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The Effect of Propofol Anaesthesia on Haematological and Biochemical Blood Profile of European Whitefish

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Abstract

Propofol (2'6-diizopropylofenol) effectively induced general anaesthesia in whitefish in the preliminary test. The aim of the study was to assess the effects of propofol on European whitefish by hematological and biochemical indices of blood plasma. A total of 60 specimens of whitefish from experimental hatchery of Inland Fisheries Institute in Olsztyn were used. Fish were exposed to the water solution of 0.5 cm^3 dm^{-3} of Diprivan (Astra Zeneca Ltd., UK) (5 mg dm^{-3} of propofol) for 10 min. Hematological and biochemical indices were analyzed immediately after anaesthesia and 24h after exposure to the anaesthetic, and compared to respective control groups. Propofol did not affect significantly red blood cells indices. Reactive granulocytosis was observed 24 h after anaesthesia. The level of stress indices like: white blood cell count, glucose, albumin and globulin concentration was recovered 24 h after propofol anaesthesia. However, elevated value of alkaline phosphatase suggested some liver disturbance.

Keywords: 2'6 diisopropylophenol, *Coregonus lavaretus*, diprivan, haematological indices, handling stress.

Introduction

European whitefish, *Coregonus lavaretus* (L.), inhabits cold and clean water bodies in the northern hemisphere. In many countries, natural whitefish populations dwindle and the continuation of the species is threatened in many areas (Thomas and Eckmann, 2007). Whitefish populations have to be supported with active protection measures including the creation of brood stocks and stocking programs which use material reared under controlled conditions (Wedekind *et al.*, 2007; Szczepkowski *et al.*, 2010). First attempt showed high potential of the species to be culture in ponds (Tourney, 2006) and recirculated aquaculture systems (Wunderlich *et al.*, 2011), however, whitefish is still non domestic species highly vulnerable to both environmental and handling stress.

Anaesthesia is a valid method of protection of an animal against the stress however, there is no one anaesthetic suitable for all fish species and all purposes. Thus, there is the need for new anaesthetics in fisheries.

Propofol (2'6 diisopropylphenol) is a powerful anesthetic used in both human and veterinary medicine. Propofol is the promising anaesthetic agent for fish. Fleming *et al*. (2003) reported propofol anaesthesia in Mexico Gulf sturgeon*Acipenser oxyrinchus desotoi*. Miller *et al*. (2005) applied propofol for anaesthetising a white spotted bamboo shark *Chiloscyllium plagiosum.* Gholipour Kanani and Ahadizadeh (2013) proved that propofol is effective anaesthetic for goldfish *Carassius auratus auratus*. Propofol rapidly induces surgical anaesthesia and is quickly metabolized in mammals. This drug is considered as very safe and thus it is the most frequently used anesthetic for intravenous infusion in humans (Murthy, 2008).

The aim of the study was to assess the influence of propofol on the haematological and biochemical blood profile, of the European whitefish.

Materials and Methods

Fish

European whitefish (n=60) *Coregonus lavaretus* were supplied by "Dgał" Experimental Hatchery of Polish Inland Fisheries Institute. Fish mean length and mean body weight (±SD) was 20.0±1.2 cm and 55.9±11.2 g respectively. Fish were reared in the "Dgał" facilities from the hatch. Fish were reared in flow trough, 0.5 m^3 tanks supplied with deep well water. Water temperature was 15.9±0.5°C, and water

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pH was 6.35±0.3, and oxygen saturation was above 80% during all the test.

Anaesthetic agent

Induction of anaesthesia was done by means of Diprivan, the anaesthetic drug widely used in both human and veterinary medicine (Barash *et al*., 2009; Flecknell, 2009). Diprivan is an intravenous preparation containing 10 mg cm^{-3} of 2'6-diisopropylphenol (propofol).

