Comparative Biochemistry and Physiology, Part C 226 (2019) 108590

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Acute exposure to the water-soluble fraction of gasoline (WSF_G) affects oxygen consumption, nitrogenous-waste and Mg excretion, and activates anaerobic metabolism in the goldfish *Carassius auratus*

Dal Pont G.[a,b,c,*], Luciana Rodrigues Souza-Bastos[a,b,e], Marina Giacomin[c], Rafael Garrett Dolatto[d], Loana Mara Baika[d], Marco Tadeu Grassi[d], Antonio Ostrensky[a], Chris M. Wood[c]

[a] Integrated Group for Aquaculture and Environmental Studies, Dept. of Animal Science, Federal University of Paraná, Curitiba, P.R. 83035-050, Brazil
[b] Programa de Pós-Graduação em Zootecnia, Universidade Federal do Paraná, Curitiba, PR ZIP 80035-050, Brazil
[c] Department of Zoology, University of British Columbia, Vancouver, BC ZIP V6T 1Z4, Canada
[d] Grupo de Química Ambiental (GQA), Dept. of Chemistry, Federal University of Paraná, PO Box 19032, Curitiba, PR ZIP 81531-970, Brazil
[e] Institute of Technology for Development – Lactec, Curitiba, PR ZIP 81531-980, Brazil

ARTICLE INFO

Keywords:
BTEX
Environmental pollution
Ionic flux rates
Mg^{2+} excretion
Lactate
PAH
Oxygen uptake
Urea-N

ABSTRACT

Contamination of aquatic environments by petroleum and its products (e.g. gasoline) is a hazard for aquatic organisms as a result of the potential toxicity of monocyclic aromatic hydrocarbons (BTEX) and polycyclic aromatic hydrocarbons (PAH). Our goal was to evaluate the acute effects of the water-soluble fraction of gasoline (WSF_G) on nitrogen excretion, osmoregulation, and metabolism of goldfish *Carassius auratus*. We first chemically characterized the WSF_G and then tested its effects on these physiological aspects of *C. auratus*, in several different exposure scenarios (0, 0.25, 5, 10 and 25% of WSF_G). The WSF_G contained high concentrations BTEX (toluene 70% and benzene 17%) relative to PAH (< 1%), and low levels of several metals (Al, Fe, Zn, Sr). Routine O_2 uptake rate (MO_2) of goldfish was inhibited by exposure to 5% WSF_G, and during post-exposure recovery, MO_2 increased in a dose-dependent fashion. Ammonia excretion was not affected by exposure to WSF_G, but urea-N excretion increased progressively with the WSF_G concentration. The same pattern of dose/response was observed for net Mg^{2+} loss rates and steadily increasing plasma lactate concentrations. Loss rates of Na^+, Ca^{2+}, K^+ and Cl^-, and plasma concentrations of Mg^{2+} and urea-N were not significantly altered. We propose that acute exposure to WSF_G inhibits aerobic metabolism and activates anaerobic metabolism, breaking down ATP such that bound Mg^{2+} is liberated and the purine ring component is metabolized to urea-N, both of which are subsequently excreted.

1. Introduction

Petroleum and refined products are primarily composed of hydrocarbons (aliphatic and aromatic) (Albers, 2003; Wang et al., 2003) that can represent up to 97% in some light products (Dupuis and Ucan-Marin, 2015). Among the hydrocarbon components, aromatics are usually associated with toxicity to aquatic organisms (Fingas, 2011; Heath, 1995).These include the monocyclic aromatic (BTEX – benzene, toluene, ethylbenzene and *o*-, *m*-, and *p*-xylenes) and the 2 to 6-ringed US EPA priority polycyclic aromatic (PAH) hydrocarbons. The low molecular weight aromatic hydrocarbons are highly toxic to aquatic organisms (Barron et al., 1999). These include BTEX and 2-ringed PAH (naphthalene) components that are soluble in water, lipophilic and thus acutely toxic (Anderson, 1979; Barron et al., 1999; Dupuis and Ucan-Marin, 2015; Harper and Liccione, 1995). In fish, BTEX and PAH are known to cause biochemical, physiological, morphological and behavioral alterations (Nikinmaa, 2014; Peakall, 1994; van der Oost et al., 2003). Gasoline and its water-soluble fraction (WSF_G) contains higher concentrations of BTEX and 2-ringed PAH components when compared to other petroleum products (Dal Pont, 2018; Dupuis and Ucan-Marin, 2015; Rodrigues et al., 2010; Saeed and Al-Mutairi, 1999; Speight, 2015). These low molecular weight constituents, especially BTEX, are also highly volatile and generally are lost rapidly from the water following a spill (Dupuis and Ucan-Marin, 2015; Neff, 1988). Therefore,

* Corresponding author at: Integrated Group for Aquaculture and Environmental Studies, Dept. of Animal Science, Federal University of Paraná, Curitiba, P.R. 83035-050, Brazil.
E-mail addresses: giorgi.dalpont@ufpr.br (D.P. G.), luciana.bastos@lactec.org.br (L.R. Souza-Bastos), giacomin@zoology.ubc.ca (M. Giacomin), mtgrassi@quimica.ufpr.br (M.T. Grassi), ostrensky@ufpr.br (A. Ostrensky), woodcm@zoology.ubc.ca (C.M. Wood).

https://doi.org/10.1016/j.cbpc.2019.108590
Received 28 March 2019; Received in revised form 26 July 2019; Accepted 8 August 2019
Available online 09 August 2019

they probably contribute more to acute than chronic toxicity (Neff, 1988). In contrast, higher molecular weight constituents, such as PAH compounds with 3 or more benzene rings, are less soluble but are also less volatile and remain longer in the environment (Hollebone, 2017; Neff, 1988; Yang et al., 2017). Thus, many studies have associated 3- to 5-ringed PAH with chronic effects in fish (Adams et al., 2014; Bornstein et al., 2014; Esbaugh et al., 2016; Incardona et al., 2014; Incardona et al., 2011; Neff, 1988).

Toxic actions of aromatic petroleum hydrocarbons include narcotic effects (Correa and García, 1990) and morphological damage to the gills (Agamy, 2013; Hook et al., 2018; Nero et al., 2006; Pal et al., 2011; Simonato et al., 2008; Solangi and Overstreet, 1982; Stentiford et al., 2003; van der Oost et al., 2003; Winkaler et al., 2001), resulting in a decline of blood O_2 content (Alkindi et al., 1996). In theory, the resulting hypoxemia could lead to ATP breakdown, the release of Mg^{2+} ions that were bound to ATP, increased anaerobic metabolism, lactic acid production, and associated metabolic acidosis (Hochachka, 1980). Although a great number of studies have investigated the effects of petroleum hydrocarbons on various physiological processes in fish (Gagnon and Holdway, 1999; Horodesky et al., 2015; Incardona et al., 2004; Rodrigues et al., 2010; Simonato et al., 2008; Spies, 1987), there are only a few reports regarding their effects on the excretion of nitrogenous wastes, and the possible interaction with metabolic regulation and ionoregulatory mechanisms (Correa and García, 1990; Engelhardt et al., 1981; Pasparakis et al., 2016).

The metabolic pathways of fish produce two major nitrogenous wastes - ammonia, the larger fraction resulting largely from the oxidative deamination of amino acids, and urea, the smaller fraction resulting largely from the breakdown of purine nucleotides (such as those in ATP) in the process of uricolysis (Randall and Wright, 1987; Wood, 1993). Both ammonia and urea are excreted mainly through the gills (Forsters and Goldstein, 1969; Wood, 2001). The presence of Rhesus (Rh) glycoproteins enhances the diffusion of NH_3 across the branchial membranes, and this process appears to be intimately associated with branchial Na^+ uptake and acid-base regulation (Wright and Wood, 2009). Therefore, if petroleum hydrocarbons acutely alter the balance between aerobic and anaerobic metabolism, we hypothesized that effects on N-waste excretion (ammonia and urea-N) and ionoregulation (e.g. Na^+, Mg^{2+}) may occur, as well as increased lactate production.

Our goal was to evaluate the effects of acute exposure to WSF_G on metabolic and ionoregulatory parameters of the goldfish *Carassius auratus*. This temperate freshwater fish species is widely used in laboratory experimentation because of its well-known physiology and high capacity to withstand hypoxic conditions (van den Thillart and Kesbeke, 1978). We first chemically characterized the WSF_G and then tested the effects of different concentrations (0, 0.25, 5, 10 and 25% of WSF_G- *v/v*) on these physiological aspects of *C. auratus*, during exposure (6 h) or recovery periods (6 h of exposure followed by 1 h of recovery in clean water). In particular, we evaluated O_2 uptake, excretion rates of ammonia and urea-N, the net flux rates of major ions with the environment, as well as plasma concentrations of these ions, urea-N, and lactate.

2. Material and methods

2.1. Animal care

Goldfish specimens (N = 100; W = 3.49 ± 1.19 g) were purchased from Noah's Pet Shop, Vancouver, British Columbia, Canada. At the laboratory, fish were held in 200-L tanks filled with dechlorinated Vancouver tap water (pH = 7.23; $[Na^+]$ = 110 μmol L^{-1}, $[K^+]$ = 3.8 μmol L^{-1}, $[Ca^{2+}]$ = 171 μmol L^{-1}, $[Mg^{2+}]$ = 7.1 μmol L^{-1} and $[Cl^-]$ = 124 μmol L^{-1}) under flow-through conditions (dissolved O_2 ≥ 75.0% saturation; room temperature = 20.0 ± 1.0 °C; controlled photoperiod = 12 h light: 12 h dark) for two weeks prior to experimentation. Commercial flakes of dry food (45% crude protein) were offered once a day and suspended 24 h before starting and during experiments.

All experimental procedures were conducted in accordance with Canada Council for Animal Care guidelines and approved by the University of British Columbia Animal Care Committee (AUP# A14-0251).

2.2. Procedure for the extraction of WSF_G

Regular gasoline was obtained in a commercial gas station (Vancouver, Canada) and stored in a 2-L autoclaving glass bottle covered with aluminum foil. Prior to use (5 days), it was kept inside a sealed flammable cabinet to shield it from light.

