of Waters.

Work was done at the histology laboratory under the surveillance of our supervisor MSc Maria Eugenia Sancho Santos.

8.3 Sampling

Squalius cephalus is used for our samples. Sampled need to be collected from freshly dead fish. The fish used for the samples mustn't be frozen before taking the samples. The samples are divided into the control group and the exposure group. The exposed fish are exposed to 1 µg/l of a representative mixture of psychoactive substances. It contains citalopram, sertraline, oxazepam, tramadol, venlafaxine, methamphetamine. These are in turn subdivided according to the period of time the fish are exposed to psychoactive substances. The first sampling takes place 7 days after exposure, the second after 21 days of exposure. The sampling was already done by the facility.

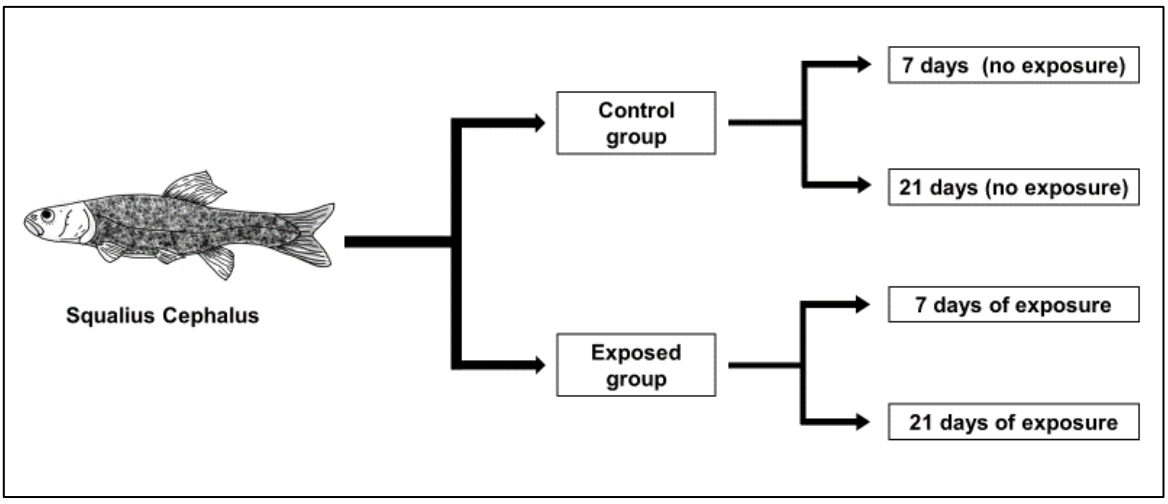


Figure 11: Collection of samples

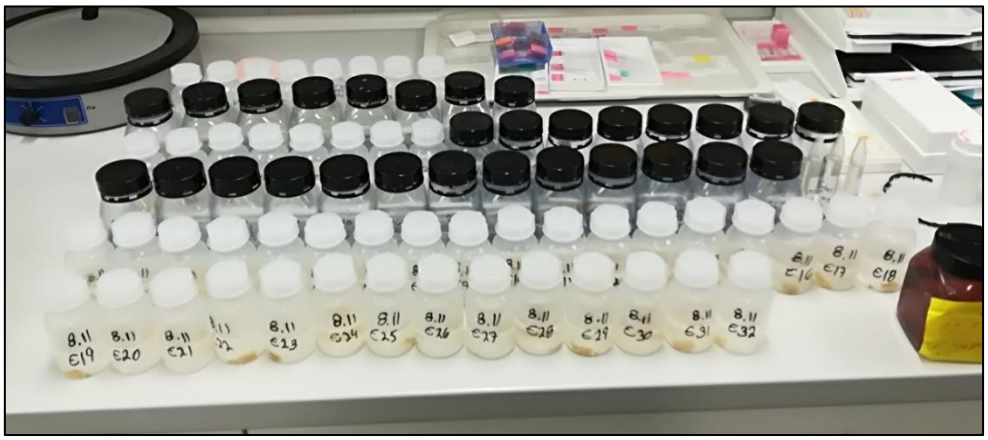


Figure 12: Listing of samples

8.4 Fixation

The fixation is very important in order to produce good histologic slides. Formalin is used for the fixation, which is the most widely used fixative. Tissues shouldn't be much thicker than 3 mm in order to achieve a good fixation. Formalin does not produce "overfixation", which means that the tissues don't harden unpredictably.

In order to fixate the tissue, the samples simply need to be placed in formalin. The formalin needs to be buffered, in order to prevent formalin pigmentation.

After 12-24 hours the samples should be transferred to 70% EtOH. This will prevent the tissue from overhardening and makes the storage and transport of the samples easier.

Davidson's shouldn't be used as a fixative in this case, because it could cause in tissue swelling and lead to the destruction of cells, which are needed for analysis.

8.5 Tissue Processing

Before the actual tissue processing the samples need to be transferred from the fixative to cassettes.

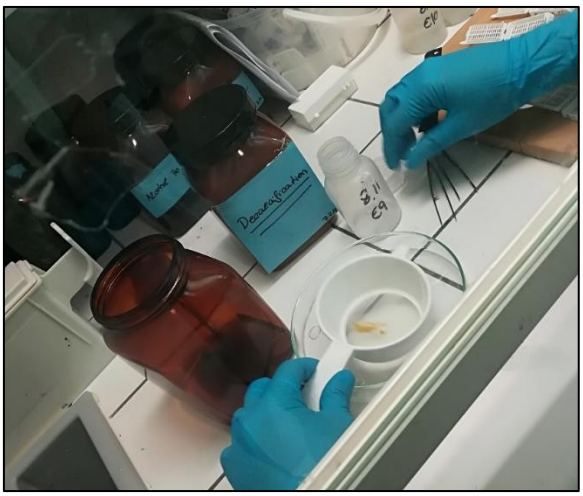


Figure 13: Transfer of samples 1

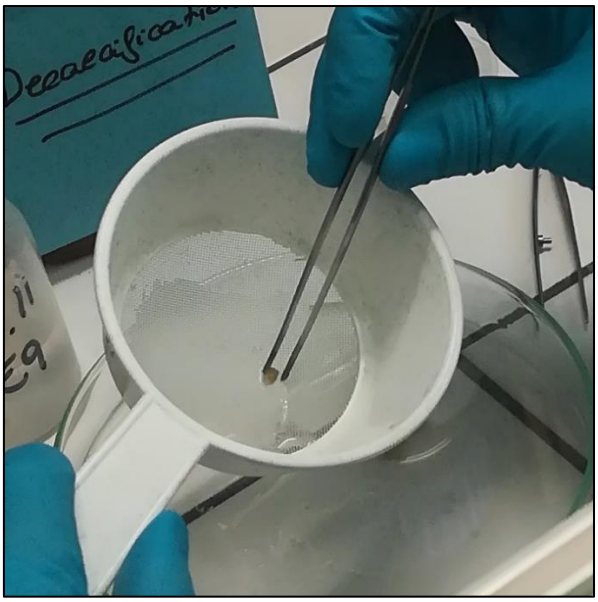


Figure 14: Transfer of samples 2

Before being placing the liver and the heart in the cassettes, the liver has to be cut, because it is connected with the intestines of the fish. The intestines are not relevant for the project and would only make it harder to detect the liver tissue when looking at it through the microscope. Another positive aspect of cutting the liver is to prevent the cassette from getting overstuffed. It is very important to not overstuff the cassettes and to handle the organs gently.

The following figure shows the liver and the intestines connected to it.

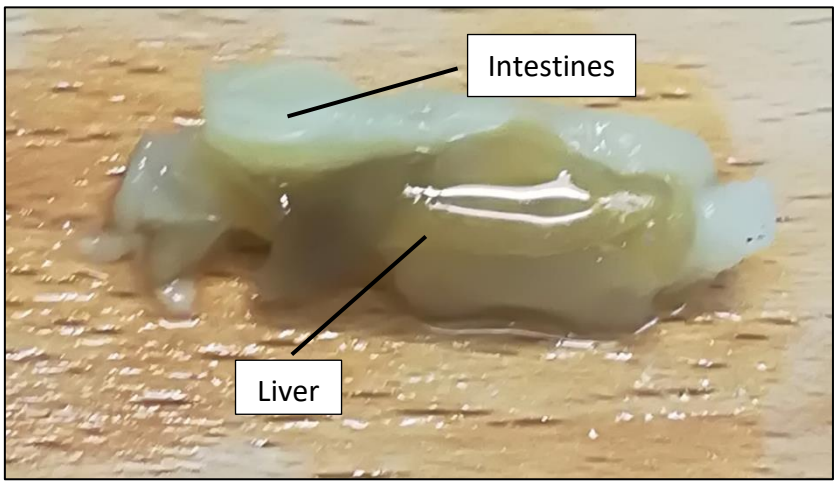


Figure 15: Liver attached to interstines

After cutting the liver there should be as little of the intestines as possible attached to the part that is used for analysis. It is recommended not to cut away too much of the sample.

After the liver is cut, both heart and liver are placed in cassettes. The cassettes must be inscribed to avoid mixing up the samples afterwards. This has to be done with pencil, since it won't dissolve in ethanol.

Figure 16: Cassette with inscription

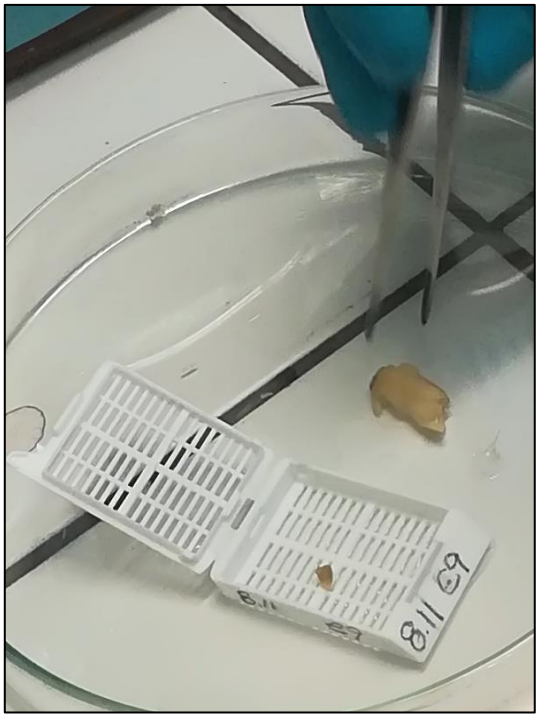


Figure 17: Placing the samples in the cassettes

In order to dehydrate and infiltrate the tissue of the samples the cassettes are placed in the tissue processor. In the processor the cassettes with the samples will be taken trough a series of water/alcohol mixtures up to full alcohol. Next, they will be taken through a clearant (makes the tissue become translucent), xylene (HistoClear) or substitute. The final step of this procedure is to put the cassettes into melted paraffin. This is also done by the tissue processor. It is recommended to let the processor work over night, because the procedure takes a lot of time.

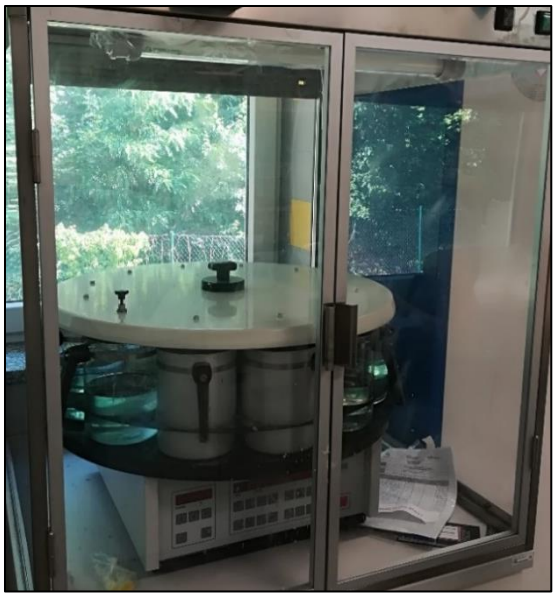


Figure 18: Closed tissue processor

Figure 19: Opened tissue processor

The dehydration of the tissue with alcohol is necessary, because water is not compatible with wax/paraffin. The clearant makes the alcohol compatible with wax/paraffine. Wax/paraffine provides a matrix that supports the tissue during sectioning.

8.6 Embedding

First the organs need to be transferred from the cassettes to a small metallic tin. The samples are oriented inside the tin, which gets filled up with paraffin afterwards. At first it is necessary to fill the tin only slightly and put it onto the freeze spot on the machine. This allows the first small layer of paraffin to harden and prevents the organs from slipping out of place during filling up the rest of the tin. It is very important to avoid having any bubbles of air inside the samples. After the tin is filled up with paraffin one half of the inscription on it is put onto the filled-up tin. After hardening the paraffin will make the piece of the cassette stick to the rest of the block. This allows the sample to have a solid bottom after removing the tin. It also provides the inscription to avoid mixing up any samples.

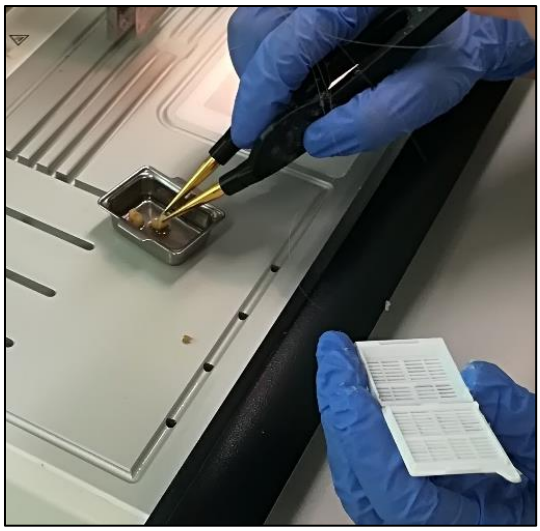


Figure 20: Embedding 1

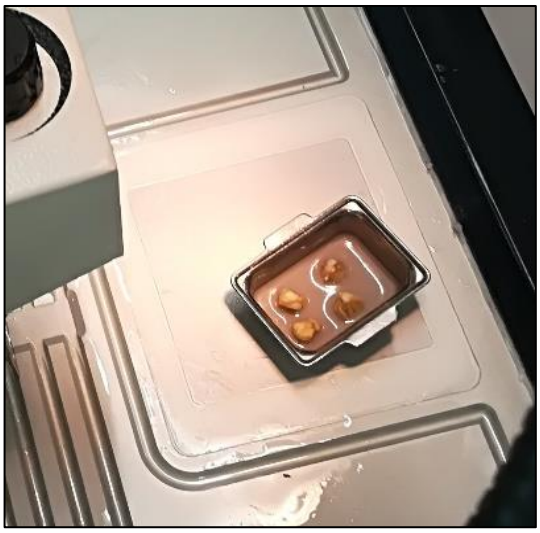


Figure 21: Embedding 2

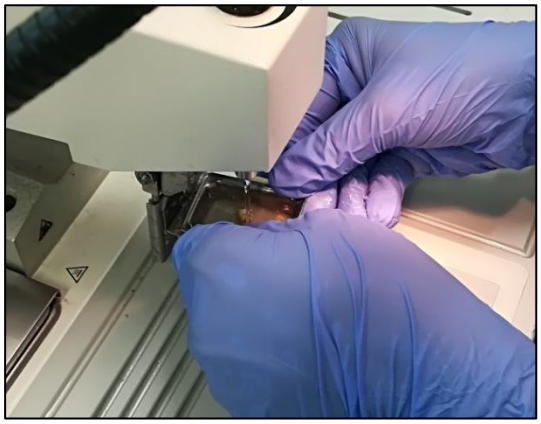


Figure 22: Embedding 3

Figure 23: Embedding 4

After the embedding is complete the blocks are placed in the refrigerator allowing them to fully harden. As soon es the block has hardened the tin gets removed from the block.

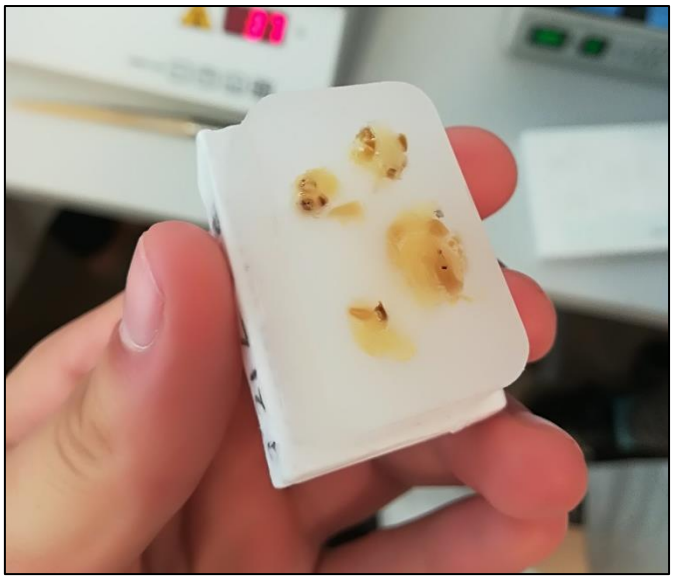


Figure 24: Paraffin block

8.7 Sectioning

In order to section the tissue the blocks are cut with the microtome into 4,5 µm thin sections. The sections shouldn't have to many holes or tears in them, because they lower the quality of the samples.

