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Can mitochondrial malondialdehyde content be a useful tool to evaluate sea lamprey juveniles' capacity to seawater acclimatization?

Abstract: The sea lamprey is an anadromous species that migrates twice during its life cycle between freshwater and seawater. Microphagous larvae generally spend 4–5 years burrowed in the substrate of rivers and streams before undergoing metamorphosis that ends with the beginning of the juvenile trophic migration. Once metamorphosis is complete, sea lamprey juvenile downstream migrants are fully tolerant to seawater salinity. Pollution resulting from industrial effluents may disturb the seawater acclimatization causing oxidative damage, and ultimately may lead to a decrease of sea lamprey population. The aim of this study was to compare salt acclimation of sea lamprey juveniles captured in river basins with different levels of aquatic pollution, using mitochondrial glutathione (GSH) and malondialdehyde (MDA) of gills and liver as markers of physiological stress and cell damage. The results showed that juveniles from the Lima basin exhibited the highest levels of mitochondrial MDA in gills, even though significant changes in the stress markers of mitochondrial gills of all animals subject to salt acclimation were not detected. In addition, an increase in the oxidative damage of hepatic mitochondria of macrophthalmia from the Vouga basin suggests the occurrence of metabolic failures with the potential to disturb the capacity to adaptation to the marine environment.

Keywords: cell damage; oxidative stress; *Petromyzon marinus*; seawater acclimation.

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1 Introduction

The sea lamprey (*Petromyzon marinus* L., 1758) is an anadromous species widely distributed along both sides of the North Atlantic, occurring in several European rivers basins, from Norway, to the north, to the Iberian Peninsula, in the south [1, 2]. During its life cycle, the sea lamprey migrates twice between freshwater and seawater for feeding and reproduction. Ammocoetes spend around 4–5 years in the substrate of rivers and streams before undergoing a period of metamorphosis with marked behavioral, morphological and physiological changes that ends with the start of the juvenile (i.e., macrophthalmia) trophic migration [3]. Once metamorphosis is complete, juvenile downstream migrants are tolerant to full-strength seawater [4–11]. In the marine environment, sea lampreys adopt a parasitic strategy, feeding primarily on the blood of its hosts for a period of approximately 1.5 years [6]. After that period, the adults return to freshwater and migrate upstream, spawn and die [4–11].

In the last decades, several authors pointed out a decline in the abundance of sea lamprey population in the Portuguese rivers where it attains a high commercial value [12–19]. Dams that block the access of adults to the spawning habitats, overfishing, poaching and the pollution

resulting from industrial effluent and agricultural industry, are frequently pointed out as the main reasons for the decrease of European sea lamprey populations [17, 18, 20–22]. In Portugal, sea lamprey is considered "vulnerable" by the Portuguese Red List of Threatened Vertebrate Species, a status that demonstrates the importance of preserving this population [4, 5, 10, 12, 23, 24]. Xenobiotics like herbicides can block the respiratory chain, and increase lipid peroxidation which could modify the gill cells or hepatocytes membrane structure and composition [25, 26]. The chemical contamination of its habitat can thus influence the capacity of mitochondria of sea lamprey to generate ATP to meet the energetic demands of tissues with continuous production of reactive oxygen species (ROS) [27, 28]. Oxidative stress arises when the balance between oxidant species, such as superoxide anion radical ($^{\cdot}O_2^-$), hydroxyl radical ($^{\cdot}OH$) or peroxide hydrogen (H_2O_2), and antioxidant species, such as glutathione (GSH) is disturbed [29–31]. Consequently, GSH and malondialdehyde (MDA) contents have been commonly used to quantify oxidative stress, providing insight into the redox status of the cell [9, 32, 33]. Studies related with changes in intracellular contents that may be indicators of exposure to xenobiotics and/or cell damage have not been conducted in juvenile sea lamprey during the trophic migration phase, in the course of which animals may be subjected to several sources of environmental stress. Results published by the Portuguese Environmental Agency (INAG 2001) and the National Information System for Water Resources (SNIRH, 2012), obtained from monitoring stations in the Minho, Lima and Vouga river basins, revealed that the Vouga basin exhibits the highest level of pollution, while the Lima watershed appears to be the more pristine system [34, 35].