The extraction of the WSF_G was conducted at room temperature (20.0 °C) according to the methodology described by Anderson et al. (1974). Briefly, one part of gasoline and nine parts of water were added to a Marriott glass bottle (1 L) and stirred on a magnetic plate. To standardise the procedure, the velocity of stirring was maintained to form a vortex corresponding to $^1/_3$ of the gasoline + water column height. After 22 h, the stirring was stopped, and the solution was allowed to rest for 30 min to permit the separation of the fuel and WSF_G phases. The obtained WSF_G was immediately used.

Samples of the WSF_G (100%) were collected for the determination of BTEX and PAH, metals (Ag, Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, P, Pb, Sr and Zn), nitrogen compounds (total ammonia and urea-N), and ions (Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}) content. Samples for PAH and BTEX were stored in 60 mL- amber vials that had been washed in an ultrasonic bath (three steps: tap, bi-distilled and Milli-Q® water, Millipore Corp., Burlington, MA, USA), dried (24 h at 105 °C) and calcinated (4 h at 400 °C). Samples were kept under refrigeration (4 °C) for later chromatographic analysis. For determination of metal concentration, water samples were stored in amber vials (60 mL) and preserved with 1% HNO_3 until analysis.

2.3. Experimental design

Thirty fish were transferred from the main acclimation tank to 260-mL individual glass containers for a 4-h settling period. This static system was continuously aerated and kept at room temperature (20 °C). After this settling period, 80% of the water volume was replaced with clean freshwater (control) or freshwater containing the four tested concentrations of WSF_G (0.25, 5, 10 and 25% v/v – N = 6 fish per treatment). The settling period (4 h), room temperature (20 °C), airflow supply (PO_2 ~ 75% saturation) and tested WSF_G concentrations were kept the same through all the experimental series described below.

2.4. Experimental series

2.4.1. Series 1 - measurement of N-waste and ion flux rates

The main goal of this series was to evaluate N-waste excretion and, also, the flux rates of ions (Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+}) during exposure to WSF_G. At the end of settling period, fish were exposed for 6 h to four concentrations of WSF_G mentioned above. Water samples (2 mL) were collected every hour during the experimental period for determination of ammonia and urea-N. Ten -mL samples were collected at the beginning and end of the 6-h period for ion determinations. Temperature (YSI® 55, Yellow Springs, OH, USA) and pH (Fisher Scientific® AB15, Toronto, ON, Canada) were measured immediately after collection. Then, the water samples were stored at −20 °C until analysis. At the end of the experiment the exposed fish were euthanized with an overdose of neutralized MS222 solution (Syndel Labs, Parksville, BC, Canada; 5 g L^{-1} neutralized to pH 7.8 with 5 M NaOH).

2.4.2. Series 2 - oxygen consumption rate during WSF_G exposure, with blood and muscle sampling

The second experimental series was designed to evaluate the routine

oxygen consumption, plasma levels of urea-N, Mg^{2+} and lactate, and muscle Mg^{2+} and lactate concentrations. Fish were exposed to the four WSF_G concentrations described above for 6 h. After exposure, 80% of the water volume in each container was replaced with WSF_G and air-saturated water. The initial oxygen partial pressure (PO_2) was measured using a handheld oxygen meter and probe (Accumet AP84A, Fisher Scientific, Toronto, ON, Canada), and the container lid was sealed with rubber latex (127 mm × 127 mm – Hygenic® dental dam, Performance Health, Akron, OH, USA) to avoid diffusion of O_2 from the atmospheric air to the water. The initial average (mean ± SEM) PO_2 was 154.4 ± 1.9 Torr. Fish were kept in this condition for 60 min, then a final PO_2 measurement was performed.

For blood and muscle sampling, fish were anaesthetized with neutralized MS222 solution (5 g L^{-1}) added directly in the experimental container. Blood samples, acquired by caudal puncture with heparinized syringes (lithium heparin, Sigma Aldrich®, St. Louis, MO, USA), were centrifuged for 6 min (6000 rpm) and the obtained plasma were immediately frozen (−80 °C) for later urea-N, Mg^{2+} and lactate determination. Fish were euthanized by medullar section and white muscle tissue was sampled and immediately frozen in liquid nitrogen, then transferred to −80 °C freezer until Mg^{2+} and lactate determinations.

2.4.3. Series 3 - oxygen consumption rate during recovery from WSF_G exposure, with blood and muscle sampling

The WSF_G exposure procedure used here followed the same protocol described above. However, for the oxygen consumption measurement, 100% of the water + WSF_G volume was replaced with clean air-saturated freshwater at the start of the recovery period. The initial PO_2 was measured, the container was sealed, and fish were kept in this condition for 60 min. Then, the final PO_2 measurement was performed. Blood and white muscle tissue were also sampled at the end of the experimental period in a similar manner as described above.

2.5. Analytical methods

2.5.1. Analytical standards and chromatographic conditions for PAH and BTEX determination

High purity analytical standards of BTEX (benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene) and the 16 priority PAH suggested by U.S. EPA (2001) [naphthalene (Naf); acenaphthylene (Aci); acenaphthene (Ace); fluorene (Flu); phenanthrene (Fen); anthracene (Ant); fluoranthene (Fla); pyrene (Pyr); benzo(*a*)anthracene (BaA); chrysene (Cris); benzo(*b*)fluoranthene (BbF); benzo(*k*)fluoranthene (BkF); benzo(*a*)pyrene (BaP); indene(1,2,3-*cd*)pyrene (Ind); dibenzo(*ah*)anthracene (Dib); benzo(*ghi*)perylene (Ben)] were used.

A mixed solution of five deuterated internal standards (IS) (AccuStandard®, New Haven, CT, USA) were also used: naphthalene (NafD8); acenaphthene (AceD10); phenanthrene (FenD10); chrysene (CrisD12) and perylene (PerD12) (Leite et al., 2008). A deuterated standard *p*-terphenyl D14 (AccuStandard®, New Haven, CT, USA) was also used as surrogate. Internal standards of PAH were used to minimize possible effects of solvent volatilization. The stock mixed standard solutions of PAH and BTEX were prepared in dichloromethane and methanol (J.T. Baker®, Phillipsburg, NJ, USA), respectively, at concentrations of 5 mg L^{-1}, and kept under controlled temperature (−18 °C) until use. Working solutions were prepared daily. All the solvents used were of HPLC grade and the water used for the preparation of standards was purified by reverse osmosis and Milli-Q system (Millipore, Simplicity UV, Molhseim, France).

For PAH determination, we used a gas chromatograph (Focus GC Polaris Q model, Thermo Fisher Scientific®, Waltham, MA, USA), equipped with a Thermo® AS3000 auto-sampler for liquid injection. The analyses of BTEX were carried out utilizing a different gas chromatograph (model 2010, Shimadzu®, Kyoto, Japan) coupled to a mass spectrometer (TQ8040) in a tandem system (GC–MS/MS), equipped with a Shimadzu® AOC-5000 autosampler for headspace analysis. Instrumental parameters used are detailed in the electronic supplementary material (ESM) (Table 1S).

For PAH extraction, samples of 100% WSF_G were submitted to a vortex-assisted liquid-liquid dispersive microextraction (VA-DLLME) procedure adapted from Braga et al. (2018). In this method, 1 mL of the extraction solution ($CHCl_3$ = 0.075 mL + C_3H_6O = 0.925 mL) is quickly added into 5 mL of the aqueous sample, or the PAH standard solution, in a glass conical tube, followed by agitation in a vortex for 1 min and centrifugation at 2800 rpm for 10 min. Thereafter, an aliquot of the highest density fraction (50 µL) was transferred to a chromatographic insert containing 10 µL of the deuterated HPA internal standards mixed solution at 600 µg L^{-1}, resulting in 60 µL of solution and a deuterated internal standard concentration of 100 µg L^{-1}. The final solution was injected into a GC–MS system.

The procedure used for BTEX extraction was based on the headspace (HS-GC–MS) methodology described by Fernandes et al. (2014). The 100% WSF_G samples (5 mL) were added into a 20-mL vial sealed with a PTFE/silicone septum and aluminum seal. The sealed vial was heated (60 °C) and stirred (300 rpm) for 5 min to promote the volatilization of the BTEX out of the aqueous phase by shifting the equilibrium to the gas phase, located in the headspace of the vial.

Analytical curves were built in triplicate and subjected to the same extraction procedure for PAH (0.25; 0.35; 0.50; 1.0; 2.0 and 4.0 µg L^{-1}) and BTEX (5; 10; 50; 100; 500 and 1000 µg L^{-1}) determination. The selected linear ranges showed R^2 values higher than 0.99. In addition, the highest relative standard deviation (RSD) value for concentration levels was 8%. The quantification limits (LOQ) were calculated based on the performance parameters of the analytical curves. The LOQ was estimated to be 10 times the ratio of the standard deviation of the intercepts of the analytical curves divided by the mean slope.

Finally, the precision and accuracy of VA-DLLME extraction method was determined by a recovery test by spiking three known concentrations of PAH (0.3, 1.2, and 2.5 µg L^{-1}) and BTEX (8, 120, and 450 µg L^{-1}) compounds into a water sample from the same source used in the experimental procedure. The accuracy values determined for PAH concentrations ranged from 62 to 122% with maximum RSD of 18%. In addition, the extractions were individually monitored by surrogate (*p*-Terphenyl D14) spiking in the standards and samples at a concentration of 2 µg L^{-1}. An average accuracy of 83% with a precision of 8% (n = 3) was recorded. For BTEX, the accuracy ranged from 60 to 110% with RSD not > 9%. Blank tests using controls were investigated and no analytical signals comparable to the PAH and BTEX standards were found.

2.5.2. Metal analyses

Metal concentrations in the 100% WSF_G were measured through inductively coupled plasma optical emission spectrometry (ICP-OES) using a Thermo Scientific® (ICAP 6500) spectrometer operating with axial vision. The instrumental parameters are shown in the ESM (Table 2S). Argon with a purity of 99.996% from White Martins-Praxair (São Paulo, Brazil) was used.