Figure 25: Microtome with paraffin block

Figure 26: Microtome + cut slides

The cut sections are put into a water bath. After inscribing the slides, they are used to take the sections out of the water. This way they are easily transferred onto the slides. Next the slides are put onto a warm surface allowing the rest of the paraffin block to melt. This will only leave the tissue on the slide.

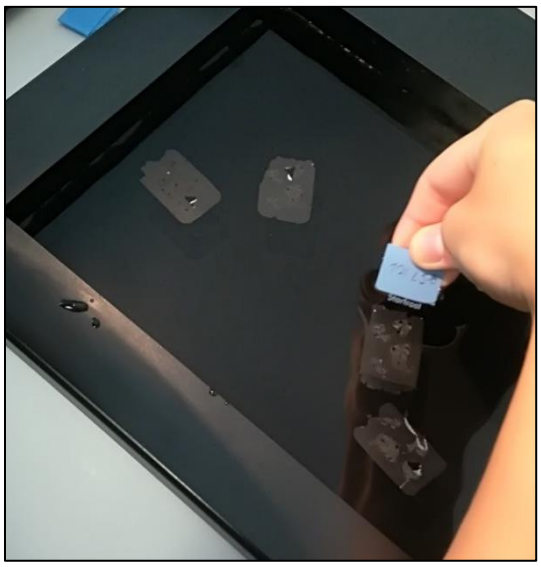


Figure 27: Taking sections from the water with slides

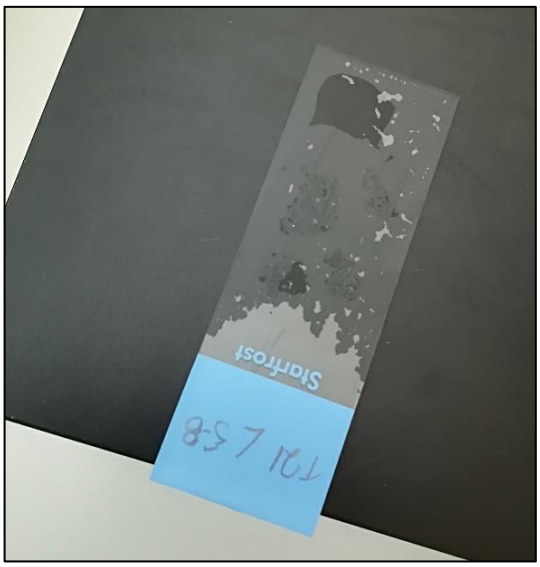


Figure 28: Melting the rest of the paraffin

8.8 Staining

The slides get re-hydrated. For each organ two different stains are used. This is necessary, to confirm certain changes in the tissue. In addition to that some changes in the tissue are only visible with a certain stain.

Haematoxylin and eosin as well as Alcian blue and PAS are used to stain the tissue of the liver. For staining the heart tissue Haematoxylin and eosin as well as Masson's trichrome are used.

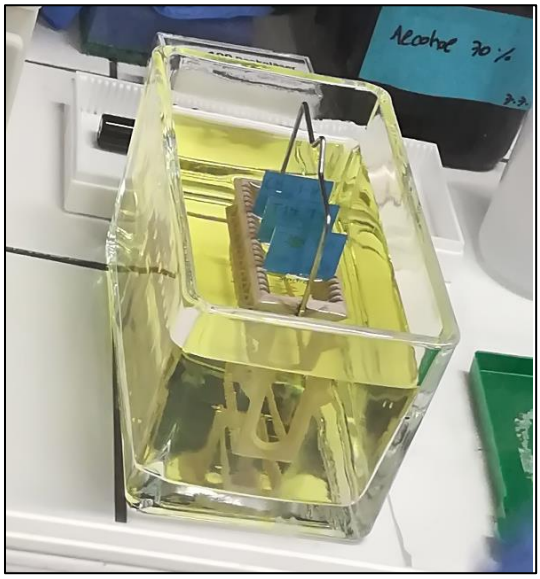


Figure 29: Staining 1

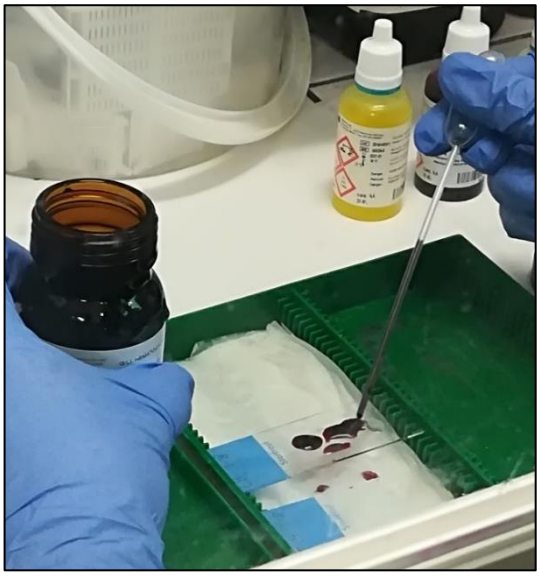


Figure 30: Staining 2

8.9 Microscopy

After the samples are stained, they are ready to be examined under the microscope. It is very important to compare both stains in order to verify changes in the tissue. The liver is scanned for fat accumulation and macro vacuoles while the heart is scanned for infiltration, fibrosis and degeneration.

After evaluating the changes in the tissue the Kruskal-Wallis test is used in order to evaluate whether the changes are significant or not. The Kruskal-Wallis test compares the control group with the group that was exposed for 21 days.

The significance for Kruskal-Wallis test is set to $p < 0,05$. The Kruskal-Wallis test tests independent samples. It checks whether the central trends of several independent samples differ. The Kruskal-Wallis test is used when the requirements for an analysis of variance are not met.

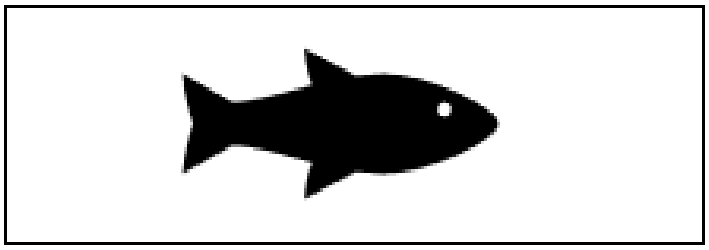
8.10 Preparation of teaching materials

The materials prepared for biology lessons held in English consist of 4 main parts.

First there is a description of fish anatomy. Second there is information about the pollution of waters and how it cannot be purified by sewage treatment plants. The next part shows how the liver and heart of fish get damaged by the pollution of waters with psychoactive substances. It also contains pictures of healthy and damaged tissue for the students to compare. The last part will be a crossword puzzle for the students to test their knowledge.

In order to visualize the structure of the worksheets some suggestions for structure plans will follow.

Fish Anatomy



1. ----------
2. ----------
3. ----------
4. ----------
5. ----------

   .....

Pollution of waters due to psychoactive substances

- ----------
- ----------
- ----------
- ----------

  .....

Figure 31: Structure plan of teaching materials page 1

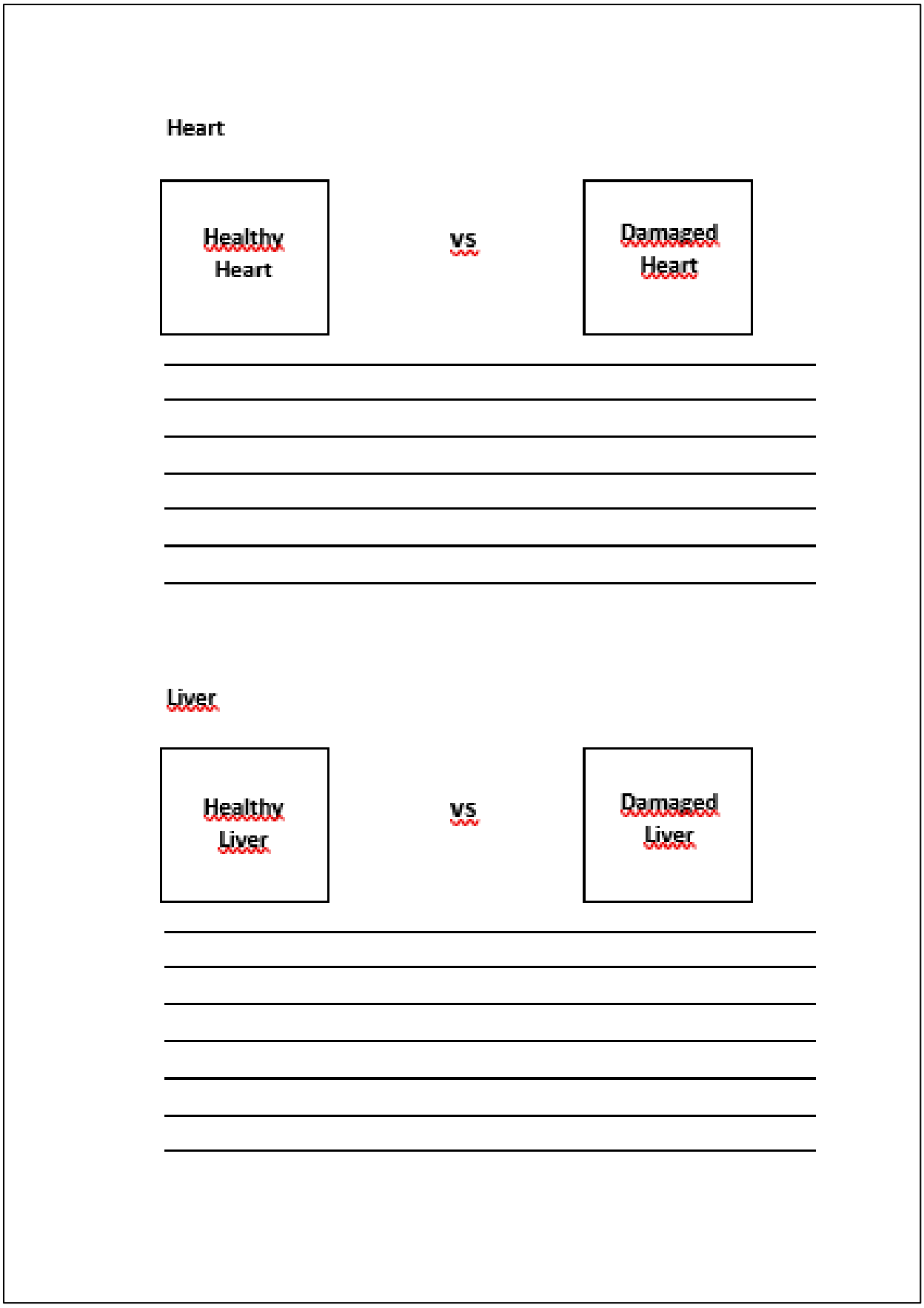
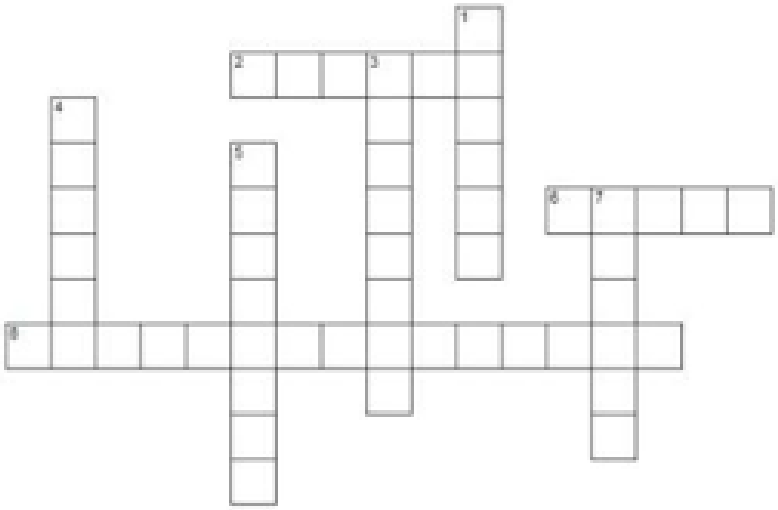


Figure 32: Structure plan of teaching materials page 2

Exercises

1) ----------
2) ----------
3) ----------
4) ----------
5) ----------
6) ----------
7) ----------
8) ----------



Solution: _ _ _ _ _ _ _ _

Figure 33: Structure of teaching materials page 3

9. Results and Interpretation

9.1 Liver

9.1.1 General information

The liver samples examined show damage. These were identified by two different col ouring methods. The staining with haematoxylin and eosin stains the cell assemblies better,but Alcian blue and PAS only serve as a comparison and stain the glycogen re ddish, which makes it possible to clearly distinguish a healthy from a damaged liver. The two most common damages are related to the accumulation of fat in the liver. The psychoactive substances reach the liver via the bloodstream with a wide variety of nutrients. Among other things, this organ is responsible for the filtration of toxic substances. The substances lead to an erroneous fail reaction, which causes fat to be stored. This is a misunderstanding between calories intake and calories consumption. It would not be necessary to store so much fat. This leads to an increased formation of neural fat in the liver. This fat is produced by the liver itself from fatty acids extracted from food. This is a normal process as this fat is converted into energy as soon as possible. However, no conversion takes place here. It leads to damages which can be irreversible. Also, the psychoactive substances are full of fatty acids which leads to a high production of neural fat.

9.1.2 Different types of damage

The following pictures show a healthy liver from the control group, the fish wasn´t exposed to any psychoactive substances. These pictures are intended to serve as reference pictures for the different damages and for explanation.

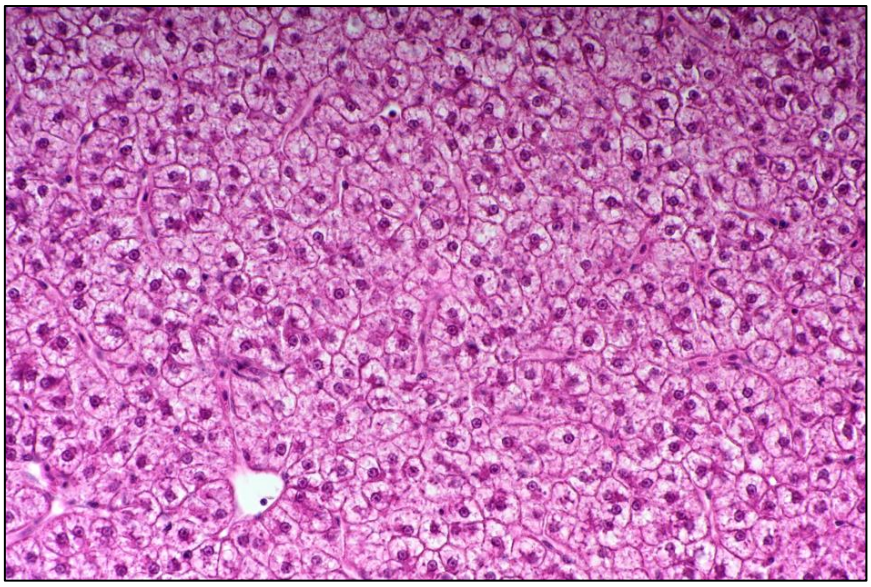


Figure 34: Healthy liver h&e (haematoxylin and eosin)

This liver got stained with haematoxylin and eosin. The nucleus as well as the membrane can be clearly seen. The whole cell structure seems to be healthy. The nucleus is mainly located in the middle of the cell.

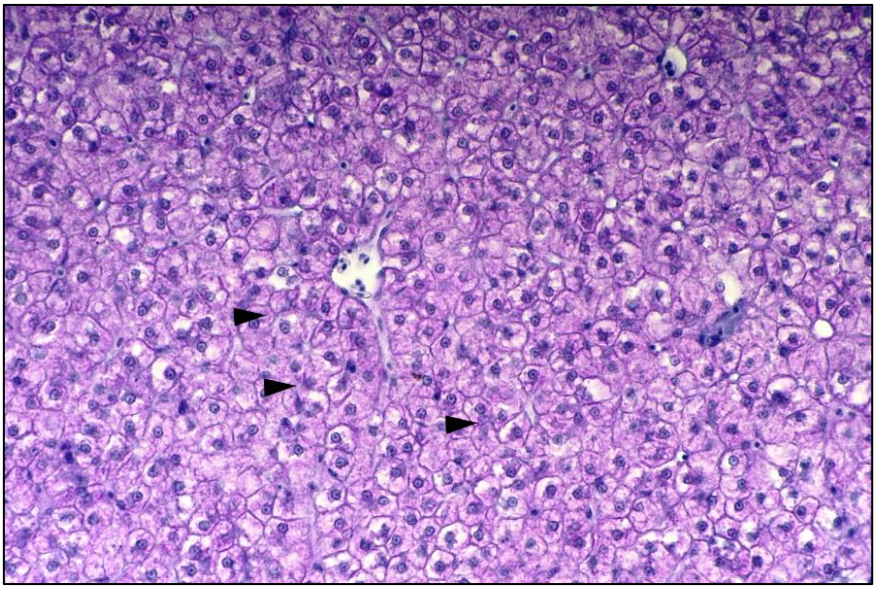


Figure 35: Healthy liver, Alcian blue and PAS

This is the same sample but stained with Alcian blue and PAS. The glycogen can be seen clearly, because it is coloured reddish. The arrows indicate granules glycogen.