The aim of this study was to compare salt acclimation of juvenile sea lampreys captured in Minho, Lima and Vouga river basins, systems with potentially different levels of aquatic pollution, using mitochondrial GSH, oxidized GSH (GSSG), MDA and ROS levels of gills and liver as markers of physiological stress and cell damage.

2 Materials and methods

Sampling occurred at the beginning of the *P. marinus* downstream migration in the Minho, Lima and Vouga Portuguese river basins (Figure 1). Sea lamprey juveniles were captured in 2011 between

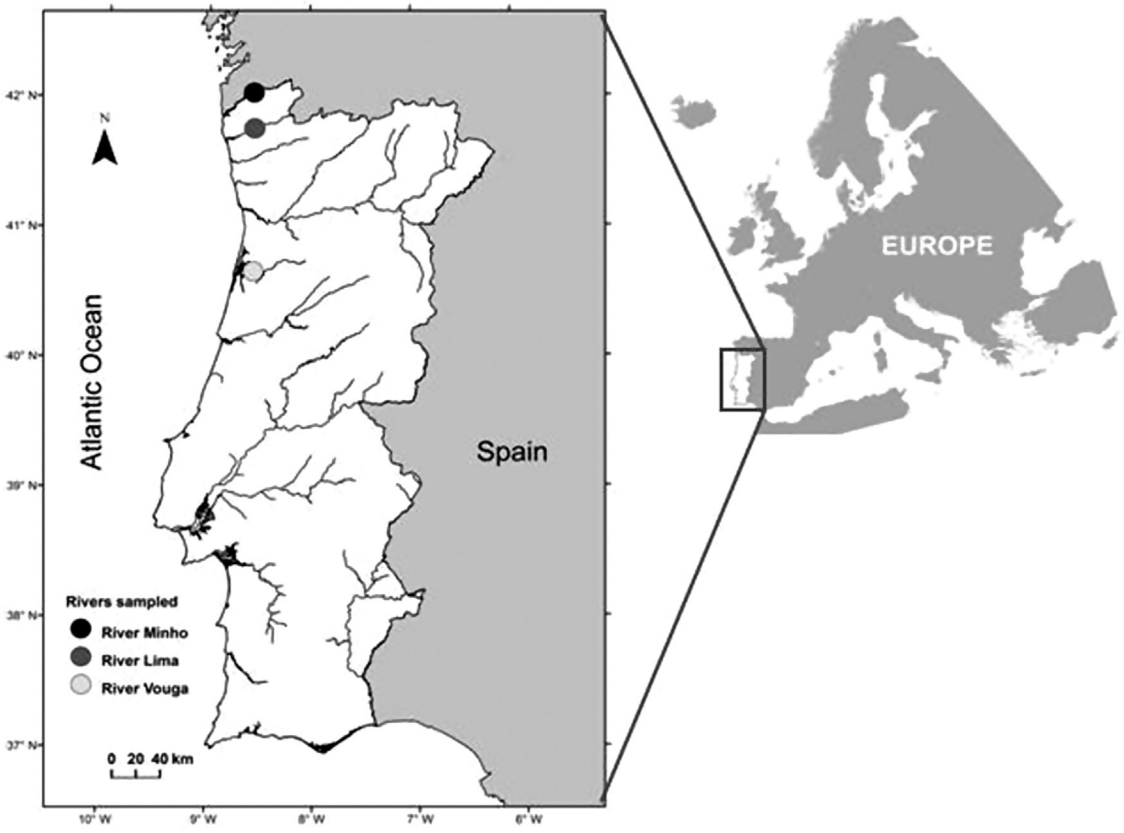

Figure 1: Geographical location of the hydrographic basins of Minho, Lima and Vouga (Portugal) where the juvenile sea lampreys were sampled.