Using the selected conditions, analytical curves were prepared from multielement standards solutions of Ag, Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn (Merck) and a stock reference solution of P (Specsol®) in 1% (m/v) HNO_3. Concentration ranged from 0.010 to 2.0 mg L^{-1}. The R^2 were > 0.99 for all elements. The limits of detection (LOD) and quantification were respectively 3 times and 10 times the standard deviation of 7 measurements of the blank divided by the analytical curve slope. The LOD and LOQ values for each metal are presented in Table S3. A Quality Control Standard Solution (UltraScientific, Kingstown, USA) was analyzed, after construction of the analytical curve. The accuracy and precision of ICP OES analysis were evaluated with 7 replicates of a sample enriched with all the analytes at the concentration of 10.0 µg L^{-1}. The recovery values varied from 90% to 108%, demonstrating good accuracy. The RSD values obtained were

below 10%, indicating adequate precision (Baika et al., 2016; Sotiles et al., 2019).

2.5.3. Total ammonia and urea-N analyses

Ammonia content in water were determined colorimetrically (read at 595 nm) according to the indophenol methodology described by Verdouw et al. (1978). Determination of urea-N in water and plasma was based on the color reaction (read at 525 nm) with diacetyl monoxime and thiosemicarbazide in the presence of acid solution (sulphuric acid, phosphoric acid and ferric chloride) under high temperature (Rahmatullah and Boyde, 1980). Both ammonia and urea-N assays were read on a SpectraMax 340_{PC} plate reader (Molecular Devices®, San Jose, CA, USA).

2.5.4. Ion analyses

Cl^- concentrations in water were colorimetrically determined (480 nm) using the methodology described by Zall et al. (1956).

Na^+, K^+, Mg^{2+} and Ca^{2+} in water were determined by atomic absorption spectrophotometry (AAS) using a Varian® AA240FS instrument (Varian Medical Systems, Palo Alto, CA, USA) operated in flame mode with acetylene as the purging gas. Plasma samples were diluted (200×) with 1% lanthanum chloride ($LaCl_3$) solution prior to AAS analysis for Mg^{2+}. Before Mg^{2+} determination in muscle, wet tissue was weighed and digested in 1 mL of nitric acid (at room temperature – 20 °C) for 24 h. Then, digested tissue samples were diluted (200×) in 1% $LaCl_3$ solution for AAS analysis. Linear absorbance-concentration curves were built for each ion using a set of six standard solutions (plus blank sample) and considered suitable if the R^2 exceeded 0.995. Reading one of the commercially manufactured standard solutions (Fisher Scientific®, Toronto, Canada) between every 15 experimental samples insured quality certification. The operation specifications and detection limits of the AAS instrument are described in the ESM (Table 4S).

2.5.5. Lactate analysis

The concentrations of lactate in plasma and white muscle were determined using a modified version of the protocol detailed by Healy and Schulte (2012). Plasma samples were diluted (1:1 ratio) with 3% PCA solution and white muscle were homogenized for 20 s in 3% perchloric acid (~ 0.8 mL of acid per 20 mg of tissue) using an ultrasonic homogenizer (Fisher Scientific®, Toronto, Canada), followed by centrifugation (10,000 *g*) for 8 min at 4 °C. The supernatants (10 µL) were added to a multi-well plate containing 125 µL of assay buffer [2 mL of glycine buffer (glycine 0.6 M and hydrazine sulphate 0.5 M – pH 9.4), 4.6 mL of water (Milli-Q®), 10 mg of βNAD^+ (Sigma Aldrich®) and 15 U mL of LDH (Sigma Aldrich®)] and incubated at 37 °C for 60 min. Absorbance was determined using a plate reader (SpectraMax 340_{PC}) at 340 nm.

2.6. Calculations

The net flux rates of ammonia-N (J_{amm} [µmol N kg h^{-1}]) and urea-N ($J_{urea\text{-}N}$ [µmol N- kg h^{-1}]) were calculated according to the Eqs. (1) and (2).

$$J_{amm} = \frac{(amm_{initial} - amm_{final}).\ V}{W.\ T} \quad (1)$$

$$J_{urea-N} = \frac{(urea_{initial} - urea_{final}).\ V}{W.\ T} \quad (2)$$

where $amm_{initial}$ (1) and $urea_{initial}$ (2) are the initial water ammonia (µmol L^{-1}) and urea-N (µmol urea-N L^{-1}; note two N's per urea molecule) concentrations; amm_{final} (1) and $urea_{final}$ (2) are the final water ammonia (µmol L^{-1}) and urea-N (µmol urea-N L^{-1}) concentrations; V is the experimental water volume (L); W is the weight of the animal (kg); and T is the duration of the flux period (h).

The net flux rates (J_{net} in µmol kg h^{-1}) of individual ions (Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}) were also assessed in an analogous fashion by measuring their gain or loss from the water to the fish, and were calculated as:

$$J_{net} = \frac{([ion]_{initial} - [ion]_{final}).\ V}{W.\ T} \quad (3)$$

where $[ion]_{initial}$ and $[ion]_{final}$ were, respectively, the initial and final ion concentrations in the experimental solution during the flux period. Note that for all these flux rates positive value indicates net uptake by the fish, and a negative value net loss.

The concentrations of O_2 (µmol O_2 L^{-1}) in the water were obtained by converting PO_2 values using the solubility constants described by Boutilier et al. (1984). Oxygen consumption rate (MO_2 [µmol O_2 kg h^{-1}]) were calculated using the Eq. (3) presented below:

$$MO_2 = \frac{(O2_{initial} - O2_{final}).\ V}{W.\ T} \quad (4)$$

The $O_{2,initial}$ and $O_{2,final}$ represent the oxygen concentrations in the water (µmol L^{-1}) at the start and end of the experiment, respectively, and the other variables (V, W and T) are as described for above equations.

2.7. Statistical analysis

Normality and homogeneity of the data were evaluated through Shapiro-Wilks and Bartlett's tests, respectively. All data were in accordance with the premises of parametric tests. Thus, one-way ANOVA analysis of variance, followed by Tukey's test, was used to determinate significant differences among treatments for all obtained data ($p < 0.05$). Data were analyzed using SigmaStat® 3.5 and graphically plotted using SigmaPlot® 11.0 (both by Systat Software Inc., San Jose, CA, USA) and presented as mean ± SEM (N).

3. Results

3.1. Characterization of WSF_G

The 100% WSF_G used in all experiments contained high concentrations of monocyclic aromatic hydrocarbon (BTEX) when compared to polycyclic aromatic hydrocarbons (PAH) compounds. Toluene (70%) and benzene (17%) were the most abundant BTEX compounds. Concentrations of naphthalene, acenaphthylene, and phenanthrene were below the quantification limit of the method (< 0.5 µg L^{-1}) and anthracene was not detected (Table 1). Aluminum, iron, strontium and zinc concentrations were detected in the 100% WSF_G (Table 2). Concentrations of Na^+ (101 µmol), Cl^- (124 µmol), K^+ (4.22 µmol), Mg^{2+} (6.72 µmol), and Ca^{2+} (168 µmol) in the WSF_G were similar to the concentrations of the ions measured in the water used for the experiments (described above in Section 2.1). Ammonia concentrations were very low (2.57 µmol) and urea was not detected on the WSF_G.

3.2. Series 1 - flux rates of nitrogenous wastes and ions in response to WSF_G exposure

The total ammonia net flux rate was not affected by the exposure to WSF_G (Fig. 1A). On the other hand, the excretion rate of urea-N, which was about 10% of the ammonia flux rate under control conditions, significantly increased along with the WSF_G concentration (Fig. 1B). When compared to the control group, the excretion of urea-N increased 1.31-fold at 0.25% and 1.92-fold at 25% of WSF_G. The same significant pattern of dose/response was observed for the Mg^{2+} net flux rate (Fig. 2), reaching a 3.5-fold increase at 25% of WSF_G when compared to the control group. The Na^+, Cl^-, K^+ and Ca^{+2} flux rates did not present significant alterations (Table 3).

Table 1
Concentrations ($\mu g\,L^{-1}$) of monocyclic aromatic (BTEX) and polycyclic aromatic hydrocarbons (PAH) in the 100% water-soluble fraction of gasoline (WSF_G) used during the exposure trials with goldfish *Carassius auratus*.

	100% WSF_G
BTEX ($\mu g\,L^{-1}$)	
Benzene	1336.1
Toluene	5562.6
Ethylbenzene	227.6
m,*p*-Xylene	429.9
o-Xylene	407.7
Σ BTEX	*7963.8*
PAH ($\mu g\,L^{-1}$)	
Naphthalene	< 0.5
Acenaphthylene	< 0.5
Acenaphthene	1.1
Fluorene	0.9
Phenanthrene	< 0.5
Anthracene	nd
Fluoranthene	3.1
Pyrene	3.0
Benz[*a*]anthracene	5.4
Chrysene	7.1
Benzo[*b*]fluoranthene	8.0
Benzo[*k*]fluoranthene	8.7
Benzo[*a*]pyrene	6.0
Indeno[1,2,3-*cd*]pyrene	7.9
Dibenz[*ah*]anthracene	7.9
Benzo[*ghi*]perylene	8.7
Σ PAH	*67.8*
Σ	*8031.5*

Limit of Quantification = BTEX < $5.0\,\mu g\,L^{-1}$ and PAH < $0.5\,\mu g\,L^{-1}$. BTEX = Monocyclic aromatic hydrocarbons.
PAH = Polycyclic aromatic hydrocarbons.
The aim is to differentiate the name of the substance from the letter indicating the position of the chemical bond.

Table 2
Metal concentrations ($\mu g\,L^{-1}$) of the 100% water-soluble fraction of gasoline (WSF_G) used during the acute exposure trials with goldfish *Carassius auratus*. Concentrations of Ag, Ba, Cd, Co, Cr, Cu, Mn, Ni, P, and Pb were below the limits of quantification (LOQ) of the analysis.