Experiment Design

To avoid environmental and handling stress, the test was performed in the hatchery facilities. The procedure proposed by Velišek and Svobodova (2004a, 2004b) and Velišek *et al*. (2006) was applied. Fish were randomly catch out of the tank and individually subjected to one of below procedures: control fish (CF) (n=10) were blood sampled immediately (within less than 2 minutes, without anaesthesia) after catch; treatment 1 fish (T1) (n=20) were exposed to water solution of 0.5 cm^3 $dm^{-3}$of Diprivan for 10 min and blood sampled immediately after exposure; treatment 2 fish (T2) (n=20) were exposed to Diprivan as above and then moved to the tank (0.5 m^3 of volume) filled with anaesthetic free water for recovery, blood was sampled 24 hours after; stress exposed control fish (SEC) (n=10) were catch out of the rearing tank and placed in exposure box filled with anaesthetic free water for 10 minutes and then moved to 0.5 m^3 tank for recovery, blood was sampled 24 hours after. Both T2 and SEC were blood sampled without anaesthesia. The exposure was done in 50 dm^3 polypropylene box. The Diprivan solution was aerated mechanically and changed every 5 fish. Bath temperature and pH was the same as in the rearing tank water.

Blood was sampled from caudal vessels by syringe covered with heparin lithium salt 50 IU cm^{-3} (Medlab Products, Raszyn, Poland). Approximately 0.8 ml of the blood was immediately centrifuged in Stat Spin centrifuge at 12000 g for 30 s at room temperature. Blood plasma was collected for biochemical analysis and immediately frozen in -24°C. The rest of collected blood was used for blood smears preparation (2 smears per each fish) and determination of haematological and biochemical indices.

After blood sampling, fish was placed in excessive propofol solution (20 mg dm^{-3}). Following the arrest of opercular movement, fish brain was destroyed with sharp scissors and the length (LC) and weight measurements were taken.

Haematological indices were determined according to standard methods given in Unified methods for haematological examination of fish (Svobodova *et al*., 1986) and covered: erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin content (MCH), leukocyte count (WBC) and the differential leukocyte count (Leukogram). Plasma samples were analyzed with VetTest Chemistry Analyzer (Idexx Lab., USA). Analysis of biochemical parameters included: inorganic phosphates (PHOS), calcium (Ca), total protein (TP), albumin (ALB) and globulin (GLOB), ammonia (NH_3), triacylglycerols (TAG), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Each plasma sample was thawed only once in room temperature and all listed above parameters were determined at one run of the chemistry analyser.

Statistical Analysis

Results were tested with Kruskall-Wallis nonparametric ANOVA and Man-Whitney test using Statistica 9.1 software. The differences were considered as significant if P≤0.05.

Table 1. Effects of propofol anaesthesia on haematological indices in European whitefish

	Treatment			
	CF (n = 10)	T1 (n = 20)	T2 (n =20)	SEC (n =10)
PCV (L L^{-1})	0.46±0.04^{ab}	0.50±0.06^{a}	0.40±0.04^{c}	0.42±0.03^{bc}
	(0.40-0.51)	(0.39-0.64)	(0.30-0.48)	(0.39-0.51)
Hb (g L^{-1})	68.6±8.3^{a}	71.4±6.4^{a}	66.5±9.0^{a}	72.8±6.1^{a}
	(52.4-79.4)	(57.3-82.9)	(41.6-79.7)	(59.7-80.5)
Er (T L^{-1})	0.94±0.16^{ab}	1.08±0.22^{a}	0.92±0.15^{b}	0.88±0.27^{b}
	(0.75-1.22)	(0.55-1.59)	(0.68-1.16)	(0.57-1.53)
MCV (fl)	504±110^{a}	491±125^{a}	448±75^{a}	505±125^{a}
	(328-640)	(317-909)	(330-608)	(333-754)
MCH (pg)	73.8±13.0^{a}	69.1±17.8^{a}	73.7±15.1^{a}	87.3±22.1^{a}
	(59.1-98.8)	(46.6-127.2)	(38.5-97.1)	(50.3-127.9)
MCHC (g L^{-1})	0.15±0.02^{ab}	0.14±0.01^{a}	0.16±0.03^{bc}	0.17±0.01^{c}
	(0.10-0.20)	(0.12-0.15)	(0.08-0.21)	(0.14-0.20)

Results expressed as mean ± SD. Values with the same letter index are not significantly different at P≤0.05.