October and December, with an electric fishing apparatus (Hans Grassl EL 62 generator DC, 600 V), and transported alive to the laboratory (Aquatic Animal Experimentation Room at the Mora Freshwater Aquarium) in a 400 l capacity tank equipped with an appropriate life support system including aeration, water quality maintenance system and temperature control and maintained in 200 l tanks during the acclimation period. In the treatment tanks, the salinity was increased gradually from 0 to 35, following a three step procedure in freshwater: at days 0, 8, 16 and 24, the salinity in the treatment tank was 0, 10, 25 and 35, respectively. The salinity in the control tank was kept at 0 during the entire experiment. Each tank was kept under constant darkness (to avoid stress related with negative phototaxis behavior), temperature was kept constant at 14°C, water parameters and mortalities were monitored daily and animals were not fed during the experiment. This study was carried out in strict accordance with the recommendations present in the Guide for the Care and Use of Laboratory Animals of the European Union – in Portugal represented by the Decree-Law n° 129/92, Portaria n° 1005/92. This work was conducted under an institutional license (Fluviário de Mora – Mora freshwater aquarium) for animal experimentation and a personal license to the authors I. Alves-Pereira, M.J. Lança, B.R. Quintella, P.R. Almeida and R. Ferreira, issued by the Direcção-Geral de Alimentação e Veterinária (DGAV), the Portuguese National Authority for Animal Health. Mitochondria obtained by centrifugation at 15,000 *g*, 30 min, 4°C, refrigerated super centrifuge, Hermle Z323 K, of gills and livers homogenates, prepared in 50 mM Tris-HCl pH 7.5 buffer, were stored at -80°C for subsequent determination of GSH, GSSG, ROS and MDA contents in a single-beam Shimadzu RF-5001PC luminescence spectrometer. The contents of GSH and GSSG were determined according the Hissin method [36] based on the reaction of o-phthalaldehyde, a fluorescent reagent with GSH at pH 8.0, or GSH disulfide, at pH 12.0 in the presence of 0.04M *N*-ethylmaleimide that scavenges GSH, using GSH or GSSG as standard, respectively. The fluorescent products were determined at λexc 350 nm and λem 420 nm, at 25°C. The determination of MDA content was based on quantification of thiobarbituric acid (TBA) oxidation products reading the fluorescence at λexc 515 nm and λem 553 nm, using as standard the MDA generated from 1,1,3,3-tetramethoxypropane by hydrolysis in acid medium [37]. The level of ROS was determined according LeBel et al. [38], using the hydrogen peroxide as standard, since this reacts with reduced 2′,7′-dichlorofluorescein (DCFH) to rapidly generate oxidized 2′,7′-dichlorofluorescein (DCF), which has been quantified reading the fluorescence at λexc 488 nm and λem 525 nm. All reagents were purchased from Sigma Chemical Co., St. Louis, MO, USA.

All values were presented as the mean of five pools for gill (#3) or liver (#8)±standard error of mean (SEM). The statistical analysis were performed by analysis of variance (ANOVA) I and the Duncan test to determine significant differences ($p<0.05$) between treatments, using SPSS® statistical software, version 22.0 (SPSS Inc., Chicago, IL, USA) for Windows®, licensed to University of Évora [39].