Metal	100% WSF_G ($\mu g\,L^{-1}$)
Ag	< 2.0
Al	19.32
Ba	< 1.0
Cd	< 1.0
Co	< 1.0
Cr	< 1.0
Cu	< 10.0
Fe	23.01
Mn	< 0.5
Ni	< 5.0
P	< 8.0
Pb	< 5.0
Sr	18.66
Zn	20.54

3.3. Series 2 –oxygen consumption rate and other physiological responses to WSF_G exposure

In contrast to the increases observed in the flux rates of urea-N and Mg^{2+}, plasma concentrations of urea-N and Mg^{2+} were not significantly affected by the WSF_G exposure (Table 4). However, the concentrations of Mg^{2+} in white muscle increased by about 30% at 10% and 25% of WSF_G (Table 4).

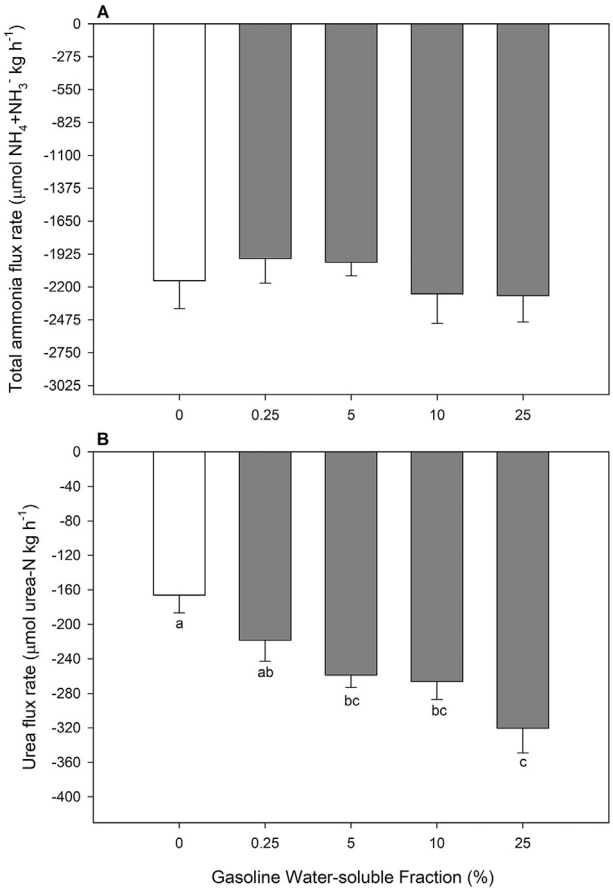

Fig. 1. Mean ± SEM (N = 6) values of (A) total ammonia and (B) urea-N flux rates of goldfish *Carassius auratus* after 6-h exposure to the water-soluble fraction of gasoline (WSF_G). Letters indicate differences among treatments (One-way ANOVA, Tukey's test, $p < 0.05$). When differences were not detected ($p > 0.05$) letters were omitted.

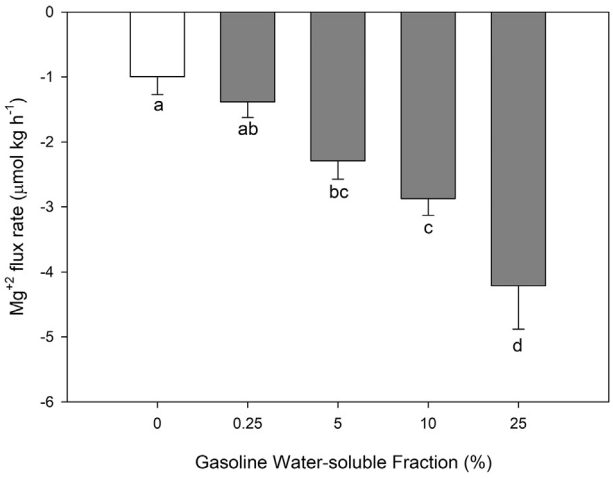

Fig. 2. Mean ± SEM (N = 6) values of Mg^{2+} flux rate of goldfish *Carassius auratus* after 6-h exposure to the water-soluble fraction of gasoline (WSF_G). Letters indicate differences among treatments (One-way ANOVA, Tukey's test, $p < 0.05$). When differences were not detected ($p > 0.05$) letters were omitted.

The routine oxygen uptake rate was significantly influenced by the WSF_G (Fig. 3). Compared to the control group, the mean MO_2 was 25% lower at 0.25% of WSF_G and reached its lowest value (a significant 31% reduction) at 5% of WSF_G. At 10% and 25% of WSF_G, the rate tended to

Table 3
Mean ± SEM (N = 6) values of Na^+, Cl^-, K^+ and Ca^{2+} net flux rates (μmol kg h^{-1}) of goldfish *Carassius auratus* during 6-h exposure to different concentrations of the water-soluble fraction of gasoline (WSF_G). There were no significant differences among treatments for each ion (One-way ANOVA, $p > 0.05$).

% of WSF_G	Flux rate (μmol kg h^{-1})			
	Na^+	Cl^-	K^+	Ca^{2+}
0	27.2 ± 9.2	14.9 ± 40.0	−7.6 ± 2.2	−3.5 ± 2.0
0.25	42.6 ± 7.0	91.8 ± 13.9	−11.0 ± 1.1	−3.3 ± 2.3
5	19.1 ± 25.5	43.7 ± 77.1	−10.5 ± 3.3	−1.1 ± 2.4
10	9.0 ± 20.1	96.4 ± 36.5	−8.1 ± 1.2	3.6 ± 1.5
25	−19.2 ± 22.9	123.2 ± 45.9	−9.8 ± 0.9	−4.2 ± 2.7

Table 4
Mean ± SEM (N = 6) values of plasma concentrations of urea-N (μmol urea-N L^{-1}) and Mg^{2+} (μmol L^{-1}) and concentration of Mg^{2+} in the white muscle (μmol g wet $tissue^{-1}$) of goldfish *Carassius auratus* after 6-h exposure to different concentrations of the water-soluble fraction of gasoline (WSF_G) (Series 2) and 6-h exposure to WSF_G followed by 1-h recovery in clean freshwater (Series 3). Letters indicate differences among treatments (One-way ANOVA, Tukey's test, $p < 0.05$). When differences were not detected ($p > 0.05$) letters were omitted.

Experimental series	% of WSF_G	Plasma		White muscle
		Urea-N (μmol-N L^{-1})	Mg^{2+} (μmol L^{-1})	Mg^{2+} (μmol g of wet $tissue^{-1}$)
Series 2	0	510 ± 58	707 ± 85	22.6 ± 1.9^{a}
	0.25	572 ± 94	632 ± 86	26.2 ± 1.4^{ab}
	5	673 ± 139	770 ± 87	25.2 ± 1.3^{ab}
	10	656 ± 90	840 ± 59	28.5 ± 1.0^{b}
	25	640 ± 102	950 ± 87	28.8 ± 0.6^{b}
Series 3	0	554 ± 131	813 ± 67	23.4 ± 1.1^{a}
	0.25	806 ± 179	729 ± 93	23.7 ± 1.2^{ab}
	5	932 ± 175	734 ± 55	26.4 ± 3.3^{ab}
	10	752 ± 134	756 ± 85	29.6 ± 2.6^{ab}
	25	683 ± 163	676 ± 62	30.0 ± 0.6^{b}

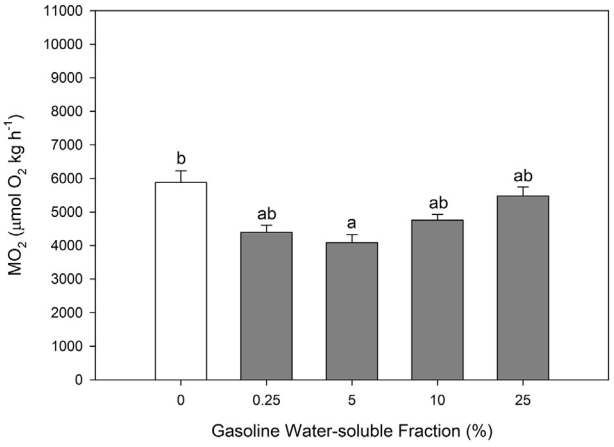

Fig. 3. Mean ± SEM (N = 6) values of oxygen uptake by the gills ($\dot{M}O_2$) of goldfish *Carassius auratus* after 6-h exposure to different concentrations of the water-soluble fraction of gasoline (WSF_G). The 1 h respirometry measurement was performed using the same WSF_G concentrations used during the exposure trial. Letters indicate significant difference among treatments (One-way ANOVA, Tukey's test, $p < 0.05$). When differences were not detected ($p > 0.05$) letters were omitted.

increase back towards the control value. Plasma lactate concentration exhibited a marked dose-response pattern of increase, which became significant at 5% of WSF_G. Thereafter lactate concentration increased further and at 25% of WSF_G reached values six times higher than in the control group (Fig. 4A). However, white-muscle lactate concentrations, on the other hand, did not present a significant variation (Fig. 4B).

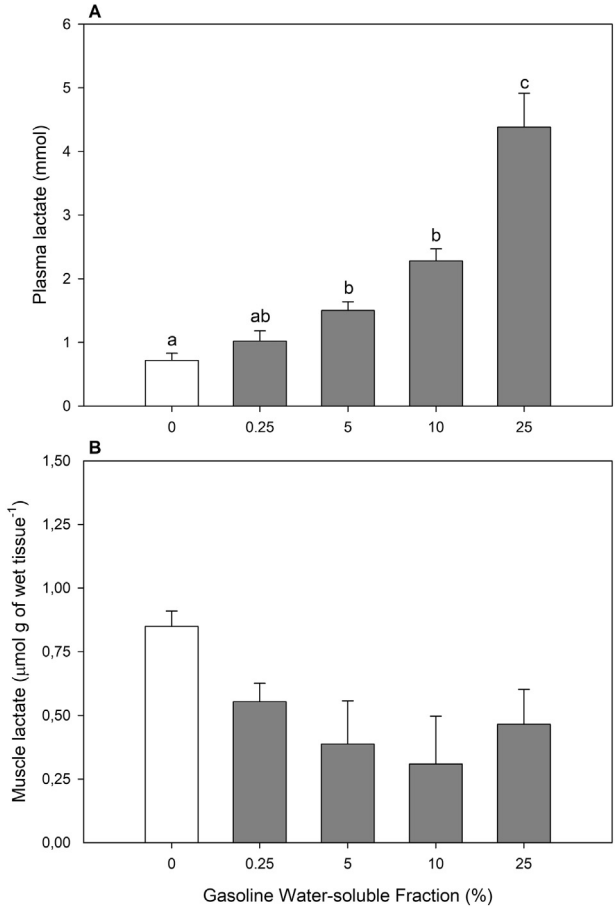

Fig. 4. Mean ± SEM (N = 6) values of lactate content in (A) plasma and (B) white muscle of goldfish *Carassius auratus* after 6-h exposure to different concentrations of the water-soluble fraction of gasoline (WSF_G) and 1 h respirometry measurement in the same WSF_G concentrations used during the exposure trial. Letters indicate differences among treatments (One-way ANOVA, Tukey's test, $p < 0.05$). When differences were not detected ($p > 0.05$) letters were omitted.

