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Research Article

Effect of water-borne cadmium toxicity on some biochemical parameters and histopathological alterations in common carp, *Cyprinus carpio*

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Abstract
Cadmium is one of heavy metals affecting aquatic biota. The current study aimed to investigate the toxic effect of cadmium on the biochemical parameters of blood (glucose, total protein, activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and the histological structure of gills and liver in common carp exposed to sublethal concentrations for 96 hours. The toxicity test was performed with sublethal cadmium chloride (CdCl2) concentration using the fixed bioassay method in static renewal conditions. Based on the results, glucose levels significantly increased in treated fish, while total protein declined significantly. Cadmium toxicity raised the activity of serum AST and ALT ($P<0.05$). The histopathological examinations showed increased alternation in the gill tissues and hepatic tissue over the 96 h of exposure. In conclusion, *Cyprinus carpio*, is affected by the toxic impact of cadmium on biochemical parameters of blood, and the alteration in the histology of gill and liver.

Keywords: Cadmium. Biochemical parameters. Histopathological changes. Carp.

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Introduction

Cadmium is a non-essential trace element and has a size and charge density similar to essential elements (McGeer et al. 2012). It is found in natural fresh water at deficient levels (less than 0.01µg/l). The cadmium in the environment is expected to be at a low to medium level (Lee 2015). Generally, heavy metals can accumulate in fish; and the consumption of these fish endanger people's health and is a health concern worldwide (Barone et al. 2021). The accumulated heavy metals have adverse effects on both ecosystems and living organisms; especially, high accumulation leads to hematotoxicity and genotoxicity. Therefore, exposure to their high concentration may harmfully affect aquatic organisms, including fish, hampering their physiological functions (Alak et al., 2018).

Many studies have proven the effect of heavy metals on the biochemical properties of fish, such as glucose, total protein, AST, and ALT (Heydarnejad et al. 2013; Mutlu et al. 2015; Aldoghachi et al. 2019). In addition, many studies have investigated histopathological changes in fish and considered them as warning signs of toxic damages of pollutants. Short-term exposure to heavy metals causes pathological conditions in gill, kidney, and liver. (Jalaludeen et al. 2012). These pathological changes in different organs tissues can be used to diagnose fish diseases and are reported as pollution biomarkers (Zeitoun & Mehana 2014; Onita (Mladin) et al. 2021). The current study aimed to study the toxic effect of cadmium on some biochemical parameters of blood and the histological structure of gills and liver in common carp, *Cyprinus carpio* exposed to sublethal concentrations for 96 hours.

Material and Methods

Experimental design: The *C. carpio* specimens

(with a mean total length of 16±1.5cm and mean weight of 150.01±2.5 g, as Mean±S.E.) were collected from the marine science center's aquariums. The fish were acclimated to aerated glass aquaria for two weeks under laboratory conditions of 22±1.5°C and photo-period of 12:12 light:dark. Fish were fed commercial food with a protein content of 30%. 20 litter of dechlorinated tap water were used in each glass aquarium (60x30x30cm) and provided with oxygen aeration using aerator system.

The toxicity test for cadmium was performed with cadmium chloride ($CdCl_2$) using the fixed bioassay method in static renewal conditions as described by APHA (2012). A preliminary test was conducted to calculate the median lethal concentration of $CdCl_2$ for a period 96 h (96hLC50) by concentration-response curves of probit transformation (1.5mg\l). The fish were divided into two groups, the first group was as control samples in which tap water was used, while the other group was exposed for a period of 96 h to 50% LC50 of cadmium (0.75mg/l). 10 fish were used for each aquarium, with three replications. During the experimental period (96 h), the fish were allowed to feed in both groups, then feeding was stopped after the end of the exposure for 24 hours (Yuanyuan et al. 2009).

Biochemical measurements: After the 96h post-exposure with 0.75mg of Cd ions, the fish feeding was stopped within 24 hours until blood samples were taken. Five fish were taken and directly anesthetized in ice water. Then blood was taken from the caudal vein (Zang et al. 2015) using a 3ml syringe and collected in 5ml glass test tubes. The serum was separated from the clot using a centrifuge at a rate of 3000rpm for 10 minutes. The serum was kept frozen at -20°C until later use. According to Pandit & Sharma (2019), glucose and total protein were calorimetrically determined. AST and ALT were measured by UV technique, as mentioned in Rahimikia (2017). A spectrophotometer (Humalayzer primus) was used to measure the absorbance at a wavelength of 546nm, and calculated as U/L.

