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Original article

Effects of propofol and carbon dioxide on acid-base balance in Siberian sturgeon

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Abstract

The aim of this study was to compare physiological responses in Siberian sturgeon (*Acipenser baeri*) induced by propofol and CO₂ anaesthesia. Two procedures were applied during the experiment. In procedure I, blood samples were collected immediately after exposure (1, 2, 5, 10 min) to the anaesthetic. In procedure II, fish were exposed to the anaesthetic for 10 minutes and then were moved to anaesthetic free water. Blood was sampled after 5, 10, 20 or 30 min of recovery time. Gasometrical and biochemical analyses were performed on collected blood.

In CO₂ anaesthetized fish strong hypercapnic acidosis was revealed. The drop of the HCO₃⁻/CO₂ ratio, from 28:1 in control fish up to 4:1 in CO₂ anaesthetized ones, proved that the compensation mechanism is not capable of preventing acidosis during CO₂ anaesthesia in Siberian sturgeon. In contrast, only moderate, respiratory acidosis occurred in sturgeons anaesthetised with propofol. Hypercapnic acidosis during CO₂ anaesthesia was followed by a fourfold increase of ammonia level in the blood. Glucose level, increasing only during recovery time, indicates that a secondary stress response occurred when awareness of anaesthetized fish had been restored.

Key words: anaesthetics, 2,6-diisopropylphenol, hypercapnic acidosis, respiratory acidosis, *Acipenser baeri*

Introduction

Handling is an unavoidable element of modern fish culture. Thousands of fish are subjected to different manipulations every day. In order to minimize handling stress and suffering, many anaesthetic agents are being used (Bell 1964, Ross and Ross 2008).

Propofol (2'6-diisopropylphenol) is a relatively new anaesthetic for fish and there are almost no reports on its influence on fish physiology (Gomulka

et al. 2012). Drugs containing propofol are commonly used in human (Biebuyck et al. 1994, Meierhenrich et al. 2010) and veterinary medicine (Branca et al. 1995, Guzel et al. 2006). Propofol has been shown to be an effective anaesthetizing agent for bamboo shark (Miller et al. 2005), silver catfish (Gressler et al. 2012) and gold fish (GholipourKanani and Ahadizadeh 2013). Propofol anaesthesia is quickly induced and followed by deep myorelaxation and suppression of gill ventilation rate (Fleming et al. 2003).

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Carbon dioxide (CO₂), unlike propofol, has been in use for many years (Ackerman et al 2005, Ross and Ross 2008). The present interest in CO₂ anaesthesia in fish is triggered by the fact that no hazardous residues remain in fish tissues following anaesthesia (Ackerman et al 2005). However, there are some limitations of CO₂ anaesthesia in fish, for example dosing difficulties, a strong excitation phase of the anaesthesia in some species, and staff safety during the anaesthesia procedure. Many field trials done by our team showed that CO₂ anaesthesia can be safe and convenient, if applied properly, for many freshwater fish species.

Anaesthesia induced by both propofol and carbon dioxide can be followed by harmful effects resulting from respiration disorders. The aim of the study was to compare physiological changes in Siberian sturgeon (*Acipenser baeri*), which occur under both propofol and CO₂ anaesthesia.

Materials and Methods

Young Siberian sturgeon (n = 90; mean total length 362±38 mm and mean body weight 163.0±57.4 g), from the „Dgał” Experimental Fish Hatchery of the Inland Fisheries Institute (Olsztyn, Poland) were used for the experiment. Fish were kept in 1 m³ flow-through tanks at 14°C of water temperature. Water pH was 7.3±0.3 and the oxygen saturation was maintained above 80%.

Compressed gaseous CO₂ was delivered by Eurogaz Ltd. (Poland). Both CO₂ and atmospheric air were mixed at a 2:8 ratio and the experimental bath water was saturated with this gas mixture until the CO₂ concentration stabilized at 400 mg dm⁻³. Water CO₂ concentration was determined with Karat apparatus following the Hartl method (Żak 2005).