3.4. Series 3- oxygen consumption rate and other physiological responses during recovery from WSF_G exposure

As observed in experimental Series 2 (see above), the plasma concentrations of urea-N and Mg^{2+} were not significantly affected by the exposure to WSF_G (6 h) followed by 1 h recovery period (Table 4). The concentrations of Mg^{2+} in white muscle of fish exposed to 25% WSF_G increased when compared to the control group (Table 4).

Goldfish exhibited a different MO_2 response pattern when the measurement was performed during recovery (1 h) in clean freshwater (Fig. 5). In this scenario, the MO_2 progressively increased with the experimental concentrations of the WSF_G to which the fish had been exposed during the preceding 6 h. After exposure to 0.25% of WSF_G, the MO_2 measured in clean freshwater was 32% higher than the control group and the increase reached 92% in the group previously exposed to 25% of WSF_G. Under this experimental condition, however, both plasma and muscle lactate concentrations were not affected by WSF_G exposure, presenting values similar to the control group (Table 5).

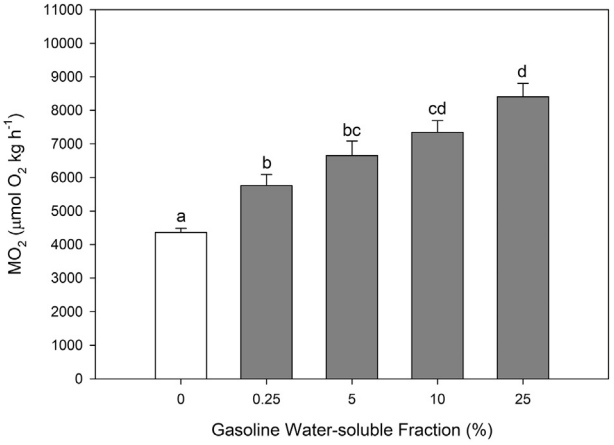

Fig. 5. Mean ± SEM (N = 6) values of oxygen uptake by the gills (MO_2) of goldfish *Carassius auratus* during recovery from 6-h exposure to different concentrations of the water-soluble fraction of gasoline (WSF_G). The 1-h respirometry measurement was performed in clean-fresh water. Letters indicate differences among treatments (One-way ANOVA, Tukey's test, $p < 0.05$). When differences were not detected ($p > 0.05$) letters were omitted.

Table 5
Mean ± SEM (N = 6) values of lactate concentrations in plasma (mmol L^{-1}) and white muscle (µmol g of wet tissue $^{-1}$) of goldfish *Carassius auratus* after 6-h exposure to different concentrations of the water-soluble fraction of gasoline (WSF_G), followed by 1-h recovery in clean freshwater. No differences were detected among the tested WSF_G concentrations (One-way ANOVA, $p > 0.05$).

% of WSF_G	Plasma lactate (mmol L^{-1})	White muscle lactate (µmol g of wet tissue $^{-1}$)
0	0.78 ± 0.17	0.76 ± 0.05
0.25	0.75 ± 0.15	0.61 ± 0.06
5	0.60 ± 0.22	0.61 ± 0.05
10	0.61 ± 0.18	0.65 ± 0.06
25	0.73 ± 0.31	0.73 ± 0.07

4. Discussion

4.1. The toxicity of WSF_G

In this study, the individual petroleum hydrocarbon concentration ranges, detected in the 100% WSF_G (Table 1), are in accordance with the international literature and can be considered as an effective source of toxic aromatic hydrocarbons (Bettim et al., 2016; Galvan et al., 2016; Pilatti et al., 2016; Rodrigues et al., 2010). The most abundant petroleum hydrocarbons were toluene (70%) and benzene (17%). These individual monocyclic aromatic hydrocarbons are responsible for a number of toxicological effects on fish including physiological (Brocksen and Bailey, 1973; Dange and Masurekar, 1981; Korn et al., 1976; Rice et al., 1977; Roubal et al., 1977; Simonato et al., 2008; Thomas and Rice, 1979), morphological (Akinsanya et al., 2019; Caldwell, 1997; Simonato et al., 2008) and, reproductive (Korn et al., 1976; Struhaaker, 1977) alterations. The toxicity of petroleum hydrocarbons to fish increases as the individual molecular weight decreases (Albers, 2003; McAuliffe, 1966; Neff, 1988). BTEX compounds are the lowest-weight molecules of aromatic hydrocarbons (Fayemiwo et al., 2017; Fingas, 2011) and are considered the most water-soluble components of gasoline (Dupuis and Ucan-Marin, 2015; Pruden et al., 2003). Due to this chemical characteristic and the lipophilicity of BTEX components (Eisler, 1987; Neff, 1988; Neff, 1979; Poulsen et al., 1992), gasoline is considered the most toxic petroleum refined product to fish (Barron et al., 1999; Harper and Liccione, 1995; Poulsen et al., 1992; Saeed and Al-Mutairi, 1999; Speight, 2015) as its monocyclic hydrocarbons are absorbed across fish gill membranes directly into the bloodstream (Meyerhoff, 1975). Rodrigues et al. (2010) acutely exposed larvae of the marine species *O. argentinensis* to the water-soluble fraction of gasoline, diesel and crude oil under similar experimental conditions. The exposure to WSF_G for 96 h resulted in a higher toxicity (LC_{50} = 5.48%) when compared to the exposure to WSF of diesel (LC_{50} = 13.46%) and crude oil (LC_{50} = 70.68%). The same pattern of toxicity (gasoline > diesel > petroleum) was observed by Dal Pont (2018) for juveniles of the freshwater species *Astyanax altiparanae* during a 96 h exposure. In this work, the LC_{50}-96 h calculated for the WSF_G, diesel, and crude oil components were: 2.65, 16.22 and 23.59%, respectively. In both studies, the concentration of BTEX, which includes toluene and benzene, in the WSF_G was higher than its concentration in the WSF of diesel and crude oil. In the present study with goldfish, the concentrations of PAH components in the 100% WSF_G corresponded only to 0.09% of the total sum of aromatic hydrocarbons (BTEX + PAH). PAH with four or more rings totaled 0.08%. This specific group of aromatic hydrocarbons presents lower water solubility and are rarely account for acute toxicity (Neff, 1988). Therefore, BTEX components were probably the main chemical substances in the tested WSF_G that induced the observed acute toxic effects to goldfish in the present study.

Although Al, Fe and Zn were detected in the 100% WSF_G, they are only considered harmful to fish in higher concentrations (Wood, 2001). The toxicity of Sr is also known to be very low for the aquatic biota (McPherson et al., 2014). Thus, we believe that metals detected in the WSF_G were unlikely to impose a significant toxicological hazard.

4.2. Responses in N-waste excretion

WSF_G shows high toxicity to marine (Rodrigues et al., 2010; Saeed and Al-Mutairi, 1999) and freshwater (Alves et al., 2017; Bettim et al., 2016; Galvan et al., 2016; Simonato et al., 2011) fish. Despite this, exposure for 6 h to this product did not alter the ammonia flux rate of *C. auratus*. This result refutes in part our hypothesis about N-waste excretion (but see below) and diverges from the result found by Correa and García (1990) with juveniles of the marine *Mugil curema* exposed to benzene (0 to 10 mL L^{-1}), where the authors detected a 40% reduction in ammonia excretion during 24 h exposure to the higher tested benzene concentration. To date, the Correa and García (1990) study has been the only one that has evaluated the effects of individual petroleum hydrocarbons on the excretion of nitrogenous products in fish, but Pasparakis et al. (2016) showed elevations in ammonia excretion in some treatments of larval marine *Coryphana hippurus* with crude oil. These differences could be attributed to the differences in the time exposure, chemical characteristics of the pollutant (individual versus complex mixtures of petroleum hydrocarbons) and to the resilient characteristic of *C. auratus* under conditions of environmental challenge (Van Waarde and De Wilde-Van Berge Henegouwen, 1982). In the same way, under anoxic conditions, the closely related teleost *Carassius carassius* L. also did not alter its ammonia flux rate (Johnston and Bernard, 1983). In both cases, the lack of ammonia alterations may be related to changes in N-waste metabolism (Campbell, 1991; Chew et al., 2005; Randall, 2011); Some fish species present the ability to reduce the ammonia toxicity through the synthesis of less toxic N-wastes when exposed to situations of environmental challenge (Randall, 2011; Souza-Bastos et al., 2014). In the present study, goldfish increased urea-N excretion in response to WSF_G exposure, thus supporting our overall hypothesis that N-waste excretion would be disturbed. Similarly, urea-N excretion was preferentially elevated relative to ammonia excretion in larval *Coryphana hippurus* exposed to crude oil (Pasparakis et al., 2016).