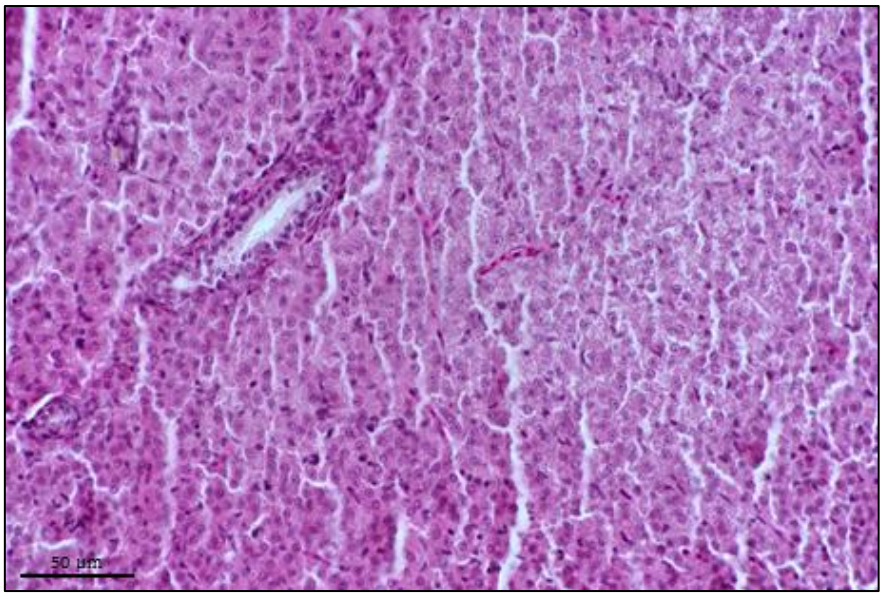


Figure 36: Liver artefacts 1

This is again a healthy liver from the control group, which is stained with haematoxylin and eosin. The white cracks are artefacts from cutting the tissue. It barely contains any fat, so it is harder.

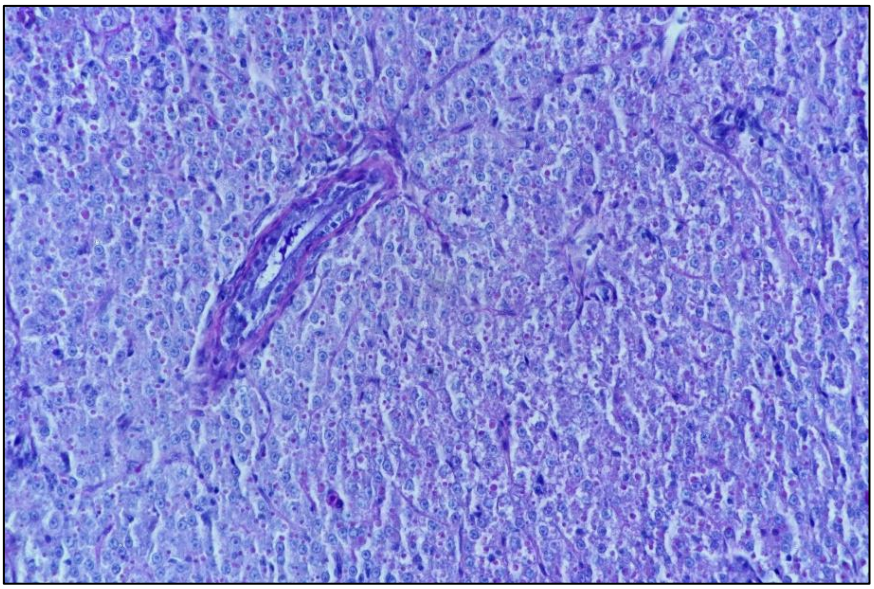


Figure 37: Liver artefacts 2

Figure 37 shows the reddish stained glycogen and a bile duct.

The following pictures show livers from fish from the exposer group.

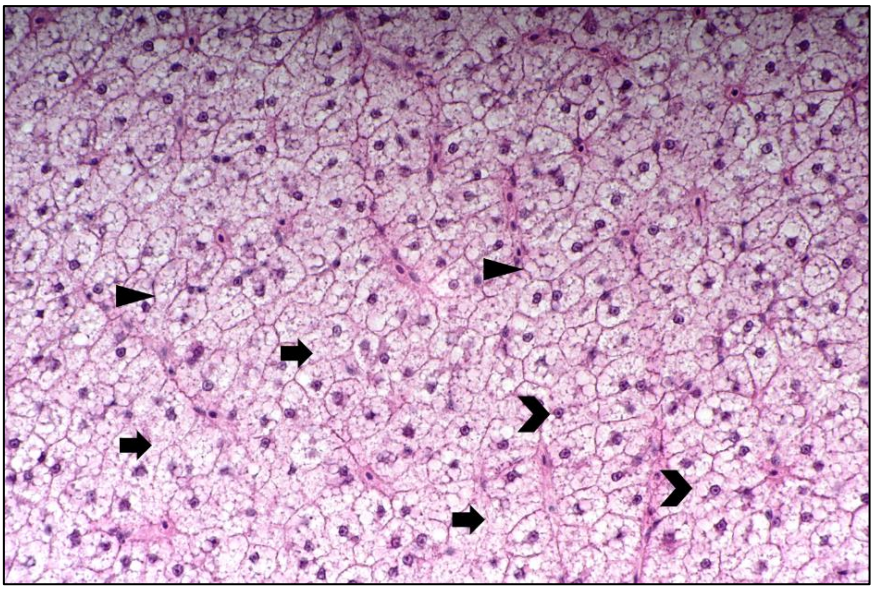


Figure 38: Damaged liver

This liver was stained with haematoxylin and eosin. It shows clear changes of the tissue. The closed arrow heads mark macrovacuole. Macrovacuole are cells in which the membrane is ruptured, this leads to an exposed nucleus. It is caused by the high accumulation of fat in the cell. The increased pressure exerted on the nucleus causes the membrane to rip apart. When a cell loses its nucleus, it cannot reproduce and loses all vital information. Consequently, the cell dies.

The arrows show a rupture of the membrane. This is caused by the high pressure in the cell, which causes the nucleus to be pressed onto the membrane, thereby breaking it up. This allows both fat and the nucleus to pass outside the cell, where they in turn exert external pressure on the cells.

The open arrow heads mark vacuoles. This damage is the precursor to macrovacuoles. Here the nucleus is pressed against the membrane by the fat but does not cause it to break. This dislocation can lead to damage of the important material contained in the nucleus, which is important for the survival of the cell. However, this damage disappears as soon as the fat in the cell is broken down. If this does not happen and an increased accumulation of fat occurs, Macrovacuoles may arise.

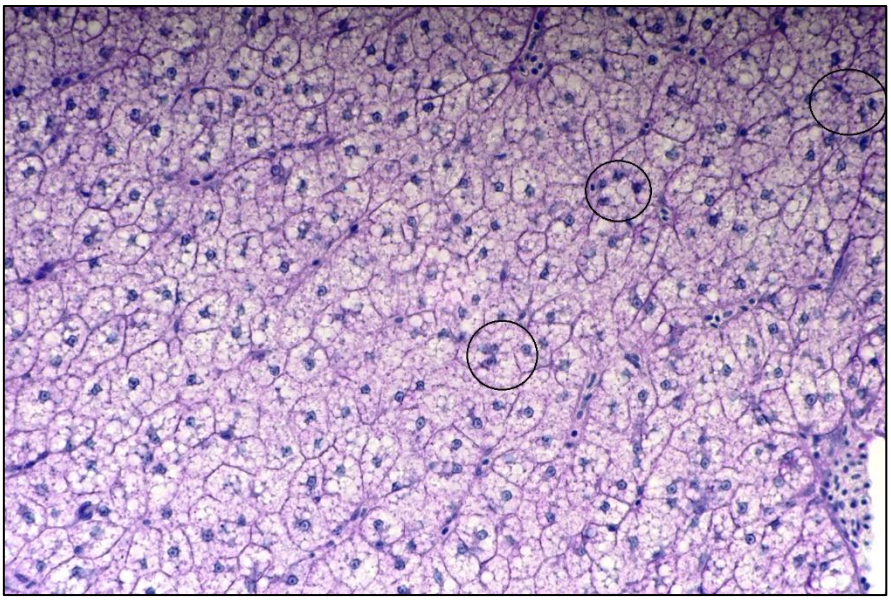


Figure 39: Liver, dislocated nucleus

This liver was stained with Alcian blue and PAS. The cells marked with a black circle show a clear dislocation of the nucleus. This staining method makes it clear that the cells are only filled with fat and no longer contain glycogen. This can be recognized by the fact that not a single reddish coloration is visible. Since such a coloration only occurs with glycogen, one can conclude that none is present.

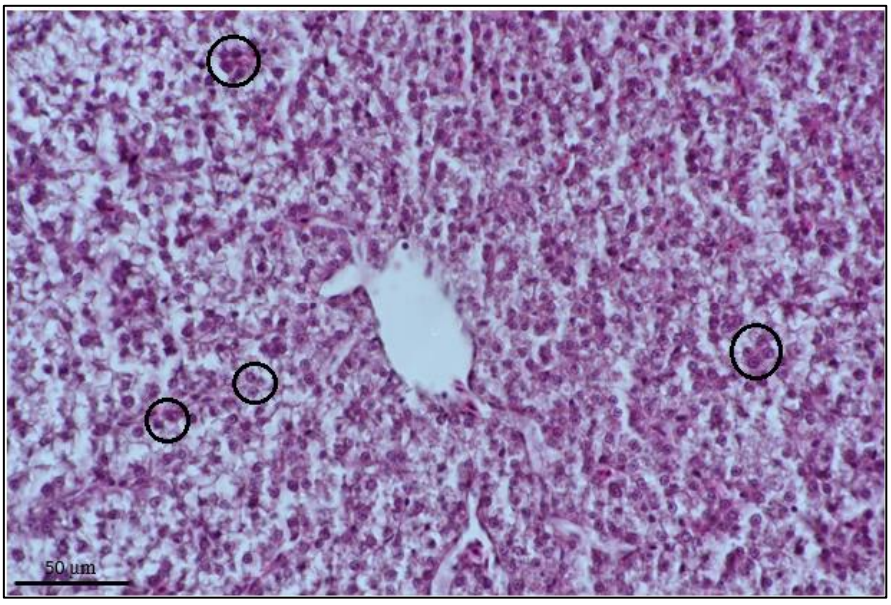


Figure 40: Liver, dislocated nucleus and artefact 1

This liver shows many dislocations of the nucleus and a round artefact in the middle of the picture. The areas marked in show only extracts of the many affected cells. Almost every cell is affected and therefore this liver shows many vacuoles. This is confirmed in the following picture, there the liver was stained with
Alcian blue and PAS and you can clearly see that there are only a few cells filled with glycogen left , because you can barely see any reddish coloration. Some of the areas where glycogen can still be seen are marked with a black circle.

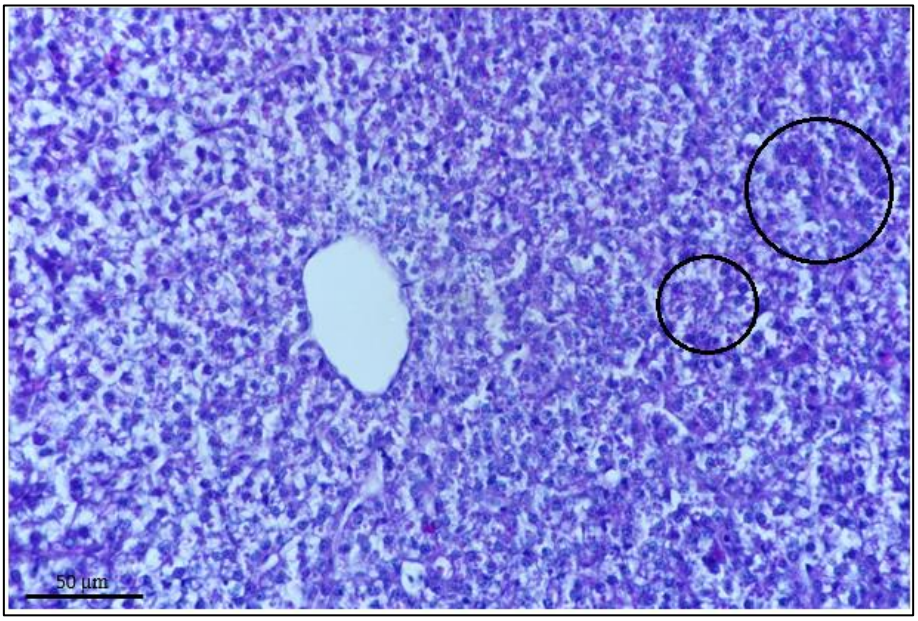


Figure 41: Liver, dislocated nucleus and artefact 2

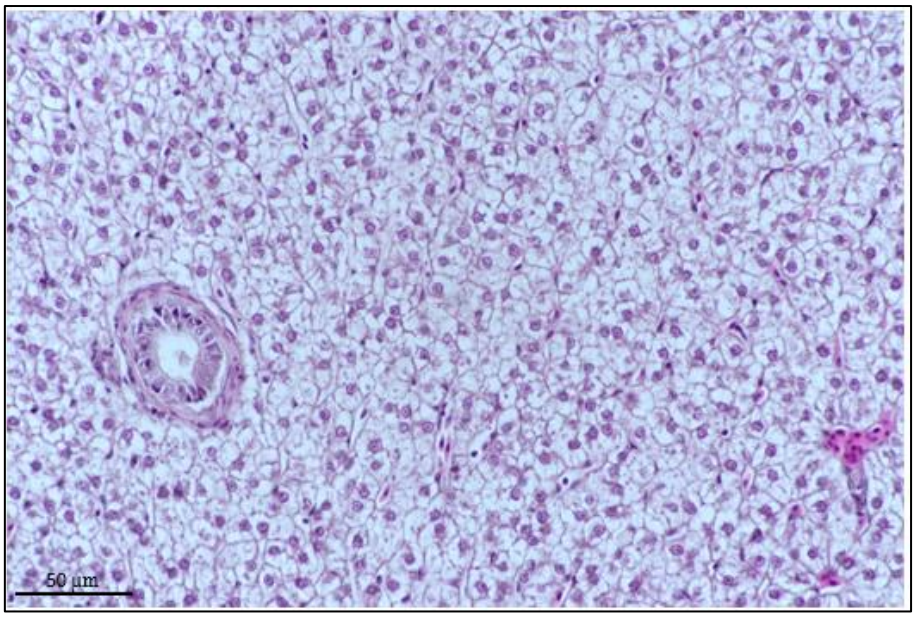


Figure 42: Liver with vacuoles and bile duct 1

In this sample it can be seen that each cell shown has a dislocated nucleus and therefore has vacuoles. Almost every nucleus is strongly pressed against the membrane. Occasionally one can see which ones are not directly on the membrane. However, they are not in the centre. If one now compares this result with the Alcian blue and PAS staining, one can clearly see that there is a high fat accumulation in the cells.

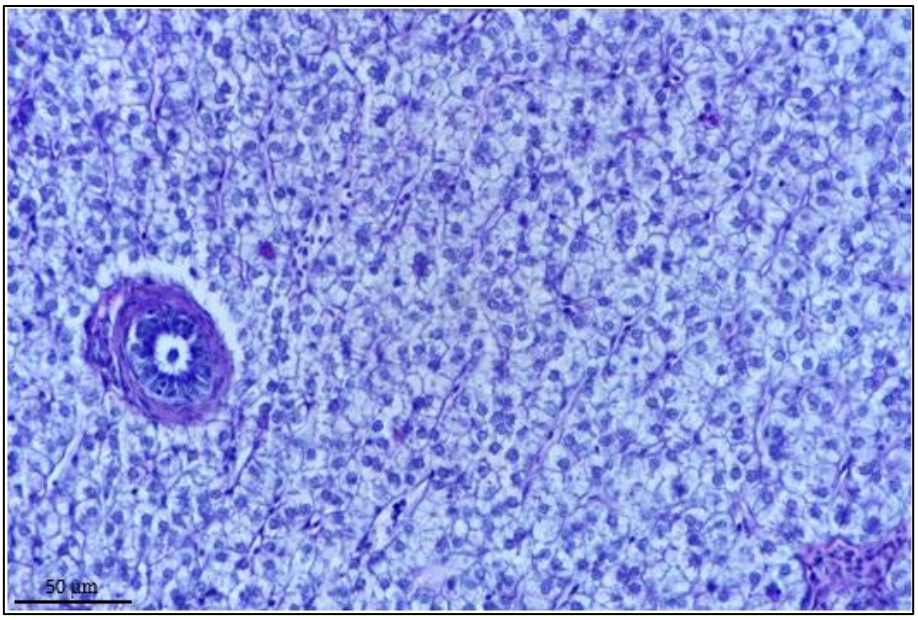


Figure 43: Liver with vacuoles and bile duct 2

9.1.3 Evaluation

In the following graphs the significance of the damage in the tissue is described on the y-axis. Therefor semi-quantitative classification was used, numbers 1 to 5 were given.