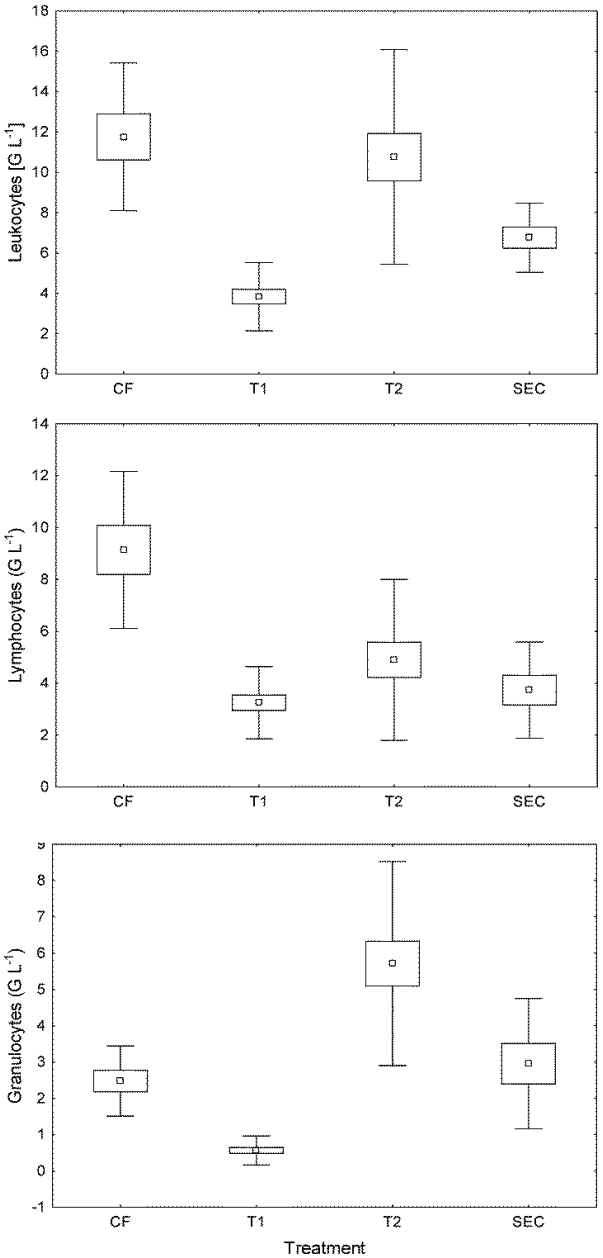

Figure 1. Effect of propofol anaesthesia on leukocytes (A), lymphocytes (B) and granulocytes (C) number in European whitefish *Coregonus lavaretus*. Points represent mean, boxes - mean ±S E and bars - mean ± SD.

Results

Propofol anaesthesia did not affect whitefish Hb, MCH and MCV significantly, however, PCV, RBC were decreased and MCHC were increased both in T2 and SEC fish (Table 1).

With exception of granulocytes, the number of leukocytes was strongly depressed in SEC fish when compared to CF. Leukopenia, occurred immediately after propofol anaesthesia (T1) and was recovered 24h later (T2) (Figure 1A). The number of all types of lymphocytes and neutrophilic granulocytes was significantly decreased in propofol group immediately after anaesthesia compared to CF (Table 2). The increase of lymphocytes count to the CF level and reactive granulocytosis was observed 24 h after anaesthesia (T2) (Figure 1B and 1C). SEC whitefish suffered severe lymphopenia (Figure 1B).

Total protein blood plasma concentration in CF fish was 38.7±4.0 g dm^{-3} and was decreased slightly in anaesthetized fish (T1) and recovered in T2 fish. TP was strongly decreased in SEC fish and reached 27.4±4.5 g dm^{-3}. This depletion was caused by the decrease of both albumin and globulin level (Figure 2C and 2D). In all tested whitefish, ALT level was under the detection limits (10 IU dm^{-3}). The activity of both AST and ALKP was not affected significantly (Table 3).