Histopathological investigation: Histopathological examination was conducted on the gills and liver of control and exposed fish. The samples were fixed in formalin for 48 hours, then a series of ethanol was used to dehydrate the tissues. The samples were embedded in paraffin, cut with a thickness of 7µm and then stained with hematoxylin and eosin (H&E). The morphological and qualitative characteristics of the tissues were examined (Triebskorn et al. 2008). The digital photos were taken using a biological microscope XSZ-H equipped with a digital camera MDCE-5C, using objective lenses of 10, 20 and 40X.

Statistical analysis: Data were analyzed using SPSS (version 18). The parameters were given in terms of Mean±standard error, and differences between means of all parameters were carried out using analysis of variance (ANOVA). Differences were considered statistically significant at $P<0.05$.

Result and Discussion

Biochemical parameters: Mean serum glucose was 60.32±1.15mg/dl in control fish, while a significant increase was found in cadmium exposed fish. In addition, serum protein in control fish was 5.70±0.43g/dl) while it showed a significant decrease in treated fish (5.528±0.02 g/dl) (Tables 1). The decrease in protein level by the effect of toxic substances that are caused metabolic stress was previously reported by Pandit & Sharma (2019). Khalesi et al. (2017) showed that exposure of *C. carpio* to cadmium and lead is caused renal injury and damage of proteins and amino acids in liver tissue. The current study also agrees with the findings of Al-Asgah et al. (2015), who reported a decrease in total protein in *Oreochromis niloticus* exposed to cadmium chloride. This reduction may be due to destruction in the subcellular structure of protein synthesis. Also, Khan et al. (2016) were documented decreased concentrations of total blood protein in grass carp (*Ctenopharyngodon idella*) exposed to acute concentrations of toxic pollutants due to gluconeogenesis of glucose.

The results showed that the activity of AST in Cd

Table 1. Biochemical parameters of *Cyprinus carpio* exposed to sublethal concentration (0.75 mg/l) of cadmium chloride for 48 and 96 hour.

Biochemical parameters (units)	Controlled value	Duration of treatment	
		48 hour	96 hour
Glucose (mg/dl)	60.32±1.15[a]	60.00±1.83[a]	69.03±0.68[b]
Total Protein (g/dl)	5.70±0.43[c]	5.60±0.38[c]	5.528±0.02[d]
AST U/L	231.4±3.02[e]	240.5±2.15[f]	441.5±1.23[f]
ALT U/L	223.9±1.33[g]	223.6±3.03[g]	211.7±0.54[h]

Values with same letter differ non-significantly (P>0.05).