3 Results and discussion

Liver and gills are the main targets of chemical changes in the natural habitat of aquatic species [40–43], such as sea lamprey [26]. Thus, the contact and possible metabolism of organic pollutants that may be present at high concentrations in the habitat of sea lamprey may induce oxidative stress in gill cells and/or hepatocytes through generated excessive ROS. This may cause changes in the structure and composition of cell membranes, damage which may compromise the success of acclimatization of juvenile sea lamprey to the marine environment [18]. The content of GSH and MDA and GSH/GSSG ratio of gills and liver of teleosts have been usually determined to compare animals at different stages of development, or exposed to different conditions of environmental stress [41, 44]. However, the quantification of these stress-response markers in the mitochondria in teleost or in agnathans species is uncommon. Thus, quantifying the levels of GSH (7.1 μmol/g tissue) and MDA (120 nmol/g tissue) in mitochondria of gills of the teleost *Anabas testudineus* [45] is one of the few examples described in the literature whose values are much higher than those found in gills of sea lamprey (≈40 and 10–30 nmol/g tissue, respectively) (Figure 2A and D). By contrast, the MDA levels determined in mitochondria of gills in tadpoles of *Rana temporaria*, another aquatic animal which undergoes metamorphosis [44], are much lower (4.4 nmol/g tissue) than those detected in sea lamprey (Figure 2D). These differences may be due to the phylogenetic evolution, development stage of each species and/or to environmental factors of the habitat, like salinity. However, the content of MDA in mitochondria of gill tissue tadpoles seems to be more close to the level determined in juvenile sea lamprey (Figure 2D). The results (Figure 2D) showed that juveniles from the Lima river basin exhibit the highest mitochondrial MDA levels of gills ($p<0.05$). This difference in the mitochondrial MDA level may suggest increasing difficulties of these individuals to captive conditions in the aquarium, even though individuals sampled in the three watersheds were all subjected to the same captive conditions. However, no significant changes were detected in markers of oxidative stress (GSH, GSSG, GSH/GSSG ratio and ROS) and cell damage (MDA) in the mitochondria of the gills of the animals captured in the three basins during salt acclimation (Figure 2A–E) ($p<0.05$). These results suggest that salt acclimation of sea lamprey juveniles caught in the river basins of Minho, Lima and Vouga did not induce an increase in the availability of ROS and consequent reducing-oxidizing transition of mitochondrial environment of gills. The estimation of GSH redox status in the liver of the flathead grey mullet (*Mugil cephalus*, L.) collected in distinct zones of the Ennore estuary (India) is one of the few examples available in literature where mitochondria GSH/GSSG ratio was used as an oxidative stress marker in fish [40]. The GSH/GSSG ratio values determined in mitochondria of hepatic tissue of sea lamprey juveniles, after