Diprivan (10 mg cm⁻³ of propofol) was supplied by AstraZeneca (UK). Diprivan is an aqueous propofol solution for intravenous injection, used in human medicine. The propofol bath concentration was 8 mg dm⁻³.

Both anaesthetic concentrations were chosen in the preliminary tests.

The experimental fish were divided into 17 groups: control (n = 10) and 16 experimental ones (n = 5). Eight experimental groups were exposed to propofol, and the others to carbon dioxide. Two procedures were applied during the experiment. In procedure I, blood samples were collected immediately after exposure to the anaesthetic. Exposure time was 1, 2, 5 or 10 minutes depending on the group. In procedure II, fish were exposed to the anaesthetic for 10 minutes and were then moved to anaesthetic free water. Blood was sampled at 5, 10, 20 and 30 min after

the end of the exposure from respective groups of fish.

Blood samples were taken by syringe from caudal vessels in less than 1 min after netting fish out of the water. To prevent blood clotting, syringes covered with heparin lithium salt (50 IU cm⁻³) were used.

The partial pressure of CO₂ (PCO₂) and partial pressure of O₂ (PO₂), bicarbonates (HCO₃⁻), total CO₂ (tCO₂), pH and haemoglobin, Na⁺, K⁺ and Cl⁻ concentration were determined using a VetStat analyzer (Idexx Inc., USA). The above parameters were determined immediately following blood samplings.

Haematocrit was determined by centrifuging heparinised blood in capillary tubes at 4000 RPM for 3 minutes. Plasma samples were obtained by centrifuging of blood samples in StatSpin centrifuge (Idexx Inc., USA) at 15 800 RPM (12000 G) for 90 seconds. Plasma supernatants were transferred to Eppendorf tubes and frozen at -24°C for further analysis. A VetTest Chemistry analyser (Idexx Inc., USA) was used for determination of ammonia (NH₃), and glucose (GLU) concentration.

Experimental data were analysed using Statistica 10 software (Statsoft, USA). Kruskal-Wallis non-parametric ANOVA and Mann-Whitney U test were used for data analyses. Differences were considered as significant when the p value was lower than 0.05 (p<0.05). Relationship of variables was analysed using non linear regression methods.

Results

Average time to reach stage IV of anaesthesia (Siwicki 1984) was approximately 3 and 5 min for propofol and CO₂, respectively. The decrease of the rate of respiratory operculum movement, progressive with the time of exposure, was visible in fish exposed to both anaesthetics.

PCO₂, HCO₃⁻ and tCO₂ were significantly higher in fish anesthetized with CO₂ when compared to control ones (Fig 1a, c and e). PCO₂ value increased over sixfold during 10 min of exposure to CO₂ saturated water (Fig. 1a). Following the transfer of the fish to CO₂ free water, PCO₂, HCO₃⁻ and tCO₂ decreased rapidly and reached levels below the initial value after 30 min of recovery (Fig. 1b, d, f).

No significant changes in PCO₂ level were found in propofol anesthetized sturgeons. Plasma HCO₃⁻ and tCO₂ levels were decreased during both the exposure and the recovery in propofol free water (p<0.05) when compared to control values. There was no significant difference for both HCO₃⁻ and tCO₂ between propofol and CO₂ anesthetized sturgeon after 30 min of recovery (Fig. 1d, f).