Propofol anaesthesia caused the decrease of ammonia level 24 h following exposure. The decrease

Table 2. Effects of propofol anaesthesia on differential leukocyte count in European whitefish

White blood cell type (G/L)	Treatment			
	CF (n = 10)	T1 (n = 20)	T2 (n =20)	SEC (n =10)
Lymphocytes –small	8.00±2.86^{a} (5.09-14.75)	2.97±1.24^{b} (1.44-6.55)	4.58±2.97^{b} (1.02-13.13)	3.01±1.81^{b} (1.21-7.47)
Lymphocytes – big	1.14±0.35^{bc} (0.36-1.56)	0.26±0.19^{a} (0.03-0.67)	0.32±0.22^{ab} (0.06-0.92)	0.71±0.54^{c} (0.17-1.89)
Monocytes	0.05±0.07^{a} (0.00-0.21)	0.003±0.007^{b} (0.00-0.02)	0.03±0.04^{a} (0.00-0.16)	0.02±0.03^{a} (0.00-0.12)
Myelocytes	0.06±0.07^{a} (0.00-0.21)	0.01±0.04^{a} (0.00-0.21)	0.09±0.14^{a} (0.00-0.53)	0.03±0.05^{a} (0.00-0.15)
Neutrophiles – rods	0.61±0.25^{a} (0.24-1.12)	0.11±0.10^{b} (0.00-0.41)	0.59±0.34^{a} (0.09-1.45)	0.64±0.39^{a} (0.21-1.33)
Neutrophiles -segments	1.86±0.83^{a} (0.72-3.11)	0.45±0.30^{b} (0.09-1.31)	5.11±2.53^{c} (1.89-9.94)	2.31±1.42^{a} (0.64-5.20)

Results expressed as mean ± SD. Values with the same letter index are not significantly different at P≤0.05.

Table 3. Effects of propofol anaesthesia on biochemical indices in European whitefish

	Treatment			
	CF (n = 10)	T1 (n = 20)	T2 (n =20)	SEC (n =10)
PHOS (mmol L^{-1})	2.9±0.5^{a} (2.1-3.8)	3.1±0.4^{a} (2.4-3.7)	2.5±0.6^{b} (1.4-4.6)	2.3±0.4^{b} (1.9-3.2)
CA (mmol L^{-1})	2.9±0.4^{a} (2.4-3.5)	2.9±0.3^{a} (2.2-3.5)	2.8±0.29^{a} (2.4-3.5)	2.4±0.3^{b} (2.0-2.8)
ALB (g L^{-1})	12.2±1.3^{a} (10-15)	10.2±2.4^{bc} (4-15)	12.1±3^{ab} (6-18)	8.8±2.3^{c} (5-13)
AST (U L^{-1})	456±105^{a} (281-616)	592±161^{a} (409-908)	491±129^{a} (230-696)	628±230^{a} (297-984)
ALKP (U L^{-1})	151±52^{a} (72-224)	122±52^{a} (58-234)	170±62^{a} (103-302)	117±49^{a} (66-197)
TRIG (mmol L^{-1})	0.88±0.23^{a} (0.56-1.15)	0.77±0.13^{a} (0.45-0.99)	0.79±0.19^{a} (0.52-1.38)	0.66±0.12^{a} (0.48-0.83)

Results expressed as mean ± SD. Values with the same letter index are not significantly different at P≤0.05.