Below we discuss the evidence for O_2 limitation caused by WSF_G exposure. The similar dose-response relationships presented by urea-N and Mg^{2+} fluxes strongly suggests that the response to acute WSF_G could be linked to the activation of anaerobic metabolism, through the breakdown of ATP and other adenylates in the muscle (Wood, 1993). ATP in tissues is bound to Mg^{2+} and when O_2 becomes limiting, ATP

breakdown leads to the production of IMP [releasing 2 mol of Mg^{2+} (Bender, 2012)], from which its purine ring components are first converted to uric acid, and then to urea in the process of uricolysis (Campbell, 1991; Randall, 2011; Wood, 1993). This anaerobic pathway is energy-expensive, as the synthesis of 1 mol of urea requires 5 mol of ATP (Hochachka, 1980; Randall, 2011). In a chronic high environmental ammonia exposure, for example, the ammonia fluxes of goldfish remained unchanged while urea-N excretion significantly increased (Fromm, 1970). The absence of significant differences in plasma urea-N (Table 4), despite significant elevations in urea-N excretion rates (Fig. 2), suggests that this breakdown product from ATP degradation was efficiently cleared from the bloodstream through branchial urea transporters (McDonald et al., 2012). Differently from urea, Mg^{2+} loss through the gills is usually low and excretion occurs almost exclusively by renal pathways (Bijvelds et al., 1998). Our results with goldfish show that while more Mg^{2+} is excreted to the water, plasma concentrations remain unchanged, yet muscle concentrations increase during exposure to WSF_G (experimental series 2) and recovery in clean freshwater (experimental series 3). The rise in white muscle Mg^{2+} concentrations at a time of increased net Mg^{2+} losses to the external water can only be explained by changes in the internal distribution of Mg^{2+} associated with exposure to WSF_G. This topic clearly deserves further investigation. In general, the mechanisms of Mg^{2+} transport/excretion are poorly understood (Flatman, 1991). The unchanged plasma Mg^{2+} observed in the present work with goldfish could be a result of an increase of red cell Mg^{2+} complexation with nucleoside triphosphates (NTP) to improve O_2 affinity to hemoglobin during challenging metabolic hypoxia (Jensen et al., 1993). In this hypothetical scenario, the extra Mg^{2+} would not be contained in the plasma portion of the blood and, thus, would not have been detected in our analyses.

4.3. Responses in aerobic and anaerobic metabolism

Exposure to WSF_G created an apparent state of metabolic hypoxia, as evidenced by the reduction of MO_2 and dose-dependent increases in plasma lactate during exposure, as well as the dose-dependent stimulation of MO_2 during recovery. In parallel, plasma lactate concentrations returned to basal levels. These results suggest that the goldfish were paying off an O_2 debt accumulated during the 6-h exposure period. After stress, hypoxia, or intensive exercise, fish focus on restoring their original metabolic state through increases of MO_2 which are associated with lactate oxidation and glycogen resynthesis, and replenishment of high energy phosphate stores (Wood, 1991). In this scenario, it would be plausible to assume that a higher MO_2 would allow fish to use accumulated lactate as an aerobic substrate. This approach was suggested by Ferreira et al. (2018) to explain the ability of *Brycon amazonicus* to present a better swimming performance when compared to *Colossoma macropomum*.

Water pollutants, such as petroleum hydrocarbons, are known to cause several acute histological alterations in the gill tissue (Agamy, 2013; Dal Pont, 2012, 2018; Simonato et al., 2008) which, consequently, could reduce the diffusion of oxygen (Heath, 1995). Also, under stressful conditions, the action of catecholamines and cortisol (Wendelaar Bonga, 1997) can modify the gill membrane permeability and affect the capacity for gas exchange through the gill (Carneiro et al., 2005). While it is possible that these effects occurred in the present exposures to WSF_G, it seems more likely that WSF_G acted on the regulatory switch from aerobic versus anaerobic metabolism, rather than on gill O_2 permeability. This is supported by the fish's ability to rapidly elevate MO_2 again during post-exposure recovery in a dose-dependent fashion. Furthermore in other petroleum exposure studies, MO_2 has generally increased rather than decreased during the exposures [e.g. Thomas and Rice, 1975; Correa and García, 1990; Pasparakis et al., 2016]. The fast-triggered anaerobic mechanism can be one of the reasons why goldfish successfully survived the toxic environmental challenge imposed by the WSF_G.

C. auratus is well known for its ability to cope with hypoxic and anoxic environments due to its capacity to efficiently extract oxygen from water and its unusual anaerobic metabolism (Regan et al., 2017; van den Thillart et al., 1980). The latter has been extensively studied (Mourik et al., 1982; Regan et al., 2017; van den Thillart and Kesbeke, 1978; van den Thillart et al., 1980; Van den Thillart et al., 1983; Van Waarde and De Wilde-Van Berge Henegouwen, 1982; Walker and Johansen, 1977). Under anoxic or hypoxic conditions, goldfish increase the activity of glucose-6-phosphatase in the hepatic tissue to mobilize glucose from glycogen and meet their energy needs (Walker and Johansen, 1977). The major anaerobic end-product produced during the anoxic utilization of glycogen is ethanol rather than lactate, which is produced to a much lesser extent. Lactate is retained in the body while ethanol (~85%) is excreted to the water (Johnston and Bernard, 1983). Differently from the hepatic tissue, white muscle glycogen was not degraded to glucose in goldfish submitted to anoxic conditions (van den Thillart et al., 1980). It appears that glycolytic flux in white muscle is rather low during anoxia. Either this, or an ethanol production route in muscle may explain why lactate did not accumulate in this tissue during either WSF_G exposure or post-exposure recovery. Furthermore, the significant rise of muscular Mg^{2+} concentrations during recovery could be associated with the restoration of muscle ATP stores.

5. Conclusions and future directions

Our results demonstrated that the acute exposure of goldfish to WSF_G affected the nitrogen-waste excretion due to alterations of anaerobic metabolism. Although WSF_G did not alter ammonia excretion, exposure for 6 h was sufficient to trigger metabolic changes and increased urea-N excretion. These changes appear to be related to an impaired ability of *C. auratus* to uptake O_2 from water, resulting in the increase of Mg^{2+} and urea-N excretion, and plasma lactate accumulation. After removal of the contaminant, goldfish can immediately (1 h) recover, exhibiting a compensatory stimulation of MO_2 and lowering of lactate concentrations to basal levels. In the future, it will be of great interest to investigate the possible role of ethanol in the metabolic responses of goldfish during contamination by petroleum hydrocarbons, and to identify if this metabolic pathway is responsible for the capacity of goldfish to endure this toxic challenge.

Transparency document

The Transparency document associated with this article can be found, in online version.

Declaration of competing interest

The authors declare no conflict of interests in this submission either financial or otherwise.

Acknowledgments

Funding was provided by Petróleo do Brasil S.A. (Petrobras) to AO (#2012/00201-3), and by a NSERC Discovery grant to CMW (RGPIN #03843-2017). We thank the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for providing a PhD (#1391555) and a *Sanduíche* (PSDE) scholarship (#88881.134304/2016-01) to GDP and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for awarding AO with a research fellowship grant (#304633/2017-8).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2019.108590.

References

Adams, J., Sweezey, M., Hodson, P.V., 2014. Oil and oil dispersant do not cause synergistic toxicity to fish embryos. Environ. Toxicol. Chem. 33, 107–114.

Agamy, E., 2013. Impact of laboratory exposure to light Arabian crude oil, dispersed oil and dispersant on the gills of the juvenile brown spotted grouper (*Epinephelus chlorostigma*): a histopathological study. Mar. Environ. Res. 86, 46–55.

Akinsanya, B., Isibor, P.O., Kuton, M.P., Saliu, J.K., Dada, E.O., 2019. Aspidogastrea africanus infections, comparative assessment of BTEX and heavy metals bioaccumulation, and histopathological alterations as biomarker response in *Chrysichthys nigrodigitatus* (Lacépède, 1803) of Lekki Lagoon, Nigeria. Sci. Afr. 3, e00060.

Albers, P.H., 2003. Petroleum and individual polycyclic aromatic hydrocarbons. In: Hoffman, D.J., Rattner, B.A., Burton, G.A., Cairns, J. (Eds.), Handbook of Ecotoxicology, 2nd ed. Lewis Publishers, New York, pp. 1315.

Alkindi, A.Y.A., Brown, J.A., Waring, C.P., Collins, J.E., 1996. Endocrine, osmoregulatory, respiratory and haematological parameters in flounder exposed to the water soluble fraction of crude oil. J. Fish Biol. 49, 1291–1305.

Alves, R.N., Mariz, C.F., Paulo, D.V.D., Carvalho, P.S.M., 2017. Toxicity of effluents from gasoline stations oil-water separators to early life stages of zebrafish *Danio rerio*. Chemosphere 178, 224–230.

Anderson, J.W., 1979. An assessment of knowledge concerning the fate and effects of petroleum hydrocarbons in the marine environment. In: Vernberg, W.B., Calabresse, A., Thurberg, F.P., Vernberg, F.J. (Eds.), Marine Pollution: Functional Responces. Academic Press, New York.

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., Hightower, G.M., 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27, 75–88.

Baika, L.M., Santos, E.J.D., Herrmann, A.B., Grassi, M.T., 2016. Simultaneous determination of As, Hg, Sb, and Se in mineral fertilizers using ultrasonic extraction and CVG-ICP OES. Anal. Methods 8, 8362–8367.

Barron, M.G., Podrabsky, T., Ogle, S., Ricker, R.W., 1999. Are aromatic hydrocarbons the primary determinant of petroleum toxicity to aquatic organisms? Aquat. Toxicol. 46, 253–268.

Bender, D.A., 2012. Nitrogen Metabolism, Amino Acid Metabolism. John Wiley & Sons, Ltd, pp. 1–65.

Bettim, F.L., Galvan, G.L., Cestari, M.M., Yamamoto, C.I., Silva de Assis, H.C., 2016. Biochemical responses in freshwater fish after exposure to water-soluble fraction of gasoline. Chemosphere 144, 1467–1474.

Bijvelds, M.J., Velden, J.A., Kolar, Z.I., Flik, G., 1998. Magnesium transport in freshwater teleosts. J. Exp. Biol. 201, 1981.

Bornstein, J.M., Adams, J., Hollebone, B., King, T., Hodson, P.V., Brown, R.S., 2014. Effects-driven chemical fractionation of heavy fuel oil to isolate compounds toxic to trout embryos. Environ. Toxicol. Chem. 33, 814–824.

Boutilier, R.G., Heming, T.A., Iwama, G.K., 1984. Appendix: physicochemical parameters for use in fish respiratory physiology. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Academic Press, pp. 403–430.