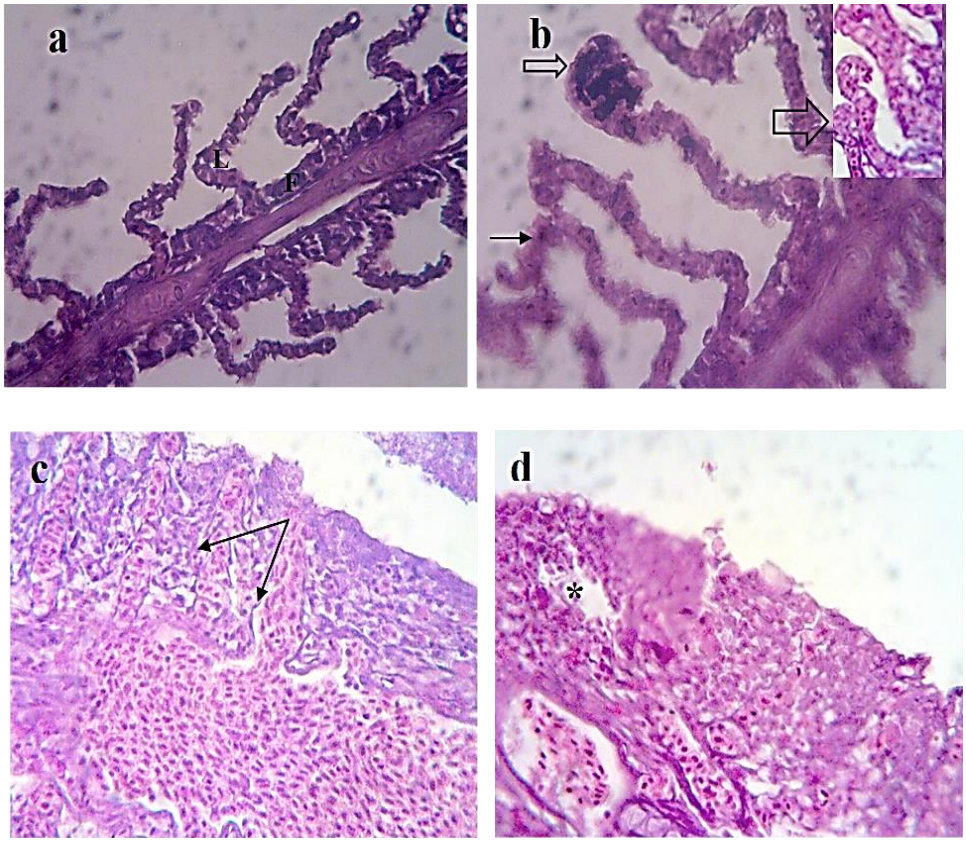

Fig.1. Photomicrographs of the gill in *Cyprinus carpio.* a: Control sample shows distinguished arrangement of gill filaments (F) and lamellae (L). b: Cadmium exposed gill viewing curling of the secondary lamellae (arrow) and formation of glob shape at the end of lamellae (arrowhead). c: Fusion of adjacent of secondary lamellae. d: Vacuolization in epithelial cells (arrowhead).

exposed fish (441.5±1.23U/L) was higher than the control group (231.4±3.02U/L) while the ALT in the treated fish (211.7±0.54U/L) was lower than the control samples (223.9±1.33U/L) (Table 1). Aldoghachi et al. (2019) showed that AST and ALT activity of *Carassius gibelio* exposed to sublethal Zn significantly elevated. Abdel-Tawwab et al. (2016) reported an increase in both AST and ALT activities in *O. niloticus* with increasing Zn exposure concentration and period due to liver damage that leads to the leakage of these enzymes from the cytosol of the liver into the bloodstream.

Histopathological examinations showed normal structure in the control group's gills (Figs. 1a). The gills showed alternations within 24 h of exposure to cadmium, and the damages increased with the increasing exposure period including some focal proliferation forming a glob shape at the end of the lamellae and curling of secondary lamellae (Fig. 1b), a fusion of adjacent secondary lamellae in some regions (Fig. 1c) and appearance of vacuoles in the epithelial cells after 96 hours of exposure (Fig. 1d).