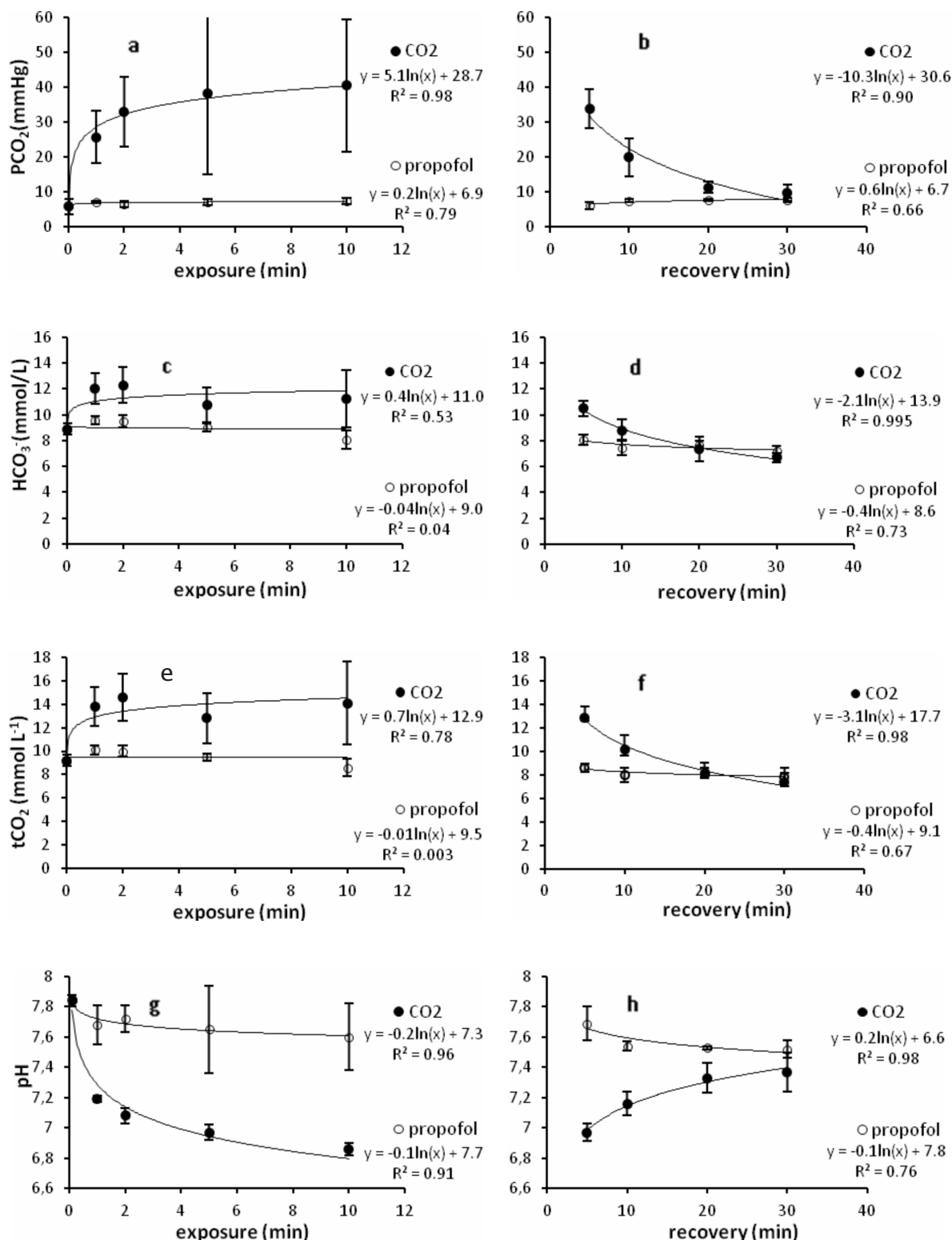


Fig. 1. Carbon dioxide, bicarbonates and pH in the blood of Siberian sturgeon exposed to both propofol and CO₂ anaesthesia. Best fit lines and regression equations are provided.

In all experimental groups significant decrease of plasma pH was noticed (Fig. 1g). In fish exposed to CO₂, pH was significantly lower compared to propofol anaesthetized sturgeons at all time points and did not recover to initial level after 30 min. The level of pH

remained below the initial value (7.84±0.04) in propofol anaesthetized fish and after 30 minutes of recovery it was as high as 7.52±0.06. A strong correlation was detected between HCO₃⁻/CO₂ ratio and pH level (R=0.992, p<0.0001) (Fig. 2).