Braga, M.A., Brauko, K.M., Vicentini, M., Salgado, L.D., Silva de Assis, H.C., Dolatto, R.G., Grassi, M.T., Sandrini-Neto, L., Lana, P.C., 2018. Cytotoxicity and enzymatic biomarkers as early indicators of benthic responses to the soluble-fraction of diesel oil. Ecotoxicol. Environ. Saf. 164, 21–31.

Brocksen, R.W., Bailey, H.T., 1973. Respiratory response of juvenile chinook salmon and striped bass exposed to benzene, a water-soluble component of crude oil. In: International Oil Spill Conference. American Petroleum Institute, pp. 783–791.

Caldwell, C.A., 1997. Aromatic hydrocarbon pathology in fish following a large spill into the Nemadji River, Wisconsin, USA. Bull. Environ. Contam. Toxicol. 58, 574–581.

Campbell, J.W., 1991. Excretory nitrogen metabolism. In: Prosser, C.L. (Ed.), Environmental and Metabolic Animal Physiology, 4° Ed. Wiley-Liss, New York, pp. 277–324.

Carneiro, P.C.F., Urbinati, E.C., Bendhack, F., 2005. Osmoregulation and fish transportation. In: Baldisserotto, B., Mancera, J.M., Kapoor, B.G. (Eds.), Fish Osmoregulation. Science Publisher, Enfield/EUA, pp. 235–248.

Chew, S.F., Wilson, J.M., Ip, Y.K., Randall, D.J., 2005. Nitrogen excretion and defense against ammonia toxicity. In: Val, A.L., Almeida-Val, V.M.F.D., Randall, D.J. (Eds.), The Physiology of Tropical Fishes. Academic Press, pp. 307–395.

Correa, M., García, H.I., 1990. Physiological responses of juvenile white mullet, *Mugil curema*, exposed to benzene. Bull. Environ. Contam. Toxicol. 44, 428–434.

Dal Pont, G., 2012. Toxicidade do óleo diesel para o peixe *Astyanax altiparanae*, Programa de Pós-graduação em Ciências Veterinárias. Universidade Federal do Paraná, Curitiba/PR 112.

Dal Pont, G., 2018. Effects of petroleum hydrocarbons to tropical and temperate fish species: a toxicity and multibiomarker approach for the assessment of environmental contamination, Zootecnia. Universidade Federal do Paraná, Curitiba/Brasil 224.

Dange, A.D., Masurekar, V.B., 1981. Toluene toxicity: effects of sublethal levels on enzyme activities in seawater adapted tilapia (*Sarotherodon mossambicus* Peters). J. Biosci. 3, 129–134.

Dupuis, A., Ucan-Marin, F., 2015. A literature review on the aquatic toxicology of petroleum oil: An overview of oil properties and effects to aquatic biota. In: F.a.O.C.C.S.A.S. (CSAS) (Ed.), Fisheries and Oceans Canada. Canadian Science Advisory Secretariat, Ottawa ON, pp. 58.

Eisler, R., 1987. Polycyclic aromatci hydrocarbon hazards to fish, wildlife and invertebrates: a synoptic review. In: Reviews, C.H. (Ed.), Biological Reports. U.S. Fish and Wildlife Service, Laurel - MD.

Engelhardt, F.R., Wong, M.P., Duey, M.E., 1981. Hydromineral balance and gill morphology in rainbow trout *Salmo gairdneri*, acclimated to fresh and sea water, as affected by petroleum exposure. Aquat. Toxicol. (Amsterdam) 1, 175–186.

Esbaugh, A.J., Mager, E.M., Stieglitz, J.D., Hoenig, R., Brown, T.L., French, B.L., Linbo, T.L., Lay, C., Forth, H., Scholz, N.L., Incardona, J.P., Morris, J.M., Benetti, D.D., Grosell, M., 2016. The effects of weathering and chemical dispersion on Deepwater horizon crude oil toxicity to mahi-mahi (*Coryphaena hippurus*) early life stages. Sci. Total Environ. 543, 644–651.

Fayemiwo, O., Daramola, M., Moothi, K., 2017. BTEX compounds in water - future trends and directions for water treatment. Water SA 43, 602–613.

Fernandes, A.N., Gouveia, C.D., Grassi, M.T., da Silva Crespo, J., Giovanela, M., 2014. Determination of monoaromatic hydrocarbons (BTEX) in surface waters from a brazilian subtropical hydrographic basin. Bull. Environ. Contam. Toxicol. 92, 455–459.

Ferreira, M.S., Aride, P.H.R., Val, A.L., 2018. Could resistance to lactate accumulation contribute to the better swimming performance of *Brycon amazonicus* when compared to *Colossoma macropomum*? PeerJ 6, e5719.

Fingas, M., 2011. Introduction to oil chemistry and properties. In: Fingas, M. (Ed.), Oil Spill Science and Technology. Gulf Professional Publishing, Boston, pp. 51–59.

Flatman, P.W., 1991. Mechanisms of magnesium transport. Annu. Rev. Physiol. 53, 259–271.

Forsters, R.P., Goldstein, L., 1969. Formation of excretory products. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology - Excretion. Ionic Regulartion and Metabolism. Academic Press, New York/USA, pp. 313–345.

Fromm, P.O., 1970. Section III - Effect of ammonia on trout and goldfish. In: Series, U.S.E.W.P.C.R. (Ed.), Toxic Action of Water Soluble Pollutants on Freshwater Fish, pp. 9–22.

Gagnon, M.M., Holdway, D.A., 1999. Metabolic enzyme activities in fish gills as biomarkers of exposure to petroleum hydrocarbons. Ecotoxicol. Environ. Saf. 44, 92–99.

Galvan, G.L., Lirola, J.R., Felisbino, K., Vicari, T., Yamamoto, C.I., Cestari, M.M., 2016. Genetic and hematologic endpoints in *Astyanax altiparanae* (Characidae) after exposure and recovery to water-soluble fraction of gasoline (WSFG). Bull. Environ. Contam. Toxicol. 97, 63–70.

Harper, C., Liccione, J.J., 1995. Toxicological Profile for Gasoline. U.S.D.o.H.a.H.S.P.H.S.A.f.T.S.a.D. Registry, Atlanta, Georgia, pp. 175.

Healy, T.M., Schulte, P.M., 2012. Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). Physiol. Biochem. Zool. 85, 107–119.

Heath, A.G., 1995. Water Pollution and Fish Physiology, 2nd Ed. Lewis Publishers.

Hochachka, P., 1980. Living without Oxygen. Harvard University Press, Cambridge.

Hollebone, B., 2017. Oil physical properties: Measurement and correlation. In: Fingas, M. (Ed.), Oil Spill Science and Technology, Second edition. Gulf Professional Publishing, Boston, pp. 185–207.

Hook, S.E., Mondon, J., Revill, A.T., Greenfield, P.A., Stephenson, S.A., Strzelecki, J., Corbett, P., Armstrong, E., Song, J., Doan, H., Barrett, S., 2018. Monitoring sublethal changes in fish physiology following exposure to a light, unweathered crude oil. Aquat. Toxicol. 204, 27–45.

Horodesky, A., Abilhoa, V., Zeni, T.D.O., Montanhini Neto, R., Castilho-Westphal, G.G., Ostrensky, A., 2015. Ecological analysis of the ichthyofaunal community ten years after a diesel oil spill at Serra do Mar, Paraná state, Brazil. Glob. Ecol. Conserv. 4, 311–320.

Incardona, J.P., Collier, T.K., Scholz, N.L., 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 196, 191–205.

Incardona, J.P., Linbo, T.L., Scholz, N.L., 2011. Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. Toxicol. Appl. Pharmacol. 257, 242–249.

Incardona, J.P., Gardner, L.D., Linbo, T.L., Brown, T.L., Esbaugh, A.J., Mager, E.M., Stieglitz, J.D., French, B.L., Labenia, J.S., Laetz, C.A., Tagal, M., Sloan, C.A., Elizur, A., Benetti, D.D., Grosell, M., Block, B.A., Scholz, N.L., 2014. *Deepwater Horizon* crude oil impacts the developing hearts of large predatory pelagic fish. Proc. Natl. Acad. Sci. 111, E1510.

Jensen, F.B., Nikinmaa, M., Weber, R.E., 1993. Environmental perturbations of oxygen transport in teleost fishes: causes, consequences and compensations. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Springer Netherlands, Dordrecht, pp. 161–179.

Johnston, I.A., Bernard, L.M., 1983. Utilization of the ethanol pathway in carp following exposure to anoxia. J. Exp. Biol. 104, 73.

Korn, S., Struhsaker, J.W., Benville Jr., P., 1976. Effects of benzene on growth, fat content, and caloric content of striped bass, *Morones saxatilis*. Fish. Bull. 74 United States.

Leite, N.F., Peralta-Zamora, P., Grassi, M.T., 2008. Multifactorial optimization approach for the determination of polycyclic aromatic hydrocarbons in river sediments by gas chromatography–quadrupole ion trap selected ion storage mass spectrometry. J. Chromatogr. A 1192, 273–281.

McAuliffe, C., 1966. Solubility in water of paraffin, cycloparaffin, olefin, acetylene, Cycloolefin, and aromatic Hydrocarbons1. J. Phys. Chem. 70, 1267–1275.

McDonald, M.D., Gilmour, K.M., Walsh, P.J., 2012. New insights into the mechanisms controlling urea excretion in fish gills. Respir. Physiol. Neurobiol. 184, 241–248.

McPherson, C.A., Lawrence, G.S., Elphick, J.R., Chapman, P.M., 2014. Development of a strontium chronic effects benchmark for aquatic life in freshwater. Environ. Toxicol. Chem. 33, 2472–2478.

Meyerhoff, R.D., 1975. Acute toxicity of benzene, a component of crude oil, to juvenile Striped Bass (*Morone Saxatilis*). J. Fish. Res. Board Can. 32, 1864–1866.

Mourik, J., Raeven, P., Steur, K., Addink, A.D.F., 1982. Anaerobic metabolism of red skeletal muscle of goldfish, *Carassius auratus* (L.). FEBS Lett. 137, 111–114.