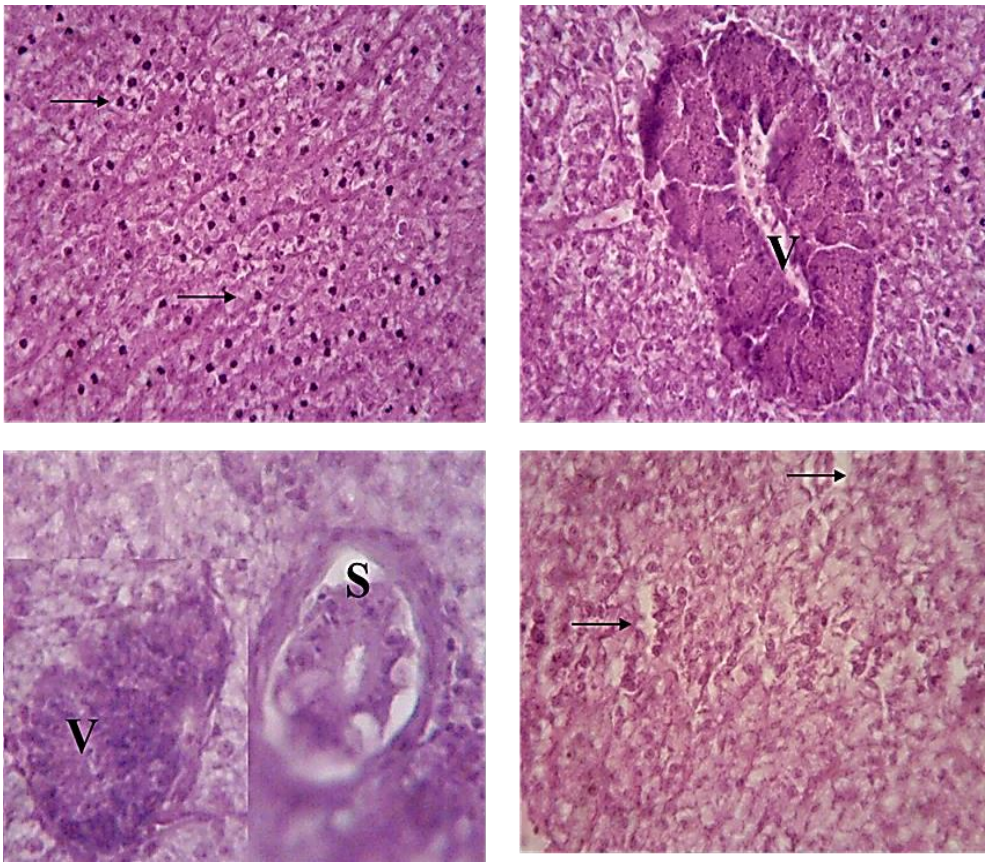

Fig.2. Photomicrographs of the liver in *Cyprinus carpio* a, b: control sample viewing central vein (V), ribbed hepatocytes with spherical nucleus in center and a spotted nucleolus (arrow), c: cadmium exposed sample viewing engorged central vein (V), sinusoid dilatation (S) and d: vacuolation in cell cytoplasm (arrow).

The fusion of the secondary lamellae reduce the entry of the toxic substance, but increasing the fusion area leads to asphyxiation and death (Hamid et al. 2015). These changes in the gill tissues in the current study agreed with Silva et al. (2012) in Hoplias malabaricus exposed to heavy metal ions that led to cell necrosis and cell hyperplasia and an increase in the secretion of mucous in the secondary gill lamellae. Fish gills perform necessary functions, including respiration, osmoregulation, nitrogenous waste elimination and acid-base balance (Evans 2005). The gill tissues are sensitive to heavy metals, leading to lamellar fusion, hyperplasia of epithelial cells, and lamellar aneurysms. Furthermore, many heavy metal ions influence the antioxidants of gill tissues (Sabullah et al. 2015).

Pereira et al. (2013) exposed several pollutants such as cadmium on *Pseudochondrostoma* sp. and *Luciobarbus bocagei* in the Portuguese rivers and observed histological alternations and oxidative stress, including epithelium proliferation of gill lamellae and then lamellar fusion, aneurysms and necrosis. Jeferson et al. (2018) in the gills of *Astyanax jacuhiensis*, who observed many histopathological abnormalities, including epithelial lifting, hyperplasia of gill filament and secondary lamella, exhibited hypertrophy which was attributed to temporal and spatial variation in the level of pollutants, which in turn lead to various changes in histological parameters.

Control fish's liver showed a normal structure of hepatocytes, central vein and the polygonal hepatic cells with a regular cytoplasm and large central or sub-central sphere-shaped nucleus and densely nucleolus (Fig. 2a, b). But in the treated fish to 72-96h Cd, the liver revealed that morphological alterations, including disorganization of hepatic tissue, joined with variations in cytoplasmic and

nuclear morphology, and severely congested sinusoids dilated central veins (Fig. 2c). In addition, the liver sections had severe lipid loss in some areas of the liver tissues characterized by low-fat vacuolation in the cytoplasm and liver necrosis (Fig. 2d).