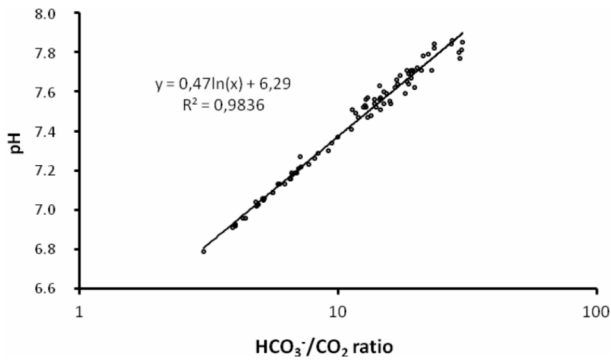


Fig. 2. Correlation between $\text{HCO}_3^-/\text{CO}_2$ ratio and blood plasma pH of Siberian sturgeon *Acipenser baeri* ($r = 0.992$). Best fit line ($p < 0.001$) and regression equation are provided.

Both CO_2 and propofol anaesthesia caused a significant decrease of PO_2 . It was recovered in CO_2 exposed fish during recovery, but in propofol exposed ones, PO_2 remained at the level below the initial value after 30 min (Fig. 3a, b).

No significant influence of both CO_2 and propofol anaesthesia on Ht and blood content of Hb, Na^+ , K^+ and Cl^- was observed.

No significant differences in glucose concentration was observed between control and CO_2 anaesthetized fish during the exposure, however, the variability of results was high (Fig. 3c) in exposed fish. The glucose level was slightly lowered in propofol anaesthetized fish during the exposure. In both propofol and