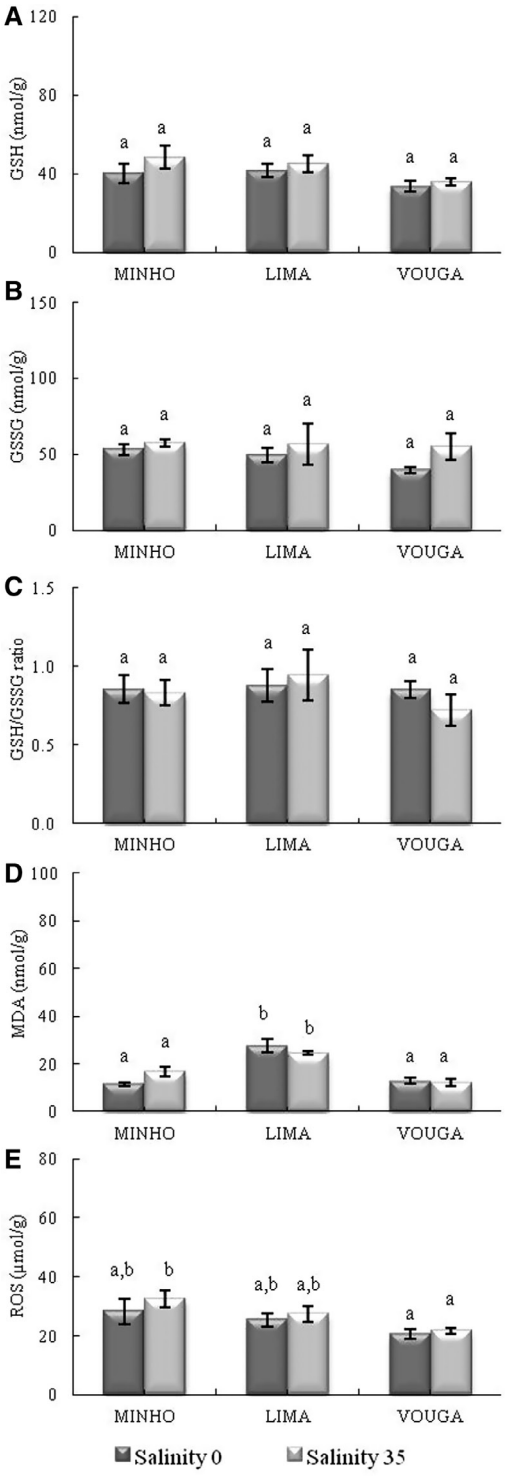

Figure 2: Mitochondrial contents in gills of (A) glutathione (GSH), (B) oxidized GSH (GSSG), (C) GSH/GSSG ratio, (D) malondialdehyde (MDA) and (E) reactive oxygen species (ROS) of sea lamprey juveniles kept for 30 days at 0 (gray bar) or gradually kept from 0 to 35 (white bar) salinity levels. Each bar represents the mean of five pools (3#)±SEM. Bars marked with different letters are significantly different ($p<0.05$).

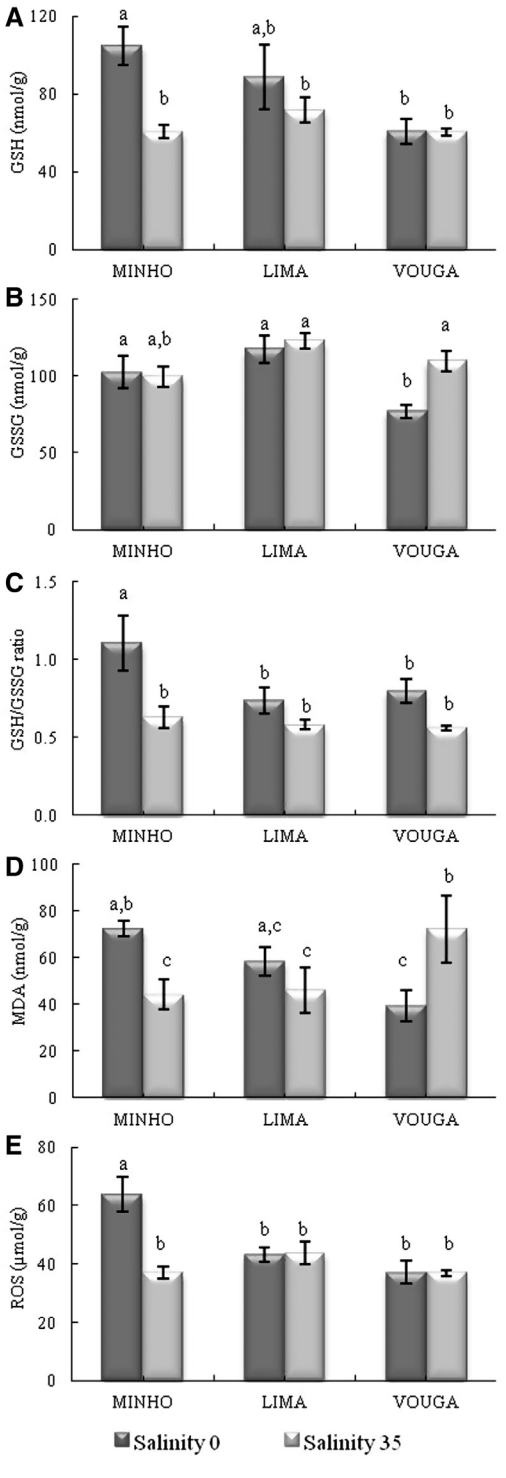

Figure 3: Mitochondrial contents in liver of (A) glutathione (GSH), (B) oxidized GSH (GSSG), (C) GSH/GSSG ratio, (D) malondialdehyde (MDA) and (E) reactive oxygen species (ROS) of sea lamprey juveniles kept for 30 days at 0 (gray bar) or gradually kept from 0 to 35 (white bar) salinity levels. Each bar represents the mean of five pools (8#)±SEM. Bars marked with different letters are significantly different ($p<0.05$).

salt acclimation (0.4), were identical to those determined in liver mitochondria of *M. cephalus* [40]. In addition, the hepatic mitochondrial contents in GSH, ROS and GSH/GSSG ratio of macrophthalmia, captured in the Minho basin, decreased to the levels detected in the animals captured in the Lima and Vouga river basins during salt acclimation (Figure 3A–C and E), although the MDA in the liver mitochondria doubled in the animals from the Vouga basin (Figure 3D) ($p<0.05$). The increase in the oxidative damage of hepatic mitochondria of individuals from the Vouga basin, considered the most polluted basin, suggests the occurrence of metabolic failures in the liver, which may reduce their adaptation capacity to the marine environment and, consequently, jeopardize the parasitic phase in the marine environment.