1 = scattered changes

2 = mild intensity

3 = moderate intensity

4 = severe intensity

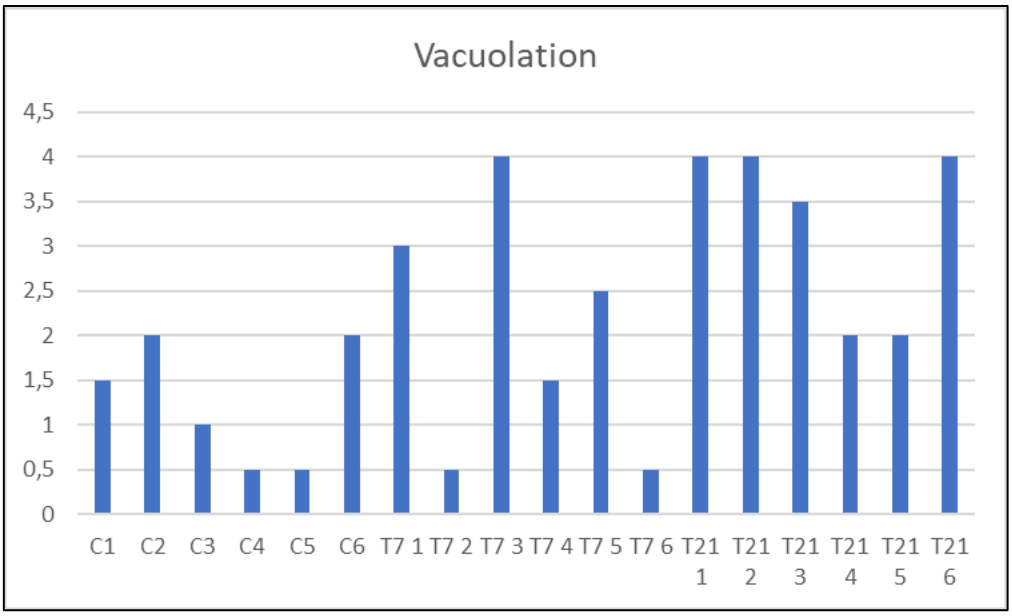


Figure 44: Vacuolation - diagram

According to the bar chart (figure 44), a rise of severity of the vaculation can be seen. In this graph, 4 (=severe intensity) stands for the worst infestation and 0 (= no changes) for no infestation at all. In the control group, it can be seen that at most a two-fold evaluation occurs - this stands for a mild intensity. This is only found in samples C2 and C6. After 7 days of exposure, a significant increase up to 4 in samples T7 3 is observed. The other samples are between a triple (3) and 0. 5 rating. After 21 days, there are three samples T21 1, T21 2 and T21 6, which have a score of four. The lowest value here is 2.

One reason for this significant increase in the intensity in the samples after 21 days is that these fish were exposed to the psychoactive substances for 14 days longer and therefore absorbed them longer than the T7 samples. More fat could thus be stored in the cells and led to vacuole.

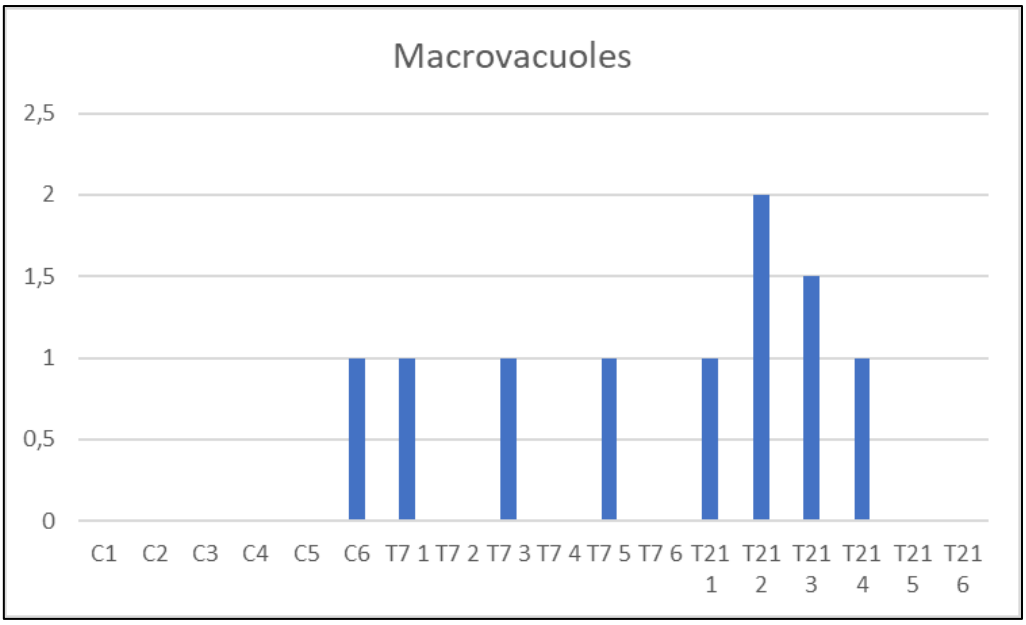


Figure 45: Macrovacuoles - diagram

In this bar chart (figure 45) the occurrence of macrovacuole is shown. The highest classification is 2 (=mild intensity) only sample T21 2 reached this figure. In the control group, only one sample showed the presence of macrovacuole. This sample is C6 and was graded with a 1 (=scattered changes). After seven days, scattered changes were detected in three of six samples.

These samples are T7 1, T7 3 and T7 5. After 21 days, a significant increase in the occurrence can be seen. Classification up to 2 are achieved. T21 1 and T21 4 are given a 1. T21 3 reaches a classification of 1. 5. The highest occurrence is shown by sample T21 2, with a classification of 2. It is noticeable that sample T21 2 already shows an increased figure in the vacuole. Due to the increased occurrence of vacuoles, the probability of macrovacuole increases. In general, it can be observed that each sample containing macrovacuole has an increased occurrence of vacuoles. This is because macrovacuoles are formed from the vacuoles. Due to the increased fat content, the pressure exerted on the nucleus can cause the membrane to break.

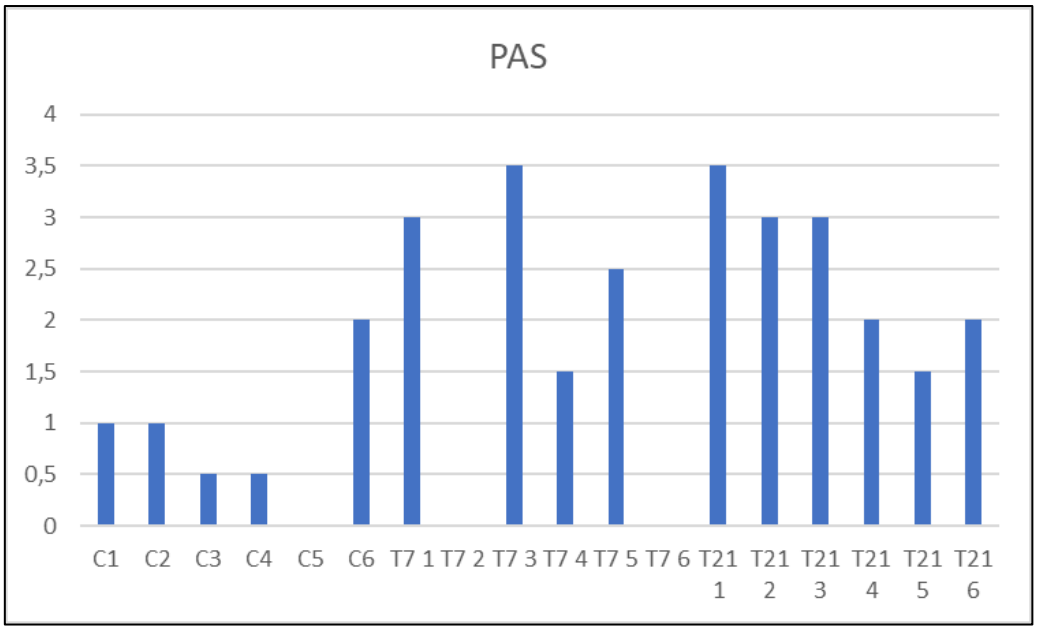


Figure 46: PAS - diagram

PAS is the second staining method used. The presence of fat in the cells is thereby made clear. This was also evaluated (figure 46). Both in the control group and in the samples of the groups with the contaminated fish, a clear occurrence is observed. While the highest classification in the control group is 2, which occurs only once in sample C6, a significant increase is observed after 7 days. T7 3 shows the highest occurrence here with a rating of 3. 5. After 21 days, fat is visible in the cells of all fish. The lowest rate here is 1. 5 and occurs at T21 5.

It is clearly visible that all samples with macrovacuoles also show an increased value in the PAS assessment.

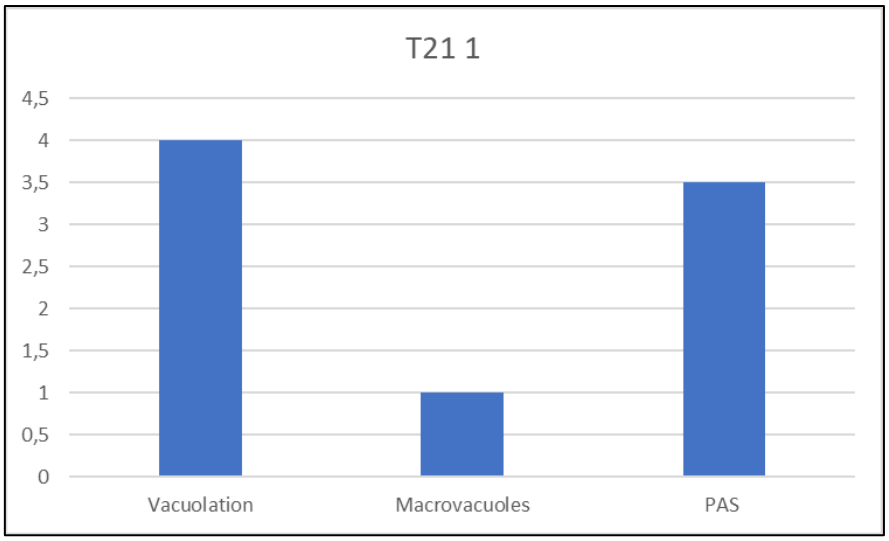


Figure 47: T21 1 - diagram

This bar chart (figure 47) represents the classification of the sample T21 1. One can clearly see a significant correlation between the formation of vacuole and macrvacuole. The vacuole which is checked against evaluation PAS shows a similar classification as PAS. An increased incidence of macrovacuoles is also clearly visible. This shows the connection between the vacuole and macrovacuoles.

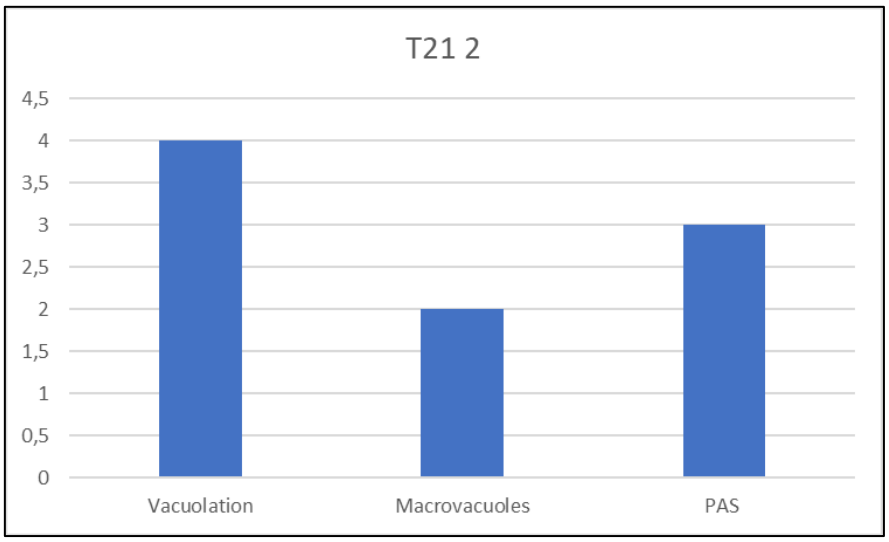


Figure 48: T21 2 - diagram

This sample (figure 48) represents the same connection as sample T21 1.

Sample	Group	Vacuolation	Macrovacuoles	PAS stain
C1	Control	1,5	0	1
C2	Control	2	0	1
C3	Control	1	0	0,5
C4	Control	0,5	0	0,5
C5	Control	0,5	0	0
C6	Control	2	1	2
T7 1	Time 7	3	1	3
T7 2	Time 7	0,5	0	0
T7 3	Time 7	4	1	3,5
T7 4	Time 7	1,5	0	1,5
T7 5	Time 7	2,5	1	2,5
T7 6	Time 7	0,5	0	0
T21 1	Time 21	4	1	3,5
T21 2	Time 21	4	2	3
T21 3	Time 21	3,5	1,5	3
T21 4	Time 21	2	1	2
T21 5	Time 21	2	0	1,5
T21 6	Time 21	4	0	2

Table 1: Results liver

The table (table 1) shows different evaluation of each sample.

The control group compared with the exposed samples after 21 days shows a significant value. For vacuolation a significant figure of $p = 0.0049$ was found. For macrovacuoles a $p > 0.05$ was found, this isn´t a significant figure. PAS shows a significant figure from $p = 0.0095$.

9.2 Heart

9.2.1. Figures

The various significant damages found in the heart tissue are infiltration, fibrosis, degeneration, oedema and pigmented macrophages. The results concerning the infiltration is divided into infiltration in the myocardium and infiltration in the pericardium.

The significance of the damages in the tissue caused by the exposition to psychoactive substances are evaluated using a semi-quantitative classification. Therefore, numbers from 1 to 5 are given.

1 = scattered changes

2 = mild intensity

3 = moderate intensity

4 = severe intensity

The following tables show the figures representing the significance of the changes in the tissue.

SAMPLE	GROUP	INFILTRATION IN THE MYOCARDIUM	INFILTRATION IN THE PERICARDIUM
C1	Control	1	1
C2	Control	0	2
C3	Control	1	1
C4	Control	0	1
C5	Control	0,5	1
C6	Control	0,5	1
T7 1	Time 7	1,5	1
T7 2	Time 7	0	1
T7 3	Time 7	2	1
T7 4	Time 7	1,5	1
T7 5	Time 7	0	1
T7 6	Time 7	2,5	1
T21 1	Time 21	2,5	1
T21 2	Time 21	1,5	1
T21 3	Time 21	3	1
T21 4	Time 21	2,5	1
T21 5	Time 21	2,5	1
T21 6	Time 21	1,5	2,5

Table 2: Results heart - infiltration

SAMPLE	GROUP	DEGENERATION	FIBROSIS	OEDEMA	PIGMENTED MACROPHAGES
C1	Control	2	0	1,5	4
C2	Control	1	0,5	1	0
C3	Control	0	0	0	4
C4	Control	1,5	2	1	2
C5	Control	1	0	0	2
C6	Control	1,5	0,5	0,5	3
T7 1	Time 7	1	1	0,5	3
T7 2	Time 7	0	0	0	2
T7 3	Time 7	0	2	2	3
T7 4	Time 7	1	3	1	1
T7 5	Time 7	1,5	2,5	3	3
T7 6	Time 7	1	2	2	2
T21 1	Time 21	1,5	2	1	2
T21 2	Time 21	4	2	0,5	4
T21 3	Time 21	2,5	0,5	1	2
T21 4	Time 21	1,5	1,5	1	4
T21 5	Time 21	2	1	1,5	2
T21 6	Time 21	2,5	1	1	2

Table 3: Results heart - other

Although all of these damages can be found in the heart tissue, not all of them show a significant change at the Kruskal-Wallis test. The following table shows the significance of each type of change that was found within the heart tissue:

	SIGNIFICANCE
INFILTRATION IN THE MYOCARDIUM	**p = 0.0014**
INFILTRATION IN THE PERICARDIUM	p > 0.05 (not significant)
DEGENERATION	**p = 0.0202**
FIBROSIS	p > 0.05 (not significant)
OEDEMA	p > 0.05 (not significant)
PIGMENTED MACROPHAGES	p > 0.05 (not significant)

Table 4: Results heart - signification

As represented by the table, only the infiltration in the myocardium as well as the degeneration show significant changes at the Kruskal-Wallis test, because the figure for p needs to be lower than 0,05 (as already described in the methods).

9.2.2 Infiltration

Infiltration is a vast array of inflammatory cells in a certain area. During an inflammatory process in the tissue defence cells are created. These have the task of eliminating the infectious agents. This causes inflammatory infiltrates to emerge.