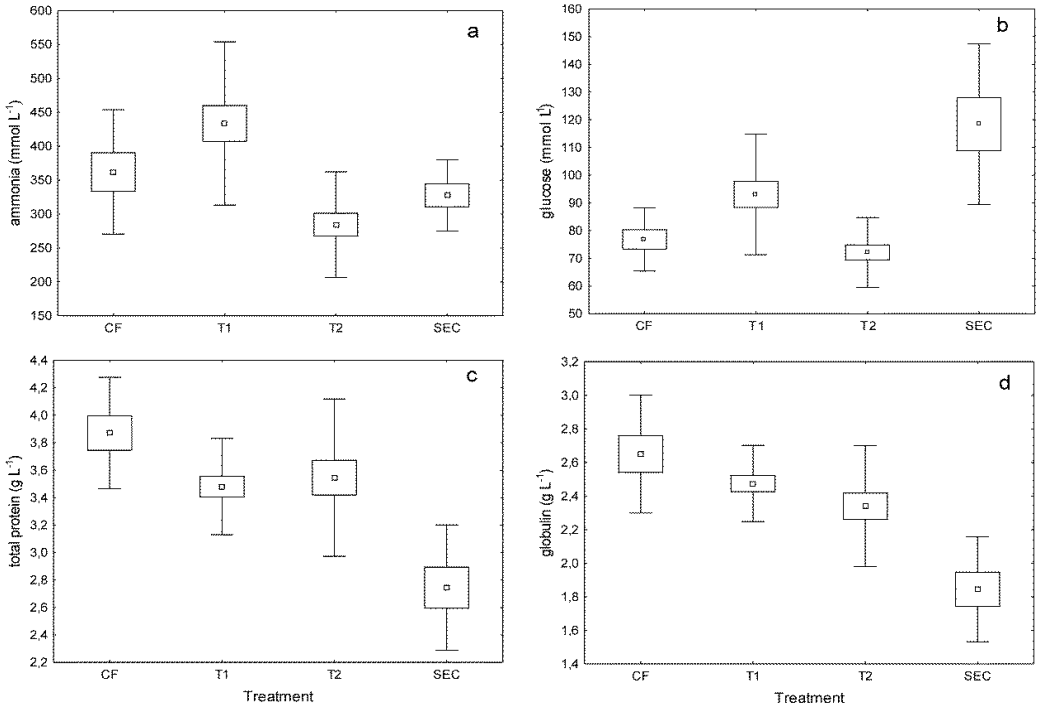

Figure 2. Effect of propofol anaesthesia on amonnia (A), glucose (B), total protein (C) and globulin (D) content in European whitefish *Coregonus lavaretus* blood plasma. Points represent mean, boxes - mean±SE and bars - mean±SD.

was also observed in SEC fish; however, it was not significant (Figure 2A).

SEC fish revealed highly elevated blood plasma glucose concentration (6.6±1.6 mmol dm^{-3}) compared to CF (4.3±0.6 mmol dm^{-3}). The increase of glucose level was observed in T1 fish, however, it was recovered within 24 h (Figure 2D). Triacylglycerols level was not affected and ranged between 0.48 and 1.38 mmol dm^{-3}.

Discussion

Both hematological and biochemical blood indices are proved to be valid measure of fish health status. However, reports regarding European whitefish blood indices are rather occasional and scarce, and thus our study could be regarded as the first preliminary but comprehensive study on whitefish blood system.

PCV values found in both control and experimental whitefish were much higher than those reported by other authors. Lappivaara (2001) found the maximum PCV value as high as c.a. 0.38 in whitefish reared in 13.0±0.5°C and pH 7.5±0.25. Siikavuopio *et al*. (2010) found 0.32, 0.31 and 0.28 PCV in fish reared in 1, 3 and 6°C respectively (pH 7.2). We suppose that higher PCV resulted from acclimation to environmental conditions.

Clove oil (CLO) and 2-phenoxyethanol (2PE) anaesthesia did not influence PCV in rainbow trout *Oncorhynchus mykiss*and common carp *Cyprinus carpio* significantly (Velišek *et al*., 2005a, 2005b, 2007a). However, in European catfish *Silurus glanis*, 2-phenoxyethanol (2PE) caused an increase of PCV (Velišek *et al*., 2007b) and CLO did not (Velišek *et al*., 2006). Gomulka *et al*. (2008) found increased PCV in Siberian sturgeon following 10 min anaesthesia in MS-222 but not in eugenol bath.

Increase of PCV can result from increased number of RBC (e.g. due to acute stress and spleen evacuation), erythrocyte swelling (e.g. due to lower blood pH, respiratory acidosis) or decreased volume of water in circulating blood (e.g. due to muscle tissue acidification following stress exposure or sever exercise). In case of whitefish exposed to propofol, RBC, MCV and MCHC were not altered. No changes in RBC, MCV and MCHC were found in rainbow trout and common carp (Velišek *et al*., 2005a, 2005b, 2007a) anaesthetised with both 2PE and CLO. Both, MS-222 and eugenol exposure resulted in significant erythrocyte swelling in Siberian sturgeon *Acipenserbaeri* (Gomulka *et al*., 2008). 2PE anaesthesia produced erythrocyte swelling in European catfish (Velišek *et al*., 2007b).