4 Conclusions

This study did not detect an induction of oxidative stress, or an increase of gills mitochondrial damage of sea lamprey juveniles captured in three Portuguese river basins. The steady pattern of stress markers detected in the hepatic mitochondria of macrophthalmia suggest that the animals from the three river basins would be metabolically prepared to move from a freshwater to a marine environment and initiate the parasitic phase. However, the increase in the oxidative damage of hepatic mitochondria of macrophthalmia from the Vouga basin suggests the occurrence of metabolic failures, with the potential to disturb the adaptation of these animals to the parasitic phase in the marine environment. These results seem to point to the potential utility of using mitochondrial MDA levels of the liver of sea lamprey as a tool to evaluate the capacity of juveniles to successfully adapt to sea water during their trophic migration.

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References

[1] Shimeld S, Donoghue P. *Development* 2012, 139, 2091–2099.
[2] Perea S, Garzón P, González J, Almada V, Pereira A, Doadrio I. *Graellsia* 2011, 67, 91–102.
[3] Quintella BR, Andrade NO, Almeida PR. *Ecol. Freshw. Fish* 2003, 12, 286–293.
[4] Pedro S, Caçador I, Quintella B, Lança M, Almeida P. *Ecol. Freshw. Fish* 2014, 23, 193–207.
[5] Araújo M, Reis P, Braga A, Antunes C. *Ann. Limnol. – Int. J. Lim.* 2013, 49, 107–112.
[6] Silva S, Servia M, Vieira-Lanero R, Barca S, Cobo F. *Aquat. Biol.* 2013, 18, 59–62.
[7] Dufour S, Rousseau K, Kapoor BG, Eds., *Metamorphosis in Fish*, 2nd ed., Science Publishers: Edenbridge, 2012.
[8] Laudet V. *Curr. Biol*. 2011, 27, 726–737.
[9] Reis-Santos P, McCormick S, Wilson J. *Exp. Biol*. 2008, 211, 978–988.
[10] Pereira S, Pinto A, Cortes R, Fontaínhas-Fernandes A, Coimbra A, Monteiro S. *Ecotoxicol. Environ. Safety* 2013, 90, 157–166.
[11] Resende P, Resende P, Pardal M, Almeida S, Azeiteiro U. *Environ. Monit. Assess.* 2010, 170, 535–544.
[12] Mateus CS, Rodríguez-Muñoz R, Quintella BR, Alves MJ, Almeida PR. *Endangered Species Res.* 2012, 16, 183–198.
[13] Beaulaton L, Taverny C, Castelnaud G. *Fisheries Res.* 2008, 92, 90–101.
[14] Cabral MJ, Almeida J, Almeida PR, Delliger T, Ferrand de Almeida N, Oliveira ME, Palmeirim JM, Queirós AI, Rogado L, Santos-Reis M. Eds., *Livro Vermelho dos Vertebrados de Portugal (Portuguese Red List of Threatened Vertebrate Species)*. Instituto da Conservação da Natureza: Lisboa, 2005.
[15] Igoe F, Quigley DTG, Marnell F, Meskell E, O'Connor W, Byrne C. *Biol. Environ. Proc. R. Ir. Acad.* 2004, 3, 43–56.
[16] Almeida PR, Quintella BR, Dias NM, Andrade N. In The biology of lampreys, symposium proceedings. International Congress on the Biology of Fish., Moser M, Bayer J, MacKinlay D. Eds., American Fisheries Society: Vancouver, 2002, p 49.
[17] Close DA, Fitzpatrick MS, Li HW. *Fishereis* 2002, 27, 19–25.
[18] Kelly FL, King JJ. *Biol. Environ. Proc. R. Ir. Acad. B.* 2001, 101, 165–185.
[19] Thomas MLH. *Fish. Res. Board Can., London, Ont., Circ.* 1961, 3, 4–7.
[20] Collares-Pereira MJ, Cowx IG, Ribeiro F, Rodrigues JA, Rogado L. *Fish. Manage. Ecol.* 2000, 7, 167–178.
[21] Kime DE. *Rev. Fish Biol. Fish.* 1995, 5, 52–96.
[22] Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, Wehrli B. *Science* 2006, 313, 1072–1077.
[23] Bartels H, Fazekas U, Youson J, Potter I. *Can. J. Zool.* 2011, 89, 538–545.
[24] Silva E, Batista S, Caetano L, Cerejeira M, Chaves M, Jacobsen S. *Environ. Monit. Assess.* 2011, 176, 331–341.
[25] Atencio L, Moreno I, Jos Á, Prieto AI, Moyano R, Blanco A, Cameán AM. *Toxicon*. 2009, 53, 269–282.
[26] van der Oost R, Beyer J, Vermeulen N. *Environ. Toxicol. Pharmacol.* 2003, 13, 57–149.
[27] Wallace KB, Datrkov AA. *Annu. Rev. Pharmacol. Toxicol.* 2000, 40, 353–388.