Neff, J.M., 1979. Polycyclic Aromatic Hydrocarbon in the Aquatic Environment: Sources, Fates and Biological Effects. Applied Science Publishers, London.

Neff, J., 1988. Composition and fate of petroleum and spill-treating agents in the marine environment. In: Geraci, J.R., Aubin, D.J.S. (Eds.), Synthesis of Effects of Oil on

Marine Mammals. Ventura, CA, pp. 2–40.
Nero, V., Farwell, A., Lister, A., Van Der Kraak, G., Lee, L.E.J., Van Meer, T., MacKinnon, M.D., Dixon, D.G., 2006. Gill and liver histopathological changes in yellow perch (Perca flavescens) and goldfish (Carassius auratus) exposed to oil sands process-affected water. Ecotoxicol. Environ. Saf. 63, 365–377.
Nikinmaa, M., 2014. Chapter 12 - bioindicators and biomarkers. In: Nikinmaa, M. (Ed.), An Introduction to Aquatic Toxicology. Academic Press, Oxford, pp. 147–155.
Pal, S., Kokushi, E., Cheikyula, J.O., Koyama, J., Uno, S., 2011. Histopathological effects and EROD induction in common carp exposed to dietary heavy oil. Ecotoxicol. Environ. Saf. 74, 307–314.
Pasparakis, C., Mager, E.M., Stieglitz, J.D., Benetti, D., Grosell, M., 2016. Effects of Deepwater horizon crude oil exposure, temperature and developmental stage on oxygen consumption of embryonic and larval mahi-mahi (*Coryphaena hippurus*). Aquat. Toxicol. 181, 113–123.
Peakall, D.W., 1994. Biomarkers: the way forward in environmental assessment. Toxicol. Ecotoxicol. News 1, 55–60.
Pilatti, F.K., Ramlov, F., Schmidt, E.C., Kreusch, M., Pereira, D.T., Costa, C., de Oliveira, E.R., Bauer, C.M., Rocha, M., Bouzon, Z.L., Maraschin, M., 2016. In vitro exposure of *Ulva lactuca* Linnaeus (Chlorophyta) to gasoline – biochemical and morphological alterations. Chemosphere 156, 428–437.
Poulsen, M., Lemon, L., Barker, J.F., 1992. Dissolution of monoaromatic hydrocarbons into groundwater from gasoline-oxygenate mixtures. Environ. Sci. Technol. 26, 2483–2489.
Pruden, A., Sedran, M., Suidan, M., Venosa, A., 2003. Biodegradation of MTBE and BTEX in an aerobic fluidized bed reactor. Water Sci. Technol. 47, 123–128.
Rahmatullah, M., Boyde, T.R.C., 1980. Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. Clin. Chim. Acta 107, 3–9.
Randall, D.J., 2011. Nitrogenous-waste balance. In: Farrell, A.P., Cech, J.J.J., Richards, J.G., Stevens, E.D. (Eds.), Encyclopedia of Fish Physiology: From Genome to Environment. Academic Press is an imprint of Elsevier, London/UK.
Randall, D.J., Wright, P.A., 1987. Fish Physiol Biochem. 3. pp. 107. https://doi.org/10.1007/BF02180412.
Regan, M.D., Gill, I.S., Richards, J.G., 2017. Calorespirometry reveals that goldfish prioritize aerobic metabolism over metabolic rate depression in all but near-anoxic environments. J. Exp. Biol. 220, 564.
Rice, S.D., Thomas, R.E., Short, J.W., 1977. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in Pink Salmon Fry. In: Venberg, F.J., Calabrese, A., Thurbeg, F.P., Verberg, W.B. (Eds.), Physiological Responses of Marine Biota to Pollutants. Academic Press, USA/NY, pp. 259–277.
Rodrigues, R.V., Miranda-Filho, K.C., Gusmão, E.P., Moreira, C.B., Romano, L.A., Sampaio, L.A., 2010. Deleterious effects of water-soluble fraction of petroleum, diesel and gasoline on marine pejerrey *Odontesthes argentinensis* larvae. Sci. Total Environ. 408, 2054–2059.
Roubal, W.T., Collier, T.K., Malins, D.C., 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). Arch. Environ. Contam. Toxicol. 5, 513–529.
Saeed, T., Al-Mutairi, M., 1999. Chemical composition of the water-soluble fraction of the leaded gasolines in seawater. Environ. Int. 25, 117–129.
Simonato, J.D., Guedes, C.L.B., Martinez, C.B.R., 2008. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicol. Environ. Saf. 69, 112–120.
Simonato, J.D., Fernandes, M.N., Martinez, C.B.R., 2011. Gasoline effects on biotransformation and antioxidant defenses of the freshwater fish *Prochilodus lineatus*. Ecotoxicology 20, 1400–1410.
Solangi, M.A., Overstreet, R.M., 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. J. Fish Dis. 5, 13–35.
Sotiles, A.R., Baika, L.M., Grassi, M.T., Wypych, F., 2019. Cation exchange reactions in layered double hydroxides intercalated with sulfate and alkaline cations $(A(H_2O)_6)[M^{2+6}Al_3(OH)_{18}(SO_4)_2]\cdot 6H_2O$ (M^{2+} = Mn, Mg, Zn; A^+ = Li, Na, K). J. Am. Chem. Soc. 141, 531–540.
Souza-Bastos, L.R., Páscoa, M.I., Freire, C.A., Wilson, J.M., 2014. Ammonia excretion and expression of transport proteins in the gills and skin of the intertidal fish *Lipophrys pholis*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 167, 15–24.
Speight, J., 2015. Gasoline, Handbook of Petroleum Product Analysis. John Wiley & Sons, Inc., pp. 104–125.
Spies, R.B., 1987. The biological effects of petroleum hydrocarbons in the sea: Assessments from field and microcosms. In: Boesch, D.F., Rabalais, N.N. (Eds.), Long-Term Environmental Effects of Offshore Oil and Gas Development, 1st ed. Elsevier-Applied Sciences, London, pp. 411–467.
Stentiford, G.D., Longshaw, M., Lyons, B.P., Jones, G., Green, M., Feist, S.W., 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar. Environ. Res. 55, 137–159.
Struhaaker, J., 1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, *Clupea harengus pallasi*. Fisheries 75 United States.
Thomas, R.E., Rice, S.D., 1975. Increased opercular rates of pink Salmon (*Oncorhynchus gorbuscha*) fry after exposure to the water-soluble fraction of Prudhoe Bay crude oil. J. Fish. Res. Board Can. 32, 2221–2224.
Thomas, R.E., Rice, S.D., 1979. The effect of exposure temperatures on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, naphthalene, and water-soluble fractions of cook inlet crude oil and no. 2 fuel oil. In: Vernberg, W.B., Calabrese, A., Thurberg, F.P., Vernberg, J.F. (Eds.), Marine Pollution: Functional Responses. Academic Press, New Yourk. NY, pp. 39–52.
U.S.EPA, 2001. United States Environmental Protection Agency. Guidance for Reporting Toxic Chemicals: Polycyclic Aromatic Compounds Category. United States Environmental Protection Agency, Washington, DC, pp. 40.
van den Thillart, G., Kesbeke, F., 1978. Anaerobic production of carbon dioxide and ammonia by goldfish *Carassius auratus* (L.). Comp. Biochem. Physiol. A Physiol. 59, 393–400.
van den Thillart, G., Kesbeke, F., van Waarde, A., 1980. Anaerobic energy-metabolism of goldfish, *Carassius auratus* (L.). J. Comp. Physiol. 136, 45–52.
Van den Thillart, G., Van Berge-Henegouwen, M., Kesbeke, F., 1983. Anaerobic metabolism of goldfish, *Carassius auratus* (L.): ethanol and CO_2 excretion rates and anoxia tolerance at 20, 10 and 5 °C. Comp. Biochem. Physiol. A Physiol. 76, 295–300.
van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Van Waarde, A., De Wilde-Van Berge Henegouwen, M., 1982. Nitrogen metabolism in goldfish, *Carassius auratus* (L.). Pathway of aerobic and anaerobic glutamate oxidation in goldfish liver and muscle mitochondria. Comp. Biochem. Physiol. B Comp. Biochem. 72, 133–136.
Verdouw, H., Van Echteld, C.J.A., Dekkers, E.M.J., 1978. Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12, 399–402.
Walker, R.M., Johansen, P.H., 1977. Anaerobic metabolism in goldfish (*Carassius auratus*). Can. J. Zool. 55, 1304–1311.
Wang, Z., Hollebone, B., Fingas, M., Fieldhouse, B., Sigouin, L., Landriault, M., Smith, P., Noonan, J., Thouin, G., 2003. Characteristics of Spilled Oils, Fuels, and Petroleum Products: 1. Composition and Properties of Selected Oils. U.S. Environmental Protection Agency - EPA, pp. 284.
Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.
Winkaler, E.U., Silva, A.G., Galindo, H.C., Martinez, C.B.R., 2001. Biomarcadores histológicos e fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná. Acta Sci. 23, 507–514.
Wood, C.M., 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. J. Exp. Biol. 160, 285.
Wood, C.M., 1993. Ammonia and urea metabolism and excretion. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press, Boca Raton, pp. 379–423.
Wood, C.M., 2001. Influence of feeding, exercise, and temperature on nitrogen metabolism and excretion. In: Wright, P.A., Anderson, P.M. (Eds.), Fish Physiology - Nitrogen Excretion. Academic Press, New York/EUA, pp. 201–238.
Wright, P.A., Wood, C.M., 2009. A new paradigm for ammonia excretion in aquatic animals: role of rhesus (Rh) glycoproteins. J. Exp. Biol. 212, 2303.
Yang, C., Brown, C.E., Hollebone, B., Yang, Z., Lambert, P., Fieldhouse, B., Landriault, M., Wang, Z., 2017. Chemical fingerprints of crude oils and petroleum products. In: Fingas, M. (Ed.), Oil Spill Science and Technology, Second edition. Gulf Professional Publishing, Boston, pp. 209–304.
Zall, D.M., Fisher, D., Garner, M.Q., 1956. Photometric determination of chlorides in water. Anal. Chem. 28, 1665–1668.