Stress is thought to be responsible for leucopoenia in fish (Wedemeyer, 1970). Although relatively low leukocyte count (11.8±3.7 g dm^{-3}) was found in control whitefish, propofol exposure resulted in strong decrease of WBC in exposed fish. It was mainly due to depletion of small lymphocytes and neutrophiles, however, the number of monocytes was also significantly decreased (Table 2). WBC returned to CF level 24 h after anaesthesia but sever reactive granulocytosis occurred and lymphocytes number remain depleted. No changes in leucocytes count followed anaesthesia in common carp, rainbow trout, European catfish and Siberian sturgeon (Velišek *et al*., 2005a, 2005b, 2007a, 2007b; Gomulka *et al*., 2008). However, in case of Siberian sturgeon, decreased WBC (mainly due to lymphocytes depletion) was observed 24 hrs after both anaesthesia and stress exposure (Gomulka *et al*., 2008).

Inorganic phosphate was decreased in both whitefish groups sampled 24 h after exposure. No changes in this parameter were found in European catfish and rainbow trout anaesthetised with CLO and 2PE (Velišek *et al*., 2005a, 2007b). Gomulka *et al*. (2008) found the decrease of PHOS immediately after exposure to both MS-222 and eugenol, and re-established levels 24 hrs later in Siberian sturgeon.

Strong acute stress is usually followed by increased of glucose level and induction of gluconeogenesis and resulted both an increase of ammonia and a decrease of protein level. Glucose concentrations between c.a 2 and 5.5 mmol dm^{-3} were reported for the species by Lappivara (2001) and Siikavuopio *et al*. (2010). Brzuzan *et al*. (2009) reported values of glucose between 3.4 and 9.02 mmol dm^{-3}, and Ernst *et al*. (2006) between 0.87 and 3.0 mmol dm^{-3}. In examined whitefish, exposure to propofol caused the increase of glucose level but it was recovered 24 hrs later, ammonia level was decreased at the same time. Elevated glucose level was found in SEC fish, but no significant changes in ammonia. TP level was slightly decreased and recovered in anaesthetised whitefish and strongly decreased in SEC whitefish. No changes in GLU, NH_3 and TP was found in European catfish and common carp exposed to 2PE and CLO respectively (Velišek *et al*., 2006; Velišek and Svobodova, 2004a). Reversible increase of glucose level, with no changes in TP and NH_3, was found in European catfish and common carp exposed to 2PE and CLO respectively (Velišek *et al*., 2005b, 2007b) and rainbow trout exposed to 2PE. CLO anaesthesia caused reversible increase of both glucose and ammonia level in rainbow trout blood (Velišek *et al*., 2005a). No changes in glucose level, decrease of NH_3 and increase of TP was found in Siberian sturgeon exposed to both MS-222 and eugenol (Gomulka *et al*, 2008). Internal ammonia can be neutralized by formation of glutamine and incorporation of this amino acid into fish protein (Wicks and Randall, 2002). The ammonia production and the ammonia capacity neutralization is increasing during fish ontogeny (Gomulka *et al*., 2011).

Propofol did not affect plasma activity of intracellular enzymes in exposed whitefish and thus one can assume that no tissue damage followed propofol exposure. No changes in AST activity was

found in European catfish, common carp exposed to both CLO and 2PE (Velišek *et al*., 2004, 2005b, 2005c, 2007b), and Siberian sturgeon exposed to both MS-222 and eugenol (Gomulka *et al*., 2008). Increased level of AST activity and no changed ALT was found in rainbow trout after exposure to both CLO and 2PE (Velišek and Svobodova, 2004b; Velišek *et al*., 2005a). No changes in ALT activity was found in CLO anaesthetised common carp (Velišek *et al*., 2005 b). Significantly elevated level of ALT activity was revealed in 2PE exposed European catfish and common carp (Velišek and Svobodova , 2004a, 2007b), and in Siberian sturgeon exposed to both MS-222 and eugenol (Gomulka *et al*., 2008).

Conclusion

Our results revealed that propofol exposure caused a moderate stress in European whitefish, however most of determined indices was recovered after 24 h. The comparison of fish exposed to stress and anaesthetised ones indicates that propofol can limit negative impact of stress to whitefish.

The wide range of biochemical and haematological indices of whitefish blood was reported for the first time in this paper.

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