[28] Paradies G, Paradies V, Ruggiero FM, Petrosillo G. *World J. Gastroenterol*. 2014, 20, 14205–14218.
[29] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. *World Allergy Organ. J.* 2012, 5, 9–19.
[30] Schafer FQ, Buettner GR. *Free Rad. Biol. Med.* 2001, 30, 1191–1212.
[31] El-Bahr SM. *Sci. Int.* 2013, 1, 111–117.
[32] Zhu L, Dong X, Xie H, Wang J, Su J, Yu C. *Environ. Toxicol*. 2011, 26, 480–488.
[33] Luo Y, Wang W, Huang Q. *Fish Physiol. Biochem*. 2013, 39, 513–521.
[34] INAG-Instituto Nacional da água (Portuguese Environmental Agency), 2001. Planos de bacia hidrográfica. <http://www.inag.pt/inag2004/port/a_intervencao/planeamento/pbh/pbh.html>. Accessed 5/3/2012.
[35] SNIR, Sistema Nacional de Informação de Recursos Hídricos (Portuguese Information System for Water Resources), 2012. <http://snirh.pt/index.php?idMain=2&idItem=1>. Accessed 5/3/2012.
[36] Hissin PJ, Hilf R. *Anal. Biochem*. 1976, 74, 214–226.
[37] Turton H, Dawes I, Grant C. *J. Bacteriol*. 1997, 179, 1096–1101.
[38] LeBel P, Ischiropoulos H, Bondys C. *Chem. Res*. 1990, 5, 227–231.
[39] Sokal RR, Rohlf FJ, Eds., *Biometry*, 2nd ed., Dover Publications. INC: New York, 2009.
[40] Padmini E, Geetha BV, Rani MU. *Sci. Total Environ*. 2009, 407, 1307–1317.
[41] Ahmad I, Pacheco M, Santos MA. *Chemosphere* 2006, 65, 952–962.
[42] Oliveira UO, Araújo ASR, Belló-Klein A, da Silva RSM, Kucharski LC. *Comp. Biochem. Physiol., Part B* 2005, 140, 51–57.
[43] Passino DRM. In Contaminant Effects on Fisheries, Cairns VW, Hodson PV, Nriagu JO, Eds., John Wiley: New York, 1984, p 37.
[44] Salin K, Luquet E, Rey B, Roussel D, Voituron Y. *J. Exp. Biol*. 2012, 215, 863–869.
[45] Saumya SM, Sreejith P, Vijayasree AS, Divya L, Manju M, Oommen OV. *Indian J. Exp. Biol*. 2006, 44, 640–646.

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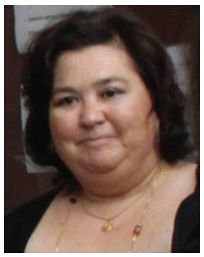

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