Upon acute exposure to Cd, it causes the loss of fatty substances stored in the hepatocytes and thus the metabolism in fish is increased. This indicates that the liver is a sensitive organ and can assess damage after exposure to pollution. Younis et al. (2013) showed that cadmium has severe effects on the structure of fish liver as augmented vacuolation, hemorrhage and permeation of sinuses with leukocytes. Ahn et al. (2020) suggested that acute exposure to waterborne cadmium (3.61mg/l) was possibly lethal to fish due to its accumulation in the liver and gills affecting antioxidant enzyme activity. Adam et al. (2019) showed that the 96-hours acute toxicity of Cd ions on Gambusia affinis has an inhibitory role in detoxifying enzymes. Shah et al. (2020) were exposed *C. idella* to some heavy metals in a short time. They showed histopathological changes with a higher level of metal accumulation in gills and the liver. Alm-Eldeen et al. (2018) in *O. niloticus* found in areas with high levels of cadmium that cytoplasmic and nuclear degeneration in the hepatocytes, many vacuoles appeared in the cell cytoplasm and pyknotic nuclei.

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References

Abdel-Tawwab, M.; El-Sayed, G. & Shady, S. 2016. Growth, biochemical variables, and zinc bioaccumulation in Nile tilapia, *Oreochromis niloticus* (L.) as affected by water-born zinc toxicity and exposure period. International Aquatic Research 8: 197-206.

Adam, M.A.; Maftuch Kilawati, Y.; Soegianto, A. & Risjani, Y. 2019. The effects of acute exposure to cadmium nitrate ($CdNO_3$) on gambusia fish (*Gambusia affinis*). IOP Conf. Series: Earth and Environmental Science 259: 012004.

Ahn, T.Y.; Park, H.; Kim, J. & Kang, J. 2020. Effects of antioxidant enzymes and bioaccumulation in eels (*Anguilla japonica*) by acute exposure of waterborne cadmium. Fisheries Aquatic Sciences 23(23): 2-10.

Alak, G.; Parlak, V.; Aslan, M.; Ucar, A.; Atamanalp, M. & Turkez, H. 2018. Borax supplementation alleviates hematotoxicity and DNA damage in rainbow trout (*Oncorhynchus mykiss*) exposed to copper. Biological Trace Element Research.

Al-Asgah, N.A.; Abdel-Warith, A.; Younis, E. & Allam, H. 2015. Hematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticus* exposed to various concentrations of cadmium chloride. Saudi Journal of Biological Science 22: 543-550.

Aldoghachi, M.A.; Al-Zaidy, F. & AlKatrani, L. 2019. Effects of sublethal zinc ions and acute crude oil exposure on serum enzymes activity of *Carassius gibelio*. Journal of Animal Behavior Biometeorology 7: 155-159.

Alm-Eldeen, A.A.; Donia, T. & Alzahaby, S. 2018. Comparative study on the toxic effects of some heavy metals on the Nile Tilapia, *Oreochromis niloticus*, in the Middle Delta, Egypt. Environmental Science and Pollution Research 25: 14636-14646.

APHA. 2012. *Standard Methods for the Examination of Water and Waste Water*. 22th (Eds.), Clesceri LS, Greenberg, A.E. & Eaton, A.D. (Eds.), American Public Health Association, Washington DC.

Barone, G.; Storelli, A.; Meleleo, D.; Dambrosio, A.; Garofalo, R.; Busco, A. & Storelli, M. 2021. Levels of mercury, methylmercury and selenium in fish: insights into children food safety. Toxics 9(39): 2-14.

Evans, D.H.; Piermarini, P.M. & Choe, K. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste, Physiological Reviews 85: 97-177.

Heydarnejad, M.S.; Khosravian-Hemamai, M. & Nematollahi, A. 2013. Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). Irish Veterinary Journal 66(11): 1-7.

Jalaludeen, M.D.; Arunachalam, M.; Raja, M.; Nandagopal, S.; Bhat, A. & Sundar, S. 2012. Histopathology of the gill, liver and kidney tissue of the fresh water fish tilapia mossambica exposed to cadmium sulphate. International Journal of Advanced Biotechnology and Research 2(4): 572-578.

Jeferson, R.B.; Gabriela, Z.; Emitério, R.; Günther, G. & Luciano, B. 2018. Analysis of histopathological abnormalities in the gills of *Astyanax jacuhiensis* (characidae) for assessment of water quality in the ijuí river, southern Brazil. Acta Toxicológica Argentina 26(3): 99-103.