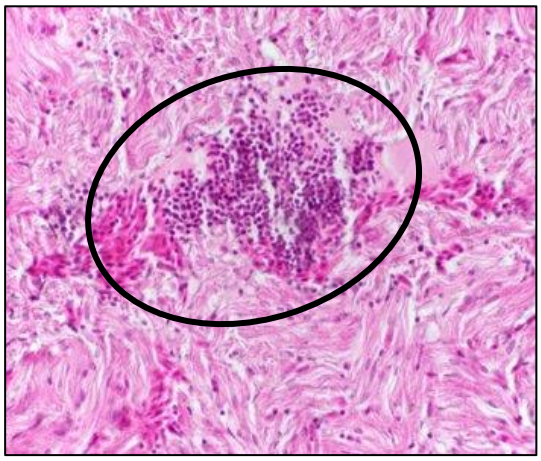


Figure 49: Infiltration - Haematoxylin and Eosin

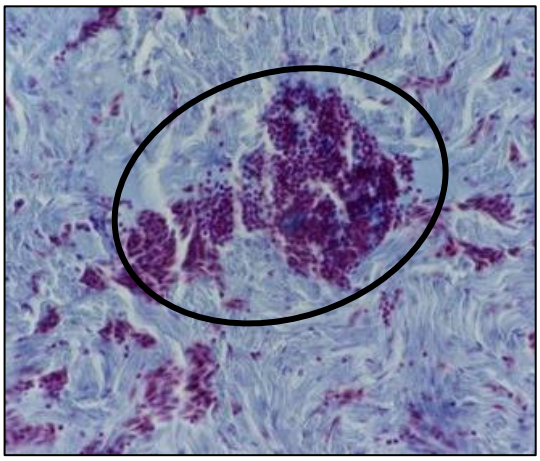


Figure 50: Infiltration – Masson's trichrome

Figure 49 and 50 show infiltration in the heart tissue. Infiltration is visible when looking at the Hematoxylin and Eosin stain (figure 49) but can further be confirmed when looking at the Masson's trichrome stain (figure 50). The Hematoxylin and Eosin stain makes the separate inflammatory cells very well visible as well. They appear as bluish/violet dots when looking at the infiltrated area (figure 51).

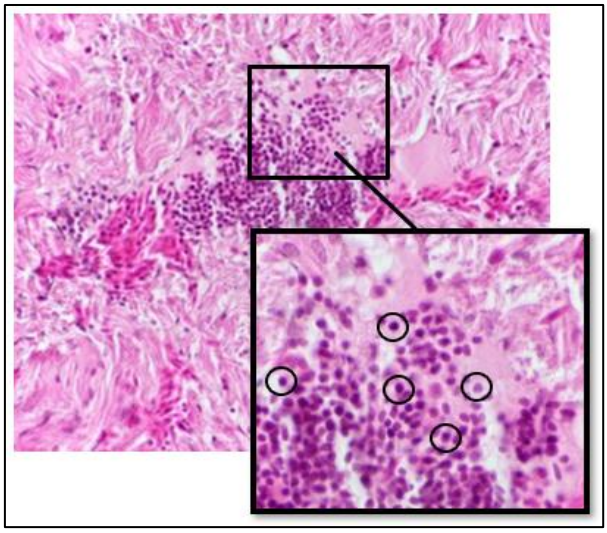


Figure 51: Inflammatory cells – Haematoxylin and Eosin

9.2.3 Degeneration

Degeneration is a kind of scar in the tissue. One speaks of degeneration when an area of the tissue deviates from the norm. In most cases, degeneration is a damage of the tissue's structure. Degeneration can be attributed to chronic damage to the organ.

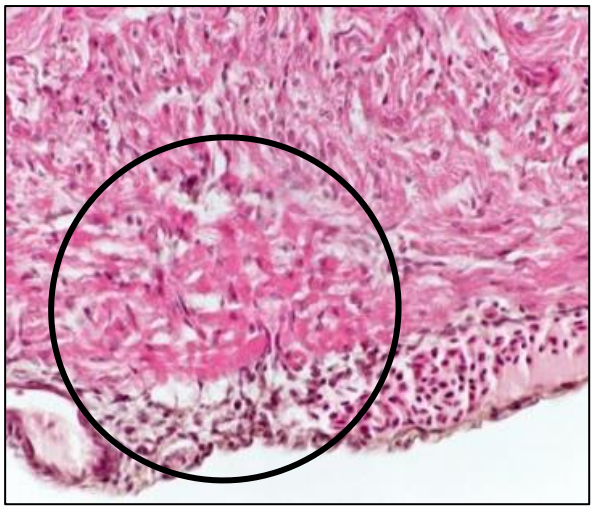


Figure 52: Degeneration – Haematoxylin and Eosin

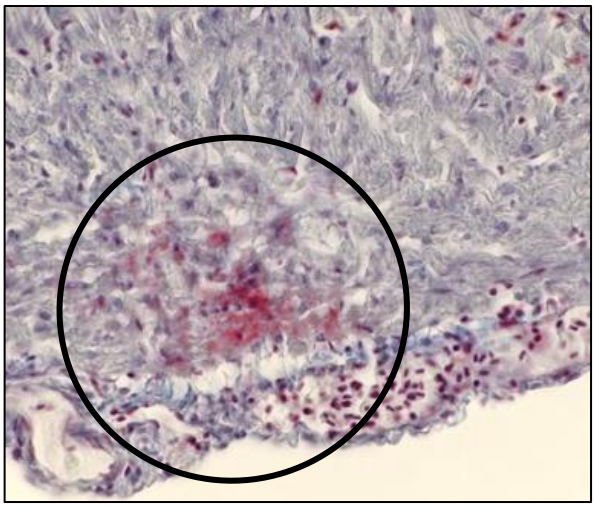


Figure 53: Degeneration – Masson's trichrome

Figure 52 and 53 show degeneration of the heart tissue. They also make clear, why two different stains need to be used. The degeneration is barely visible when looking at the Haematoxylin and Eosin stain (figure 52). It can only be identified when looking at the Masson's trichrome stain, which makes it appear red. The damaged structure of the tissue is also visible. The damage of structure is the only aspect of degeneration that is noticeable when looking at the Haematoxylin and Eosin stain.

9.2.4 Fibrosis

The cause of fibrosis is an existing metabolic disorder. Fibrosis is a pathological proliferation of the connective tissue. This happens due to the excessive formation of collagen. This process hardens the affected tissue and impairs the heart in its function.

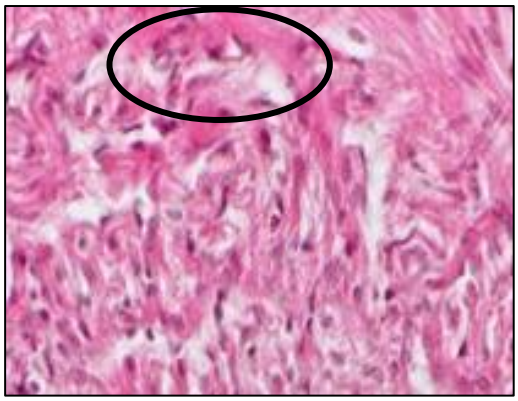


Figure 54: Fibrosis – Haematoxylin and Eosin

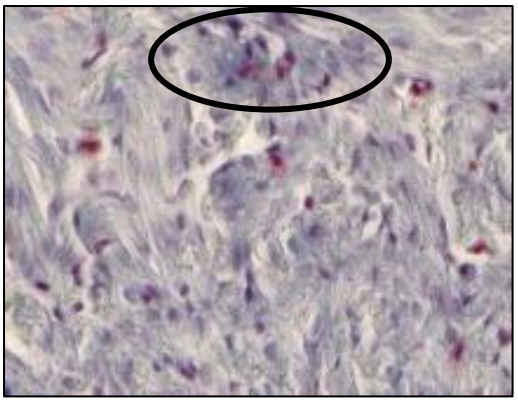


Figure 55: Fibrosis – Masson's trichrome

Like degeneration, fibrosis is hardly recognizable when looking at the Haematoxylin and Eosin stain (figure 54). When looking at the Masson's trichrome stain (figure 55) fibrosis appears dark blue.

9.2.5 Oedema

Oedema is accumulation of fluid in the tissue. It is located outside of cells and blood vessels. Oedemas are caused by an increase in pressure in the capillaries, a reduced concentration of albumin or damage to the vessel sheath. The reduced concentration of albumin can be tracked back to damage in the liver, because albumin is a protein, that is produced by the liver. Oedema leads to swelling in the tissue and subsequently to heart failure.

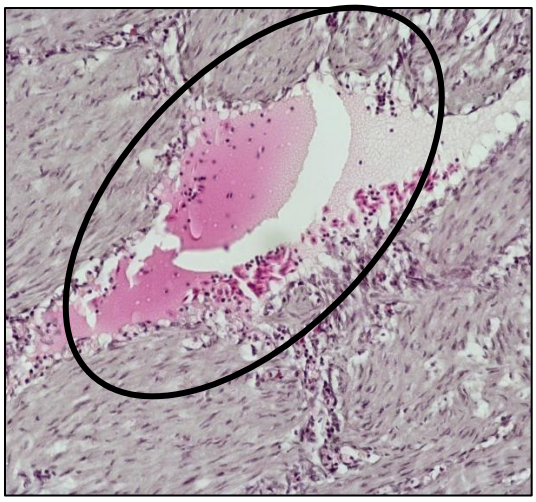


Figure 56: Oedema – Haematoxylin and Eosin Eosin

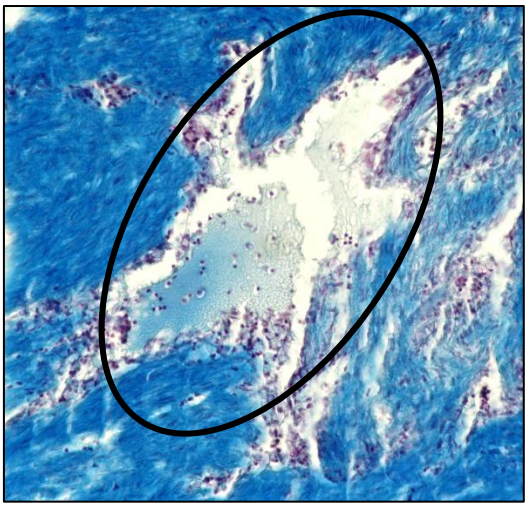


Figure 57: Oedema – Haematoxylin and

Oedema is well visible in both stains. It appears in a reddish/rose colour without cell structures in it when looking at the Haematoxylin and Eosin stain (figure 56). The Masson's trichrome stain (figure 57) makes it appear in a light blue colour. It can be found all over the tissue.

The white crack that can be seen in the oedema is an artefact. Artefacts they can arise during the creation of the samples.

9.2.6 Pigmented macrophages

Pigmented macrophages are scavenger cells. They serve as the body's immune defence. An increased number of pigmented macrophages suggests that the body tries to fight damage caused by external influences.

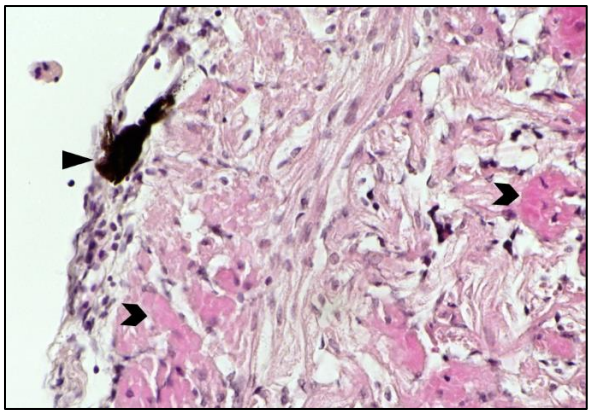


Figure 58: Pigmented macrophages – Haematoxylin and Eosin

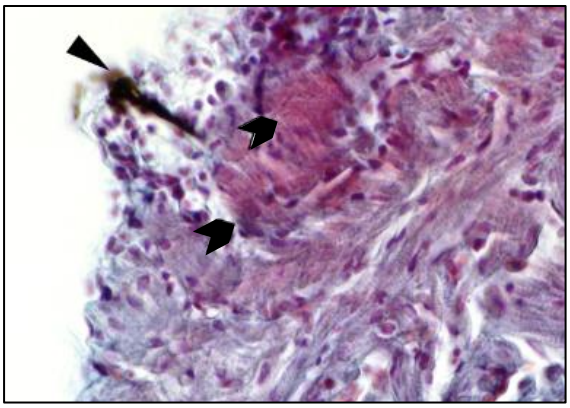


Figure 59: Pigmented macrophages – Masson's trichrome

Figure 58 and 59 show samples with a huge number of pigmented macrophages (closed arrowheads). They appear as a very dark black/brownish area in the tissue. They can easily be spotted with both stains. There is also some more degeneration visible (open arrow heads).

9.2.7 Interpretation

The following diagrams illustrate the development of the tissue health status over the time of exposition. They show the areas in which all classifications of the samples for each time of exposition occur.

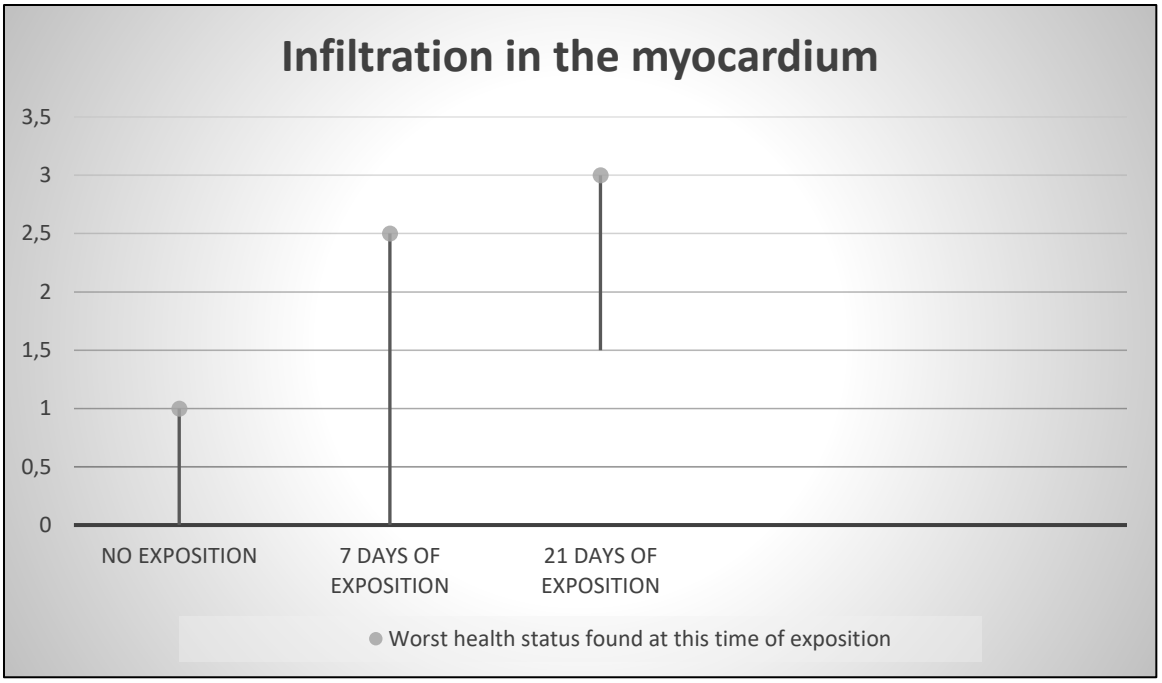


Figure 60: Infiltration myocardium – diagram

Figure 60 shows the development of the infiltration of the myocardium. It can be seen that none of the samples who were not exposed to psychoactive substances showed a worse classification than 1. After 7 days of exposition low classifications like 0 or 1 can still be found but they also reach up to 2,5. After 21 days of exposition none of the samples shows an entirely healthy status of the tissue. Damages up to moderate intensity (classification 3) can be found.

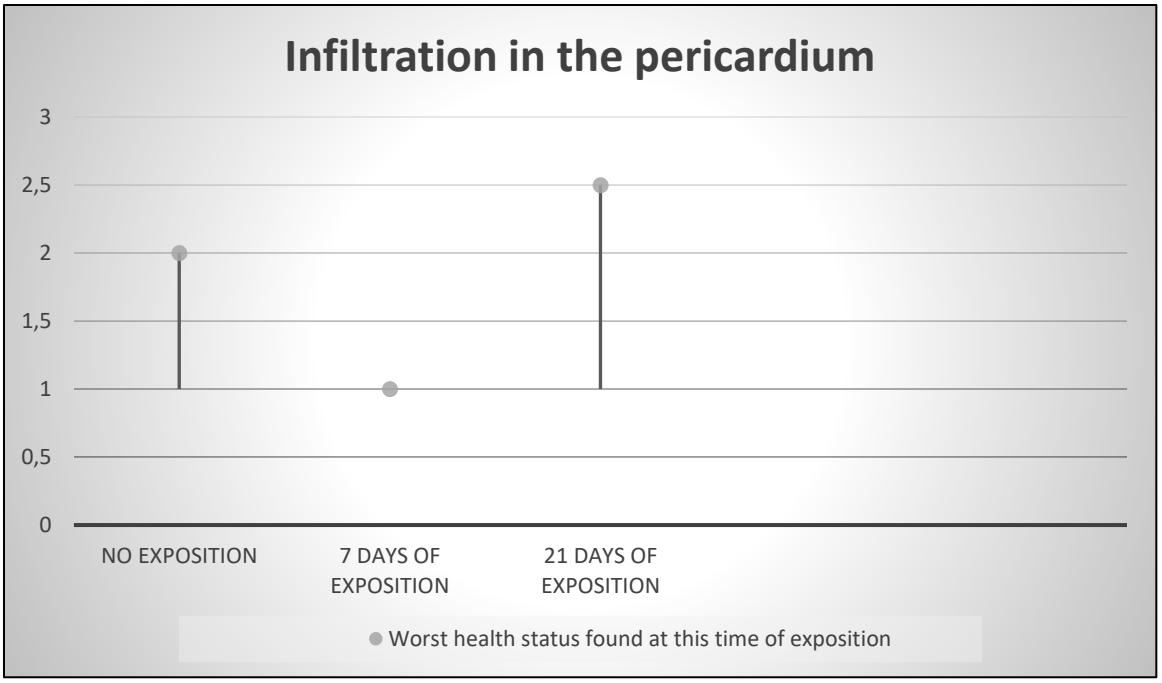


Figure 61: Infiltration pericardium – diagram

As seen in figure 61 the infiltration in the pericardium on the other hand hardly shows any changes at all. The reason for this might be the location of the pericardium. It is an external part of the heart. This may lead to it being less affected than the myocardium.