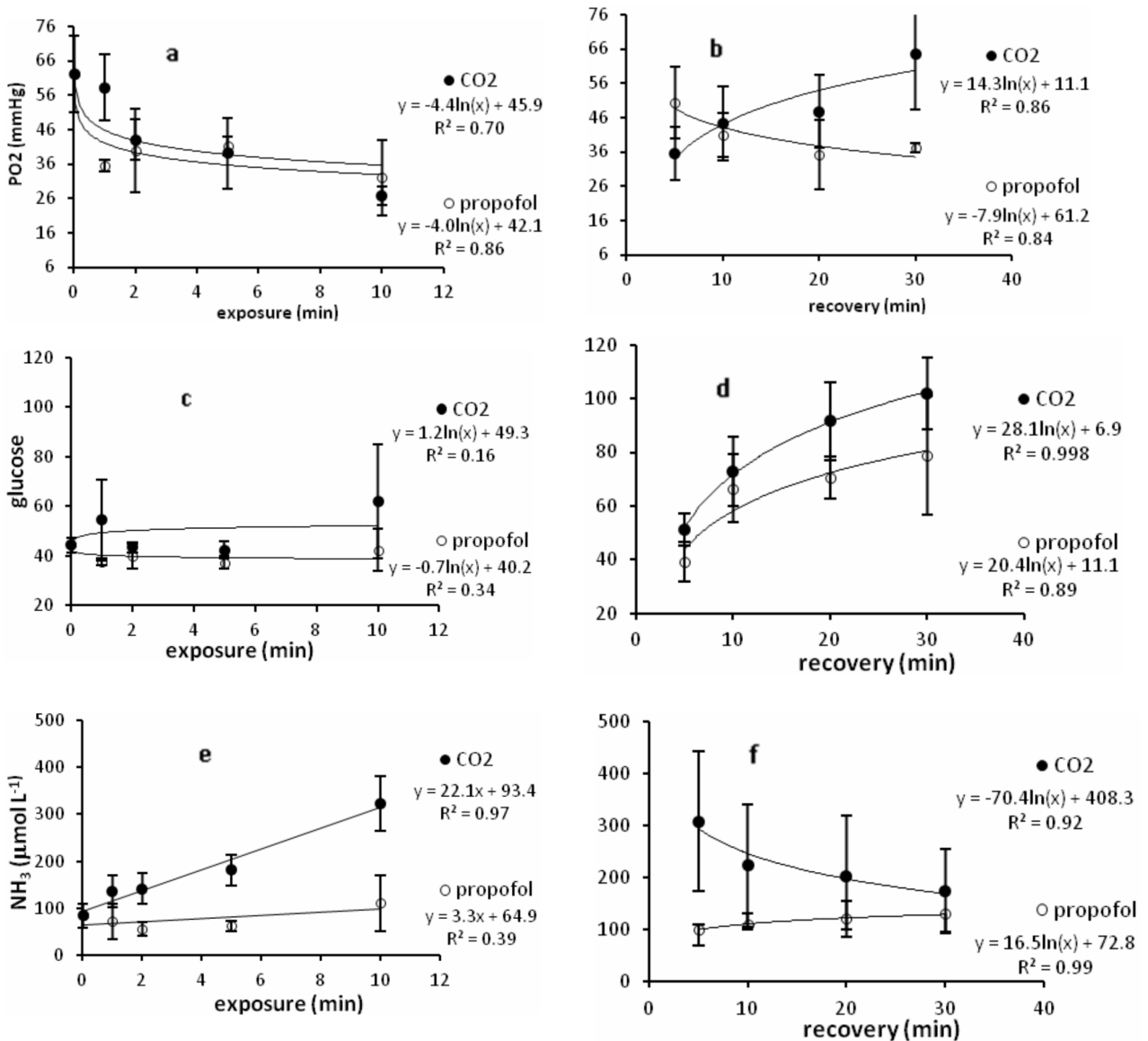


Fig. 3. Oxygen, glucose and ammonia level in the blood of Siberian sturgeon exposed to both propofol and CO_2 anaesthesia. Best fit lines and regression equations are provided.

CO₂ anaesthetized fish, the glucose level increased in time from the fifth minute of recovery, and reached values almost double of those measured at the end of exposure. However, glucose concentration was significantly lower in propofol anaesthetized groups at all time points when compared to CO₂ exposed fish ($p < 0.05$).

A significant increase ($p < 0.05$) was observed in blood NH₃ level in all CO₂ anaesthetized fish submitted to both procedure. Ammonia level (323 ± 59 mmol dm⁻³) was almost fourfold higher after 10 min of exposure than the initial one (84 ± 26 mmol dm⁻³) (Fig. 3e, f). No significant differences were found in blood ammonia in propofol anaesthetized fish during exposure. However, the ammonia level was significantly increased after 20 min and 30 min of recovery.

Discussion

Anaesthetic agents usually cause myorelaxation and decrease of respiration rate in fish. It is followed by a decrease in respiratory gases exchange rate in gills. This can result in an acid-base disorder known as respiratory acidosis – toxic accumulation of protons due to the absence of CO₂ excretion (MCO₂). Formation of H⁺ and HCO₃⁻, catalyzed by the enzyme carbonic anhydrase, plays the key role in both MCO₂ and control of acid-base equilibrium. It is believed, that respiratory compensation can be achieved through exchange of H⁺ and HCO₃⁻ for environmental Na⁺ and Cl⁻, respectively (Perry and Gilmoure 2006).

Strong acidosis revealed in CO₂ anaesthetized fish obviously followed the inflow of environmental CO₂ caused by a concentration gradient between water and blood plasma (hypercapnic acidosis). However, the plasma value of tCO₂ was still much lower than water level of dissolved CO₂ (14.1 ± 3.53 mmol dm⁻³ and 9.09 ± 0.91 mol dm⁻³ respectively).