Khalesi, M.K.; Abedi, Z. & Behrouzi, S. 2017. Hematological, blood biochemical and histopathological effects of sublethal cadmium and lead concentrations in common carp. Bulgarian Journal of Veterinary Medicine 20(2): 141-150

Khan, A.; Shah, N.; Muhammad, M.; Khan, M.; Ahmad, M.; Farooq, M.; Adnan, M. & Yousafzai, A.M. 2016. Quantitative determination of lethal concentration Lc_{50} of atrazine on biochemical parameters; total protein and serum albumin of freshwater fish grass carp (*Ctenopharyngodon idella*). Polish Journal of Environmental Studies 25(4): 1555-1561.

Lee, G. 2015. Environment and sustainability; Effect of water and nitrogen application rates on form change and effectiveness of heavy metals Cd (293-301). WIT Press, UK. 701 p

McGeer, J.; Niyogi, S. & Smith, D. 2012. Cadmium. In: Wood, C.M.; Farrell, A.P. & Brauner, C.J. (Eds.), *Homeostasis and Toxicology of Non-essential Metals.* Academic Press, London, pp. 126-184

Mutlu, E.; Aydın, S. & Kutlu, B. 2015. Alterations of growth performance and blood chemistry in Nile tilapia (*Oreochromis niloticus*) affected by copper sulfate in long-term exposure. Turkish Journal of Fisheries and Aquatic Sciences 15: 481-488.

Albu, P.; Herman, H.; Balta, C.; Lazar, V.; Fulop, A.; Baranyai, E. & Dinischiotu, A. 2021. Correlation between heavy metal-induced histopathological changes and trophic interactions between different fish species. Applied Sciences 11(9): 3760.

Pandit, D.N. & Sharma, S. 2019. Evaluation of short term stress of lead on certain hematological biomarkers in an Indian air breathing fish, *Channa punctatus* (Bloch). Trends in Fisheries Research 8(3): 2319-4758

Pereira Pinto, A.; Cortes, R.; Fontaı´nhas-Fernandes, A.; Coimbra, A. & Monteiro, S. 2013. Gill histopathological and oxidative stress evaluation in native fish captured in Portuguese northwestern rivers. Ecotoxicology and Environmental Safety 90: 157-166.

Rahimikia, E. 2017. Analysis of antioxidants and serum biochemical responses in goldfish under nickel exposure by sub-chronic test. Journal of Applied Animal Research 45: 320-325.

Sabullah, M.K.; Ahmad, S.; Shukor, M.; Gansau, A.; Syed, M.; Sulaiman, M. & Shamaan, N. 2015. Mini Review Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. International Food Research Journal 22(2): 435-454.

Shah, N.; Khisroon, M. & Shah, S. 2020. Metals distribution, histopathological alterations, and health risk assessment in different tissues of fish (*Ctenopharyngodon idella*). Biological Trace Element Research 199: 2730-2752

Triebskorn, R.; Telcean, I.; Casper, H.; Farkas, A.; Sandu, C.; Stan, G.; Colarescu, O.; Dori, T. & Kohler, H-R. 2008. Monitoring pollution in river Mures, Romania, part II: metal accumulation and histopathology in fish. Environmental Monitoring and Assessment 141: 177-188.

Younis, E.M.; Abdel-Warith, A.; Al-Asgah, N.; Ebaid, H. & Mubarak, M. 2013. Histological Changes in the Liver and Intestine of Nile Tilapia, *Oreochromis niloticus*, exposed to sublethal concentrations of cadmium. Pakistan Journal of Zoology 45(3): 833-841

Yuanyyuan, W.; Qixing, Z.; Shengwei P.; Lena, M. & Xiaowei, N. 2009. Toxic effects of crude-oil-contaminated soil in aquatic environment on *Carassius auratus* and their hepatic antioxidant defense system. Journal of Environmental Sciences 21: 612-617

Zang, L.; Shimada, Y.; Nishimura, Y.; Tanaka, T. & Nishimura, N. 2015. Repeated blood collection for blood tests in adult zebrafish. Journal of Visualized Experiments 102: 53272.