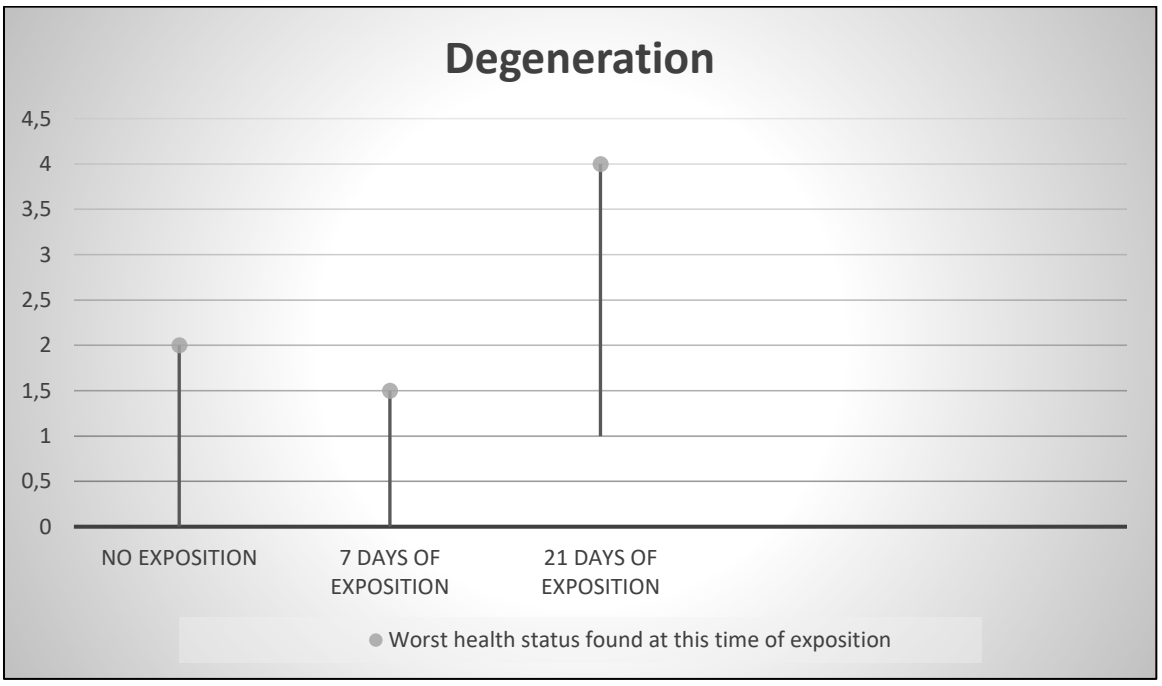


Figure 62: Degeneration – diagram

Until the 7th day of exposure there are no noticeable deteriorations as seen in figure 62. In fact, the classifications after 7 days of exposure show a lower peak than those with no exposure at all. This is possible because the fish inspected after 7 days of exposure are not the same fish as used for the control group. After 21 days of exposure some deteriorations can be found. Sample T21 2 even shows changes with severe intensity (classification 4). Also, all samples after 21 days of exposure show at least scattered changes in the tissue (classification 1).

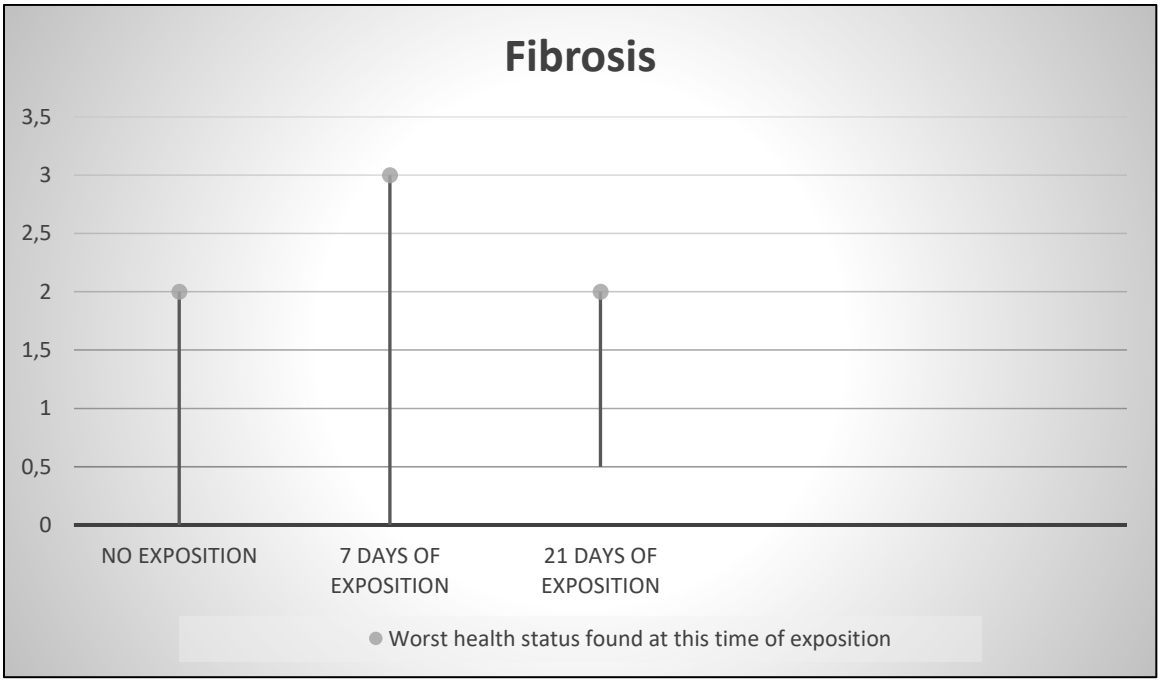


Figure 63: Fibrosis – diagram

Figure 63 shows that the state of health of the tissue with regards to fibrosis does not show any ascent of damage. However, the number of samples with completely healthy tissue decreases.

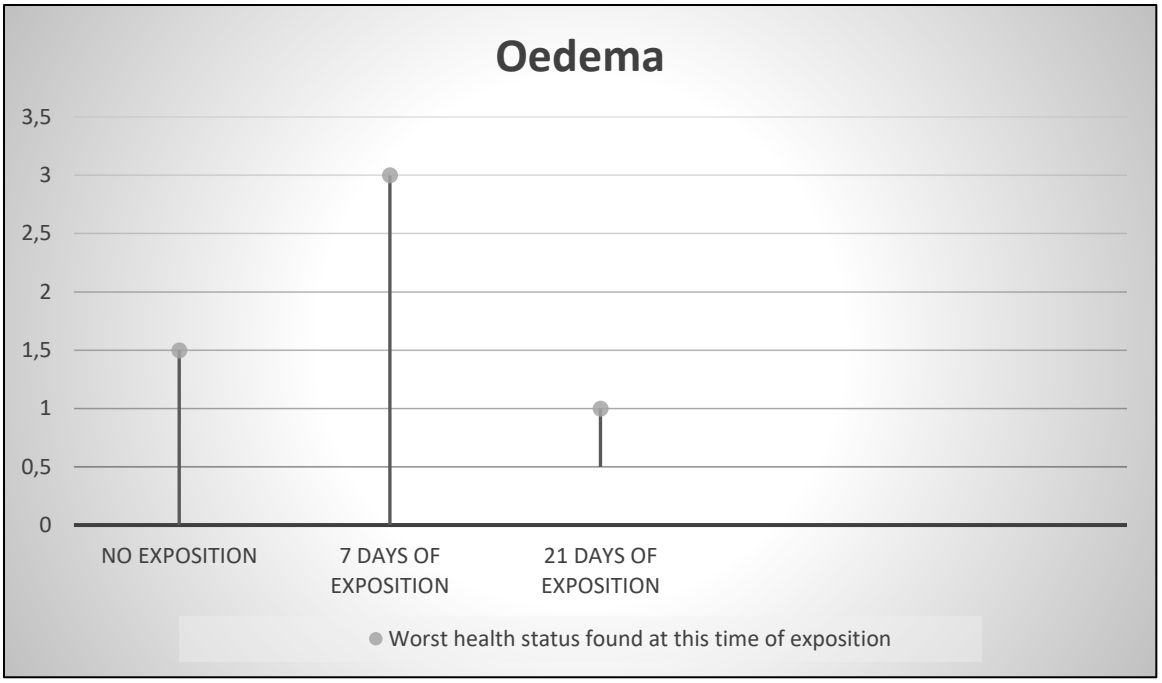


Figure 64: Oedema – diagram

Figure 64 shows that most oedema was found in samples after 7 days of exposure. However, after 21 days of exposure none of the samples shows no changes concerning oedema.

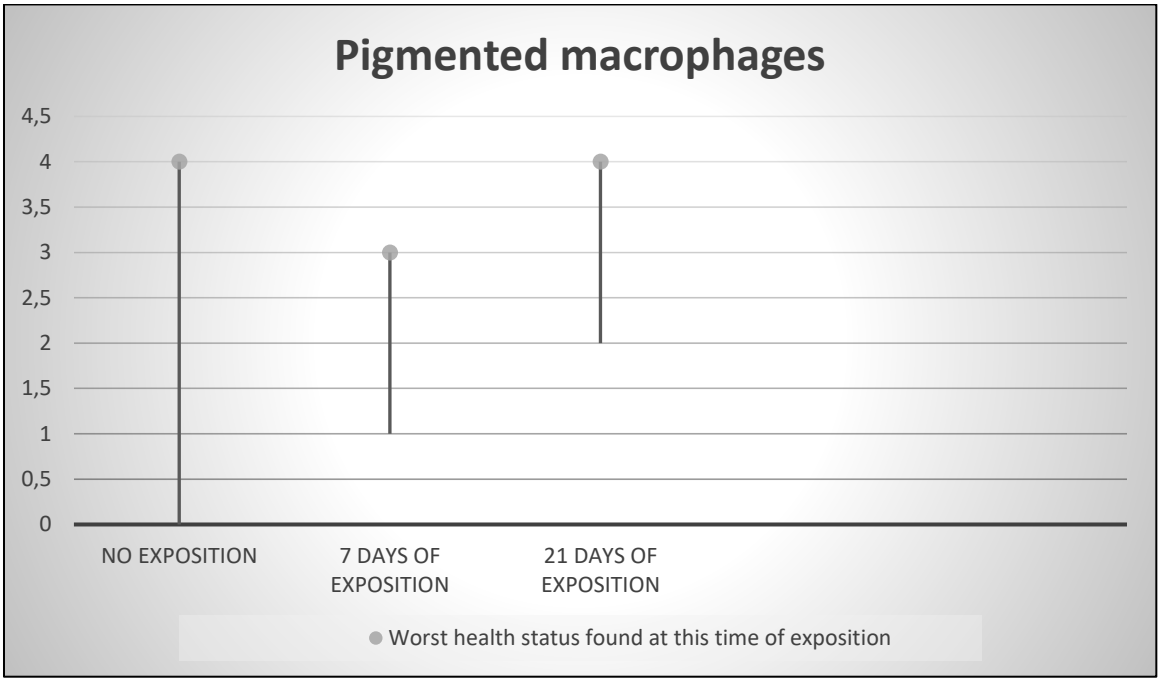


Figure 65: Pigmented macrophages – diagram

As seen in figure 65 even samples from the control group (no exposition) show pigmented macrophages with the classification 4 (severe intensity). These are sample C1 and C3. This could mean that the immune system of these fish was already struggling with harmful influences before exposure to psychoactive drugs. However, after the exposition to psychoactive substances the number of samples with none to less affected tissue decreases rapidly. After 21 days of exposure none of the samples shows changes with less than mild intensity (classification 2).

The following table, as well as the following diagram, deal with the average course and classifications of each sign of damage evaluated in the tissue.

Average classification for every evaluated change in the tissue for each time of exposition:

	Control group	*7 days of exposure*	*21 days of exposure*
Infiltration in the myocardium	0,5	1,25	2,25
Infiltration in the pericardium	1,17	1	1,25
Degeneration	1,17	0,75	2,33
Fibrosis	0,5	1,75	1,33
Oedema	0,67	1,42	1
Pigmented macrophages	2,5	2,33	2,67

Table 5: Average classifications

Average course of health status referring to each evaluated change in the tissue:

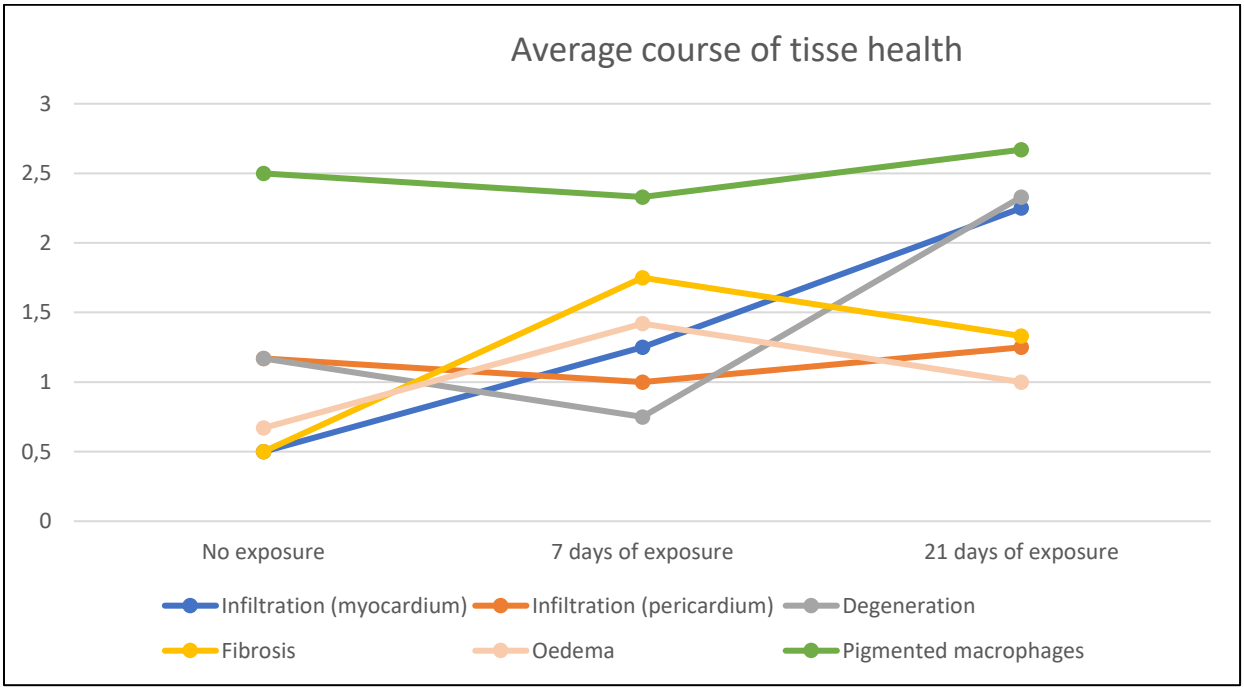


Figure 66: Average course of tissue health

The graph (figure 66) shows that the pigmented macrophages show the highest classification out of all other evaluated damage signs at all times of exposure. As already mentioned, the high number of pigmented macrophages found in the tissue of the control group might be the cause of the immune system struggling with other harmful influences in the tissues. It can be seen that degeneration and infiltration in the myocardium show major changes after an exposure of 21 days. This creates the conclusion that degeneration and infiltration in the myocardium are the two main damages found in the hearts tissue after the exposition to psychoactive substances.

The variation that can be seen in samples after 7 days of exposure is due to the fact that after each exposure period the fish specimens are different. Since each fish is individual, fluctuations can occur. However, it is important to note that every evaluated change in tissue deteriorated until after 21 days of exposure. This shows that the heart health of fish has visibly deteriorated over time due to the intake of psychoactive substances.

9.2.8 Conclusion

The heart of fish takes damages from the pollution of waters with psychoactive substances. However, fish, unlike us humans, are able to regenerate their heart. While some of these damages found in the tissue could be life-threatening for humans, fish are able to gain back their health after some time in unpolluted clean waters. Unfortunately, fish are permanently exposed to pollution by psychoactive substances, which makes it impossible for them to regenerate their heart. Therefore, it is just as dangerous for fish as it would be for us humans.