The increase of HCO₃⁻ level in CO₂ anaesthetized fish resulted from the compensation of the acidosis (Perry and Gilmoure, 2006). However, the fall in the HCO₃⁻/CO₂ ratio, from 28:1 in control fish up to 4:1 in CO₂ anaesthetized fish, proved that the compensation mechanism is not capable of preventing acidosis during CO₂ anaesthesia in Siberian sturgeon. The pH decrease observed in CO₂ anaesthetized fish was very marked (6.86 ± 0.22).

It is known that in humans blood pH decreased by one unit can cause seizures, heart arrhythmia and death (Andersen et al. 1967). Although no significant changes in HCO₃⁻ and tCO₂ were detected, pH was decreased significantly in propofol anaesthetized fish. A similar pH decrease, below 7.5, was observed by Nonnotte et al. (1993) and Maxime et al. (1998) in

Acipenser baeri in experimental hypoxia conditions. The initial pH value was 7.85 in both cases. We did not record any deaths but we can suspect fatal effects in the case of longer exposure. Brenier and Randall (1998) recorded deaths of experimental rainbow trout exposed to 1575.6 mg of CO₂ after 10 min of exposure.

It is estimated that 90% of ammonia excretion (J_{Amm}) is done by the diffusion of unionized ammonia (NH₃) through the gills epithelium. The diffusion of NH₄⁺ ion is limited due to the electric charge of the particle. According to the model of ammonia diffusion proposed by Randall and Wright (1987, 1989), MCO₂ allows for „trapping” of the ammonia in the environment, in the form of NH₄⁺ (not permeable through a biological membrane), by decreasing the pH of the water layer surrounding the gills. This mechanism is very efficient although only a small part of total ammonia (T_{Amm}) exists in the form of NH₃ in fish blood plasma under normal pH and temperature conditions. In the examined control sturgeons, NH₃ consisted approximately 1.73% of T_{Amm} (blood pH 7.84; temperature 14°C; pK=9.59). However, the drop of blood pH to 6.86 during 10 min of CO₂ anaesthesia decreased the NH₃ share to 0.2%. Thus, despite a high environmental concentration of CO₂, J_{Amm} was decreased due to a low level of NH₃ in the blood plasma. We can conclude that hypercapnic acidosis is followed by ammonia autointoxication during CO₂ anaesthesia.

On the other hand, only moderate, respiratory acidosis occurred in sturgeons anaesthetised with propofol. The low level of HCO₃⁻ may indicate that propofol did not affect MCO₂ efficiency greatly. However, more than the double decrease of the HCO₃⁻/CO₂ ratio sustained the drop of blood pH. Gomulka et al. (2012) obtained similar results in orfe exposed to propofol. The HCO₃⁻ level in this study was significantly lowered during exposure and still decreasing during recovery. The HCO₃⁻ level was under detection limits in one hour of recovery.

Two types of responses to stress can be distinguished in fish; the primary and secondary stress response. The primary stress response includes neuroendocrine responses, the release of catecholamines and the activation of the hypothalamus-pituitary-interrrenal axis leading to release of corticosteroids to circulating blood (Mommsen et al. 1999). The secondary response includes changes in blood concentration of electrolytes, glucose, triacylglycerols, proteins, calcium and phosphates etc. The respiratory and immune function is also modified during the stress response (Mazeaud et al. 1977, Reubush and Heath 1996, Barton 2002). Anaesthesia is used in fish to minimize the stress response and protect fish against

pain and negative stress impact. Both primary and secondary stress response indicators can be used to assess the protective properties of fish anesthetics.

Both propofol and CO₂ anaesthesia seem to moderate the secondary stress response during exposure, however, growing glucose level during recovery indicates that stress occurred when anaesthetized fish awareness has been restored. This remark is important since it indicates the need for research on how recovery conditions influence the secondary stress response and how we can manage the recovery to minimize the stress impact.

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