9.3 Teaching materials

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12. Appendix

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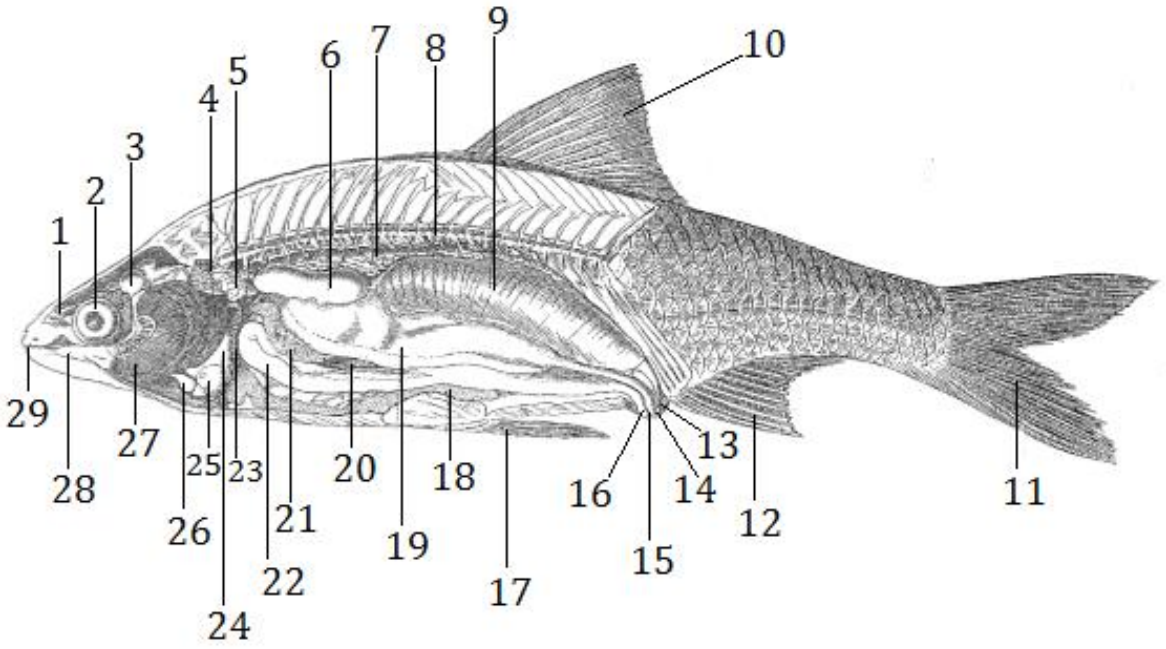
- Teaching materials results
- References
- Cooperation agreement
- Declaration of consent
- Curriculum vitae
- Projekthandbuch

Fish anatomy/histology

Anatomy



1) Lobus olfactorius:

→ Also known as olfactory brain

→ Processes odours

2) Eye:

→ Serves the perception of visual stimuli

3) Brain:

→ Central organ of the nervous system

→ Processes sensory perceptions

→ Coordinates behaviour

4) Pharynx:

→ Makes the connection between the oral cavity and the oesophagus

→ Makes the connection between the nasal cavity and the larynx

5) Pronephros:

→ Also known as head kidney

→ No longer present in all fish species

→ May be involved in blood formation

6) Swim bladder:

→ Helps fish to stay at their current water depth
→ Helps fish to save energy while swimming

7) Kidney:

→ Most fish species have several kidneys
→ Excretes waste products from the body
→ Produces urine

8) Ribs:

→ Part of the skeleton of bony fish

9) Swim bladder

→ See 6)

10) Dorsal fin:

→ Stabilization against rolling and assistance in sudden turns

11) Caudal fin:

→ Also known as tail fin
→ Serves the propulsion

12) Cloacal fin:

→ Also known as anal fin
→ Stabilises the fish while swimming

13) Bladder:

→ Temporary storage of urine

14) Ureteral opening:

→ Serves the excretion of urine

15) Genital Opening:

→ Used for reproduction (female)

16) Anus:

→ Outlet of the intestinal canal
→ Used for defecation

17) Ventral fin:

→ Also known as pelvic fin
→ Assistance in going up or down through the water, turning sharply, and stopping quickly

18) Liver:

→ Production of biochemicals necessary for digestion and growth
→ Detoxification of various metabolites
→ Protein synthesis

19) Testicle:

→ Used for reproduction (male)

20) Liver:

→ See 18)

21) Liver:

→ See 18)

22) Intestines:

→ Absorption of nutrients and water
→ Production of faeces

23) Peritoneum:

→ Serous membrane

24) Atrium:

→ Part of the heart
→ Chamber through which blood enters the ventricles of the hear

25) Ventricles:

→ Part of the heart
→ Pumps blood to the rest of the body

26) Aorta:

→ Transports oxygenated blood to the body periphery

27) Gills:

→ Serves for breathing under water
→ Supplies oxygen to the blood

28) Tongue:

→ Used in the act of swallowing
→ Manipulates food for mastication

29) Mouth opening:

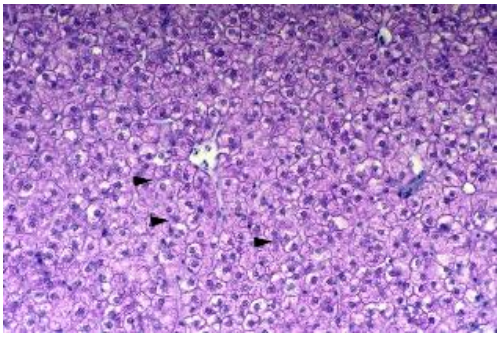
→ Used for food intake

Pollution of waters due to psychoactive substances

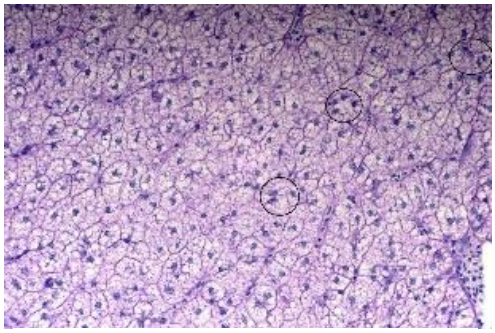
Water pollution is becoming a growing environmental problem, which can cause damage on aquatic organisms. Traces of psychopharmaceuticals and illegal drugs have been reported in effluents, influents and surface waters. These psychoactive substances cause damages, which can be detected in the tissue of the organs. These substances got filtered by the liver. Psychoactive substances are released by humans into wastewater. However, they cannot be completely filtered from water by sewage treatment plants, which leads to a residual content of psychoactive substances in water. Manly the following substances are found in the wastewater:

- Citalopram
- Sertraline
- Oxazepam
- Tramadol
- Venlafaxine
- Methamphetamine

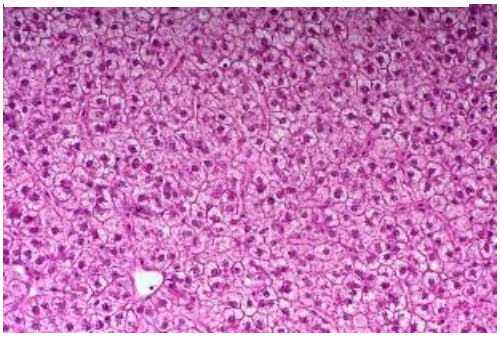
Liver tissue



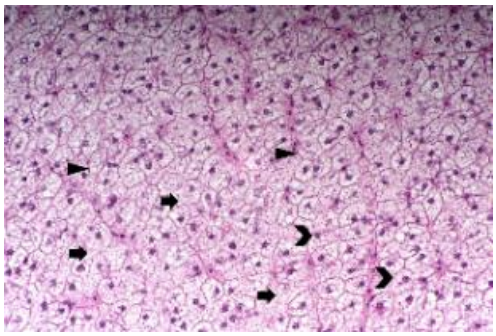
Healthy liver - PAS stain



Damaged liver - PAS stain



Healthy liver – Haematoxylin & Eosin stain

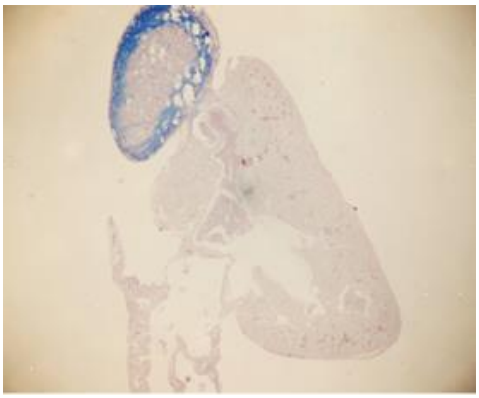


Damaged liver – Haematoxylin & Eosin stain

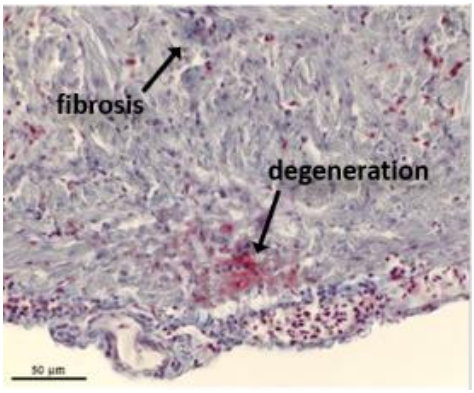
The pictures of the healthy liver show structures of healthy cells. The nucleus is located in the middle of the cell and is surrounded by glycogen. In order to be able to detect the glycogen more precisely, the additional stain Alcian blue and PAS (upper picture) is applied. This stain colours the glycogen reddish.

On the right a damaged liver is shown. In the figure below, which is stained with Hematoxylin and eosin, changes are clearly visible. The open arrow heads mark vacuoles. Due to misinformation, which is caused by psychoactive substances in this case, increased accumulation of neutral fat produced by the liver arises. The pressure in the cell becomes stronger and the glycogen decreases (compared to the upper picture, no reddish coloration can be seen, which means there is no glycogen in the cell). This leads to a delocalization of the nucleus. If the fat concentration and thus the pressure increases, a rupture of the membrane may occur (marked by arrows). This can lead to macrovacuoles (closed arrow heads) where the nucleus exits through the ruptured membrane, this process is irreversible.

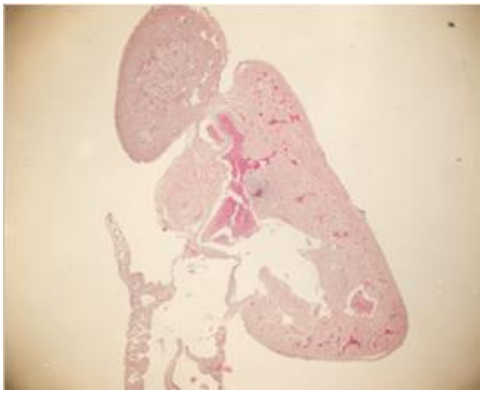
Heart tissue



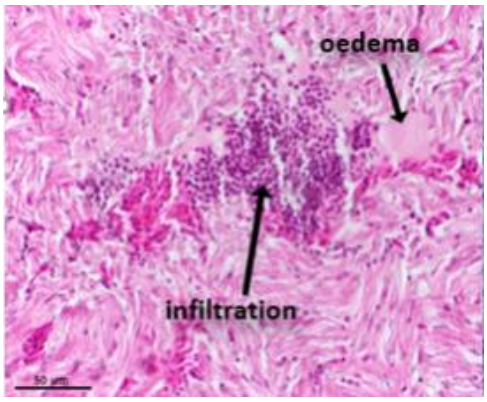
Healthy heart – Masson's stain



Damaged heart – Masson's stain



Healthy heart – Haematoxylin & Eosin stain



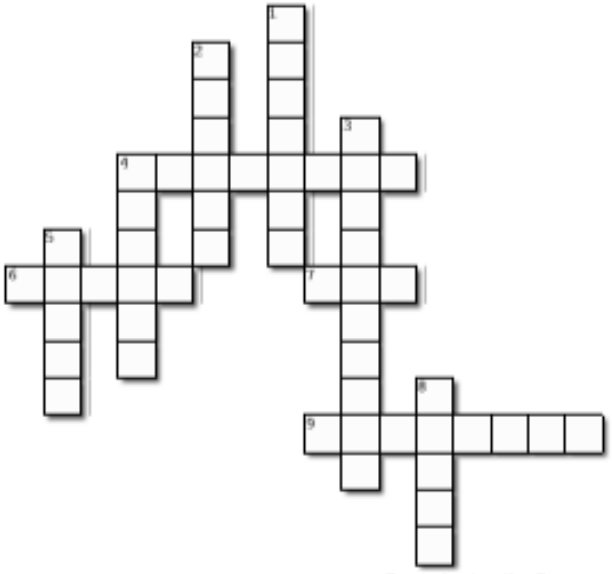
Damaged heart – Haematoxylin & Eosin stain

The heart was stained with two different staining methods. The upper bluish images show the Masson's trichrome stain, the lower images show the Hematoxylin and Eosin stain. The Masson's trichrome stain confirms that there is infiltration in the tissue. It also shows degeneration and fibrosis in the heart.

Fibrosis, degeneration, infiltration and oedema are damages often found in the tissue due to harmful impacts. Fibrosis leads to scars in the tissue, in an advanced stage it can lead to a restriction of the heart function. Degeneration refers to a regression of the tissue and thus also its function. Another damage that is clearly visible in the tissue of the sick fish is oedema. Oedema is an accumulation of fluid that leads to swelling of the tissue. Infiltration refers to the penetration of various substances, the so-called infiltrate, into the tissue.

These mentioned damages are fatal for humans, but a fish can regenerate its heart very quickly. For this regeneration it needs clean water.

Complete the crossword puzzle below



Created using the Crossword Maker on TheTeachersCorner.net

Horizontal

4. Name a psychoactive substance starting with the letter O.
6. In the tissue of which organ do macrovacuoles arise when exposed to psychoactive substances?
7. What accumulates in a livers cell leading to vacuolation?
9. What leads to scars in the hearts tissue?

Vertical

1. What is located in the middle of a cell?
2. Who releases psychoactive substances into wastewater?
3. Which fin is also known as tail fin?
4. What fluid can be found in the hearts tissue after exposition to psychoactive substances?
5. What do fish use for breathing under water?
8. What is the central organ of the nervous system?

Good luck! 

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KOOPERATIONSVEREINBARUNG

Zwischen

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   Zátiší 728/II, 389 25 Vodňany
   (Projektpartner)

   vertreten durch: **Assoc.Prof. Ing. Vladimír Žlábek, Ph.D. – Vice-Dean For Foreign Relations**

und

2. Kastner Francesca, Klatzl Lea (Projektteam)

PRÄAMBEL

Das Projektteam und der/die Projektpartner/in beabsichtigen gemäß der Verordnung über die abschließenden Prüfungen in den berufsbildenden mittleren und höheren Schulen, BGBL II, Nr.70/2000 vom 24.2.2000, die Planung und Durchführung eines Diplomprojektes, welches die Auswirkungen von psychoaktiven Substanzen auf die Organe von Fischen als Ziel hat.
Durch die Zusammenarbeit soll insbesondere den Mitgliedern des Projektteams die Möglichkeit eingeräumt werden, im Rahmen ihrer schulischen Ausbildung bei der Durchführung eines Diplomprojektes an die Verhältnisse im technischen Berufsleben herangeführt zu werden, um dabei die in der Schule erworbenen theoretischen Kenntnisse und Fähigkeiten in der Praxis anzuwenden bzw. zu erweitern. Hingewiesen wird in diesem Zusammenhang auf den unentgeltlichen Charakter dieser Vereinbarung.

§1
Gegenstand

Gegenstand ist die Erstellung von Arbeitsergebnissen zum Thema des Diplomprojektes. Das Thema des Diplomprojektes ist der Projektbeschreibung und dem Pflichtenheft zu entnehmen, welches der Kooperationsvereinbarung beiliegt.
Der/die Projektpartner/in wird jedoch darauf hingewiesen, dass es sich um ein Projekt im Zusammenhang mit der schulischen Ausbildung handelt und daher jede Haftung des Projektteams, insbesondere in Hinsicht auf die Unentgeltlichkeit des Vertrages, ausgeschlossen ist. Nutzungs- und Verwertungsrechte von im Rahmen dieser Vereinbarung erstellten Arbeitsergebnissen stehen dem/der Projektpartner/in sowie dem Projektteam gemeinsam zu.

§2
Laufzeit

Die vorliegende Kooperation tritt am 30. Juni 2019 in Kraft und wird bis zum Ende der Reife- und Diplomprüfung der HLUW Yspertal abgeschlossen.

§3
Rechte und Pflichten des Projektteams

Die Mitglieder des Projektteams haben das Recht, die Räumlichkeiten des/der Projektpartners/in samt Infrastruktur und EDV-Infrastruktur im für die Projektabwicklung erforderlichen Ausmaß nach vorheriger schriftlicher Genehmigung durch den/die Projektpartner/in mitzubenutzen.
Das Projektteam verpflichtet sich, die im Gegenstand genannten Arbeiten sorgfältig und unter möglichster Schonung der Interessen des/der Projektpartners/Projektpartnerin durchzuführen.

Das Projektteam unterliegt der Betriebsordnung des/der Projektpartners/Projektpartnerin. Das Projektteam verpflichtet sich zur Geheimhaltung aller ihm zur Kenntnis gelangenden Geschäfts und Betriebsgeheimnisse.

§4
Rechte und Pflichten des/der Projektpartners/Projektpartnerin

Der/die Projektpartner/in verpflichtet sich, dem Projektteam beratend zur Verfügung zu stehen und alles zu unterlassen, was der Vollendung des Projekts entgegensteht. Der/die Projektpartner/in verpflichtet sich, dem Projektteam folgende Hilfsmittel zur Verfügung zu stellen:

Sollte das Projektteam im Rahmen dieser Kooperationsvereinbarung eine Erfindung machen, die nach dem Gebrauchsmustergesetz bzw. dem Patentgesetz (PatG) schützbar ist, gilt diese Erfindung als Diensterfindung im Sinne des PatG und die §§ 6-19 PatG (in der geltenden Fassung) entsprechend.
Das Projektteam verpflichtet sich, den/die Projektpartner/in von einer im Rahmen der Kooperationsvereinbarung gemachten Erfindung unverzüglich in Kenntnis zu setzen. Der/die Projektpartner/in hat daraufhin das Recht, binnen vier Wochen ab dieser Bekanntgabe zu erklären, dass er/sie das Patentrecht für sich beansprucht. In diesem Fall steht dem Projektteam eine entsprechende Vergütung nach den einschlägigen Bestimmungen des PatG (in der geltenden Fassung) zu.
Sollte das Projektteam im Rahmen dieser Kooperationsvereinbarung ein Werk schaffen, dem Schutz im Sinne des Urheberrechtsgesetzes zukommt, verpflichtet es sich, den/die Projektpartner/in davon unverzüglich zu informieren. Der/die Projektpartner/in hat daraufhin die Möglichkeit, binnen vier Wochen ab dieser Bekanntgabe, mit dem Projektteam einen Werknutzungsvertrag abzuschließen.

§5
Einsicht und Präsentation

Da die Tätigkeit des Projektteams auch Inhalt bzw. Grundlage der an der HLUW Yspertal zu erstellenden Diplomarbeit ist, berechtigt der/die Projektpartner/in die zuständigen Organe des Bundes zur Einsicht und Kontrolle, um die in der Verordnung über die abschließenden Prüfungen an den berufsbildenden mittleren und höheren Schulen genannten Aufgaben zu erfüllen.

Das Projektteam ist auch berechtigt, Ergebnisse der Diplomarbeit bei der mündlichen Reifenprüfung zu präsentieren. Die zuständigen Organe des Bundes sind ihrerseits wiederum gegenüber jedermann zur Geschäfts- und Betriebsgeheimnisse des/der Projektpartners/Projektpartnerin verpflichtet.

Vodňany, am 8.2.2019

Projektpartner/in

University of South Bohemia
in České Budějovice
Faculty of Fisheries and Protection of Waters
Zátiší 728/II
389 25 Vodňany, Czech Republic (1)

Yspertal, am 29.1.2019

Projektteam

Declaration of consent

I, Maria Eugenia Sancho Santos, with ID 70871989L, hereby confirm that **Lea Klatzl** and **Francesca Kastner** are entitled to use contents (as pictures or information) for their diploma thesis which are subject to my copyright.

In Vodňany, 22nd January 2020,

Maria Eugenia Sancho Santos

europass Curriculum vitae

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WORK EXPERIENCE

13/1/2014–24/1/2014

Labor Oberwart - IMCL Institut für medizinische und chemische Labordiagnostik G.m.b.H.

Labor Oberwart - IMCL Institut für medizinische und chemische Labordiagnostik G.m.b.H, Oberwart (Austria)

- assistance at the hematology laboratory

Kronen-Apotheke Oberwart, Oberwart (Austria)

- assistance whenever it is necessary

1/6/2018–30/6/2018

Wells House & Gardens, Wexford, Ireland

1/9/2018–30/9/2018

Jil Silk – Ing. Heinrich Rabl GmbH, Dietmanns (Austria)

1/8/2018–30/8/2018

Bio Forschung Austria, Vienna (Austria)

PERSONAL SKILLS

Mother tongue(s) German

Foreign language(s)

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	C1	C1	B2	B2	B2
Latin	A1	A1			A1
Italian	A2	A2	A2	A2	A2

Levels: A1 and A2: Basic user - B1 and B2: Independent user - C1 and C2: Proficient user
Common European Framework of Reference for Languages - Self-assessment grid

Communication skills

- excellent communication skills gained through a leadership Workshop, participation in various speech contests and many presentations of projects at school
- excellent contact skills with all kinds of people gained through my family and many projects

Organisational / managerial skills

- leadership
- good team-leading skills gained through my siblings and many team-projects

Digital skills

SELF-ASSESSMENT				
Information processing	Communication	Content creation	Safety	Problem-solving
Proficient user	Independent user	Independent user	Independent user	Independent user

Digital skills - Self-assessment grid

europass Curriculum vitae

PERSONAL INFORMATION

Francesca Marie Kastner

Buchenstraße 5, 3300 Winklarn (Austria)

+43 650 990 3442

fkastner@hluwyspertal.ac.at

WORK EXPERIENCE

01/07/2016–30/07/2016	**Supermarket manager** Eurospar Euratsfeld
01/07/2017–30/07/2017	**Zookeeper** Umdash/Doka, Amstetten (Austria)
01/05/2018–30/07/2018	**assistant of the project manager** Polar Permaculture, Longyearbyen (Norway)
01/08/2018–14/09/2018	**laboratory assistant** Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig (Germany)
01/10/2018–Present	**Zookeeper** Umdash/Doka, Amstetten (Austria)
01/08/2019–31/08/2019	**Zookeeper** Umdash/Doka, Amstetten (Austria)

EDUCATION AND TRAINING

2007–2011	**Primary school** Volksschule Winklarn, Winklarn (Austria)
2011–2015	**Secondary school** Private neue Mittelschule Amstetten, Amstetten (Austria)
2015–Present	**higher vocational school** Höhere Lehranstalt für Umwelt und Wirtschaft, Yspertal (Austria)
01/07/2019–20/07/2019	**scientific international summer school** University of South Bohemia, Vodnany (Czechia)

PERSONAL SKILLS

Mother tongue(s) German

Effects of psychoactive substances on the organs of fish
Kastner, Klatzl

Foreign language(s)

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	B2	B2	B2	B2	B2
Italian	A2	A2	A2	A2	A2

Levels: A1 and A2: Basic user - B1 and B2: Independent user - C1 and C2: Proficient user
Common European Framework of Reference for Languages - Self-assessment grid

Communication skills

Part of the jounior eco export project 2017

good communication skills in english gained trough my traineeships abroad

Job-related skills

Laboratory: independent work in science (pH- meter, AAS, different typs of analytic laboratory methodes)

Independent work in microbiology (analysis of food and drinking water)

Practical work in a workshop (wood and metal work)

Practical work in biology: plant cultivation, plant identification

Theoretic topics like pets control and basics of adequate animal husbandry

Practical work in biology: plant cultivation, plant identification

Theoretic topics like pets control and basics of adequate animal husbandry

sound knowledge in group or single work activities

16 hour First Aid course

Knowledge of business management in: accounting, economic and social viewpoints, lead and found a company, create a business plan, tasks and creation of a waste management concept

It supported accounting and use of electronic and handwritten accounting in areas like payroll, cost accounting and full cost accounting.

Specialist knowledge of drinking water and sewage

Digital skills

Sound knowledge of data processing (Microsoft Office, AutoCad)

1. Eckdaten

Projekt Diplomarbeit	
Projektstart: • 10.01.2019 • First meeting with our supervisor in Vodnany	
Projektende • Diplomarbeitspräsentation (ca. 30.03.2020)	
Ziele der Diplomarbeit: • Identification of effects of psychoactive substances on the health of fish through histological examination	
Nicht-Ziele der Diplomarbeit: • Longer stay in Vodnany, because of erroneous results	
DiplomarbeitsbetreuerInnen: • OStR Mag. Gortan Gunter • Mag. Urban Isabel	

2. Projektorganisation

PROJEKTORGANISATION PROJEKTAUFTRAG PROJEKTUMFELD		
Projektrolle	**Aufgabenbereiche/ Skills**	**Name**
ProjektauftraggeberIn	Environment and Economy	HLUW Yspertal
Projektteam-mitgliederInnen	Evaluation of histological changes in the tissue of fish hearts Evaluation of histological changes in the tissue of fish livers	Lea Klatzl Francesca Kastner
ProjektpartnerInnen	Project manager	University of South Bohemia in České Budějovice HLUW-Yspertal
ProjektmitarbeiterInnen (Falls es Personen gibt, die zusätzlich bei der Arbeit mitwirken)	Supervisor, PhD student of Aquaculture and Veterinary	Maria Eugenia Sancho Santos
Sonstige Personen oder Organisationen im Umfeld des Projektes		

3. Projektmeilensteinplan

(beinhaltet wichtige Termine bei deren Nichteinhaltung das ganze Projekt verzögert wird oder scheitert)

PROJEKT-MEILENSTEINPLAN				
Meilenstein	**Plantermin * Fertigstellung**	**Ist- Termin Fertigstellung**	**Wurde der Termin eingehalten?**	**Wer ist für die Termineinhaltung verantwortlich?**
Approval of the application	20.12.2019	20.12.2019	Yes	Kastner, Klatzl
Meeting with the supervisor	10.01.2019	10.01.2019	Yes	Kastner, Klatzl
Cooperation agreement	14.01.2019	15.01.2019	No	Kastner, Klatzl
Arrival in Vodnany	01.07.2019	01.07.2019	Yes	Kastner, Klatzl
Working on the project	01.-19.07.2019	01.-19.07.2019	Yes	Kastner, Klatzl
Departure from Vodnany	19.07.2019	19.07.2019	Yes	Kastner, Klatzl
First correction	17.02.2020	17.02.2020	Yes	Kastner, Klatzl
Submission raw version	27.02.2020	27.02.2020	Yes	Kastner, Klatzl
Final release and presentation				

*Termine chronologisch nach Planterminen reihen!

4. Projektfunktionendiagramm (Verantwortlichkeitsmatrix)

PROJEKT-FUNKTIONEN-DIAGRAMM					
Rollen und Umwelten Meilenstein-Bezeichnung	Externer AuftraggeberIn University of South Bohemia in České Budějovice	ProjektauftraggeberIn HLUW Yspertal	DiplomarbeitsbetreuerIn OStR Mag. Gortan Gunter Mag. Urban Isabel	Lea Klatzl	Francesca Kastner
Approval of the application	-	-	M	D	D
Meeting with the supervisor	M	M	M	D	D
Cooperation agreement	M	M	M	D	D
Arrival in Vodnany	I	I	I	D	D
Working on the project	I	I	I	D	D
Departure from Vodnany	I	I	I	D	D
First correction	-	-	M	D	D
Submission raw version	I	I	I	D	D
Endabgabe					

Funktionen (in Tabelle „D“ oder „M“ oder „I“ eintragen)

D Durchführung, Verantwortliche/r
M Mitarbeit
I Information

5. **Aufzeichnungen über den Arbeitseinsatz**

Name des Projektteammitgliedes: Francesca Kastner Eingesetzte Arbeitszeit in Stunden Seite 1 von 3 Seiten		
Datum	**Art der Tätigkeit**	**Dauer**
20.11.2018	Meeting with the teachers, talk about the project idea	1 hours
08.01.2019	Meeting with the teachers, write the cooperation contract	1 hours
10.01.2019	Excursion to Vodnany, Meeting with our supervisor	8 hours
01.07.2019	Instruction of the security measures, discussion of the course of the project	8 hours
02.07.2019	Preparation	8 hours
03.07.2019	Preparation	8 hours
04.07.2019	Preparation	8 hours
05.07.2019	Studying	9 hours
06.07.2019	Studying	5 hours
07.07.2019	Studying	5 hours
08.07.2019	Fixation with formalin, cutting paraffin blogs	8 hours
09.07.2019	Fixation with formalin, fixation in paraffin	8 hours
10.07.2019	Fixation in paraffin, cutting paraffin blogs, colouring the samples	8 hours
11.07.2019	Cutting paraffin blogs, colouring the samples	8 hours
12.07.2019	Cutting paraffin blogs, colouring the samples	8 hours
13.07.2019	Studying	6 hours
14,07,2019	Studying	7 hours
15.07.2019	Watching and identify samples from the heart	8 hours

Name des Projektteammitgliedes: Francesca Kastner Eingesetzte Arbeitszeit in Stunden Seite 2 von 3 Seiten		
Datum	**Art der Tätigkeit**	**Dauer**
16.07.2019	Watching and identify samples from the liver	8 hours
17.07.2019	Taking photos of the samples	8 hours
18.07.2019	Controlling and discuss our results	8 hours
19.07.2019	Controlling and discuss our results, hold a presentation about our project in front of a committee	8 hours
19.09.2019	Meeting with the teachers, giving a short update	1 hours
08.10.2019	Meeting with the teachers, transfer information to the Diplomarbeitsdatenbank	1 hours
23.10.2019	Meeting with the teachers, giving an update, talk about further steps	1 hour
13.11.2019	Creating the structure plan	2 hours
02.12.2019	Writing the foundation and literature research	4 hours
05.12.2019	Writing the foundation and literature research	5 hours
10.12.2019	Writing the foundation and literature research	4 hours
14.12.2019	Writing the foundation and literature research	8 hours
15.12.2019	Writing the foundation and literature research	9 hours
19.12.2019	Drawing chemical structures with ChemSketch	3 hours
20.12.0219	Writing the foundation and literature research	2 hours
02.01.2020	Interpretation of results	7 hours
07.01.2020	Meeting with the teacher, talk about the first correction and the next steps	1 hour
07.01.2020	Correction	3 hours

Name des Projektteammitgliedes: Francesca Kastner Eingesetzte Arbeitszeit in Stunden Seite 3 von 3 Seiten		
Datum	**Art der Tätigkeit**	**Dauer**
08.01.2020	Preparation of teaching materials	4 hours
09.01.2020	Revision of the presentation	4 hours
14.01.2020	Correction and writing the results	2 hours
15.01.2020	Writing the results	2 hours
17.01.2020	Writing the results	3 hours
18.01.2020	Writing the results	4 hours
19.01.2020	Writing the results	6 hours
21.01.2020	Writing the results	2 hours
12.02.2020	Interpretation of results	5 hours
13.02.2020	Coarse formatting	5 hours
24.02.2020	Meeting with Ms. Urban, talking about the correction	2 hours
24.02.2020	Correction	2 hours
25.02.2020	Correction and structuring	4 hours
26.02.2020	Meeting with teachers, talking about the structure and the correction	2 hours
26.02.2020	Finalization of the diploma thesis	10 hours
	Arbeitszeit bisher	268 hours

Name des Projektteammitgliedes: Lea Klatzl Eingesetzte Arbeitszeit in Stunden Seite 1 von 3 Seiten		
Datum	**Art der Tätigkeit**	**Dauer**
20.11.2018	Meeting with the teachers, talk about the project idea	1 hours
08.01.2019	Meeting with the teachers, write the cooperation contract	1 hours
10.01.2019	Excursion to Vodnany, Meeting with our supervisor	8 hours
01.07.2019	Instruction of the security measures, discussion of the course of the project	8 hours
02.07.2019	Preparation	8 hours
03.07.2019	Preparation	8 hours
04.07.2019	Preparation	8 hours
05.07.2019	Studying	9 hours
06.07.2019	Studying	5 hours
07.07.2019	Studying	5 hours
08.07.2019	Fixation with formalin, cutting paraffin blogs	8 hours
09.07.2019	Fixation with formalin, fixation in paraffin	8 hours
10.07.2019	Fixation in paraffin, cutting paraffin blogs, colouring the samples	8 hours
11.07.2019	Cutting paraffin blogs, colouring the samples	8 hours
12.07.2019	Cutting paraffin blogs, colouring the samples	8 hours
13.07.2019	Studying	6 hours
14,07,2019	Studying	7 hours
15.07.2019	Watching and identify samples from the heart	8 hours

Name des Projektteammitgliedes: Lea Klatzl Eingesetzte Arbeitszeit in Stunden Seite 2 von 3 Seiten		
Datum	**Art der Tätigkeit**	**Dauer**
16.07.2019	Watching and identify samples from the liver	8 hours
17.07.2019	Taking photos of the samples	8 hours
18.07.2019	Controlling and discuss our results	8 hours
19.07.2019	Controlling and discuss our results, hold a presentation about our project in front of a committee	8 hours
19.09.2019	Meeting with the teachers, giving a short update	1 hours
08.10.2019	Meeting with the teachers, transfer information to the Diplomarbeitsdatenbank	1 hours
23.10.2019	Meeting with the teachers, giving an update, talk about further steps	1 hour
13.11.2019	Creating the structure plan	2 hours
02.12.2019	Writing the methods	4 hours
05.12.2019	Writing the methods	5 hours
10.12.2019	Writing the methods	4 hours
14.12.2019	Writing the methods	8 hours
15.12.2019	Writing the methods	9 hours
19.12.2019	Preparation of teaching materials	3 hours
20.12.0219	Writing the methods	2 hours
02.01.2020	Interpretation of results	7 hours
07.01.2020	Meeting with the teacher, talk about the first correction and the next steps	1 hour

Name des Projektteammitgliedes: Lea Klatzl Eingesetzte Arbeitszeit in Stunden Seite 3 von 3 Seiten		
Datum	**Art der Tätigkeit**	**Dauer**
08.01.2020	Preparation of teaching materials	4 hours
09.01.2020	Revision of the presentation	4 hours
29.01.2020	Correction	2 hours
30.01.2020	Correction	2 hours
04.02.2020	Writing the results	6 hours
05.02.2020	Writing the results	4 hours
06.02.2020	Writing the results	6 hours
07.02.2020	Writing the results	4 hours
12.02.2020	Interpretation of results	5 hours
13.02.2020	Coarse formatting	5 hours
24.02.2020	Meeting with Ms. Urban, talking about the correction	2 hours
24.02.2020	Correction	2 hours
25.02.2020	Correction and structuring	4 hours
26.02.2020	Meeting with teachers, talking about the structure and the correction	2 hours
26.02.2020	Finalization of the diploma thesis	10 hours
	Arbeitszeit bisher	270 hours