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Settleable atmospheric particulate matter affects cardiorespiratory responses to hypoxia in Nile tilapia (*Oreochromis niloticus*)

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1 **Settleable atmospheric particulate matter affects cardiorespiratory responses to**
2 **hypoxia in Nile tilapia (*Oreochromis niloticus*)**

3

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29

30

31 **Abstract**

32 Atmospheric particulate matter (APM) emitted by iron ore processing industries has a
33 complex composition, including diverse metallic particles and nanoparticles. Such an
34 APM causes air to water cross-contamination and has recently been demonstrated to have
35 harmful sublethal impacts on fish, eliciting stress responses, affecting the immune system,
36 and reducing blood oxygen-carrying capacity. These findings imply potential
37 consequences for fish aerobic performance and energy allocation, particularly in their
38 ability to tolerate respiratory challenges such as aquatic hypoxia. To assess that potential
39 limitation, we analyzed metabolic, cardiorespiratory, and morphological responses to
40 progressive hypoxia in tilapia, *Oreochromis niloticus*, exposed for 96h to an
41 environmentally relevant concentration of settleable particulate matter (SePM). This level
42 of SePM contamination initiate detectable gill damage, which reduced respiratory
43 efficiency, increased ventilatory effort and reduced fish capacity to deal with hypoxia.
44 The critical O₂ tension was increased (from 26 to 34% of O₂ / from 1.84 to 2.76 mg O₂ ·
45 L⁻¹). The resting normoxic respiratory frequency was elevated, limiting ventilatory
46 adjustments throughout hypoxia. Such ventilatory inefficacy is likely to increase
47 ventilatory cost and cause relevant alterations in energy allocation. We point up that a
48 potential progression in gill damage is potentially problematic in the long term as it could
49 result in infection, blood loss, ion imbalance, changes in the overall energy allocation and
50 hypoxia tolerance. Such an effect might not cause immediate lethality, but it might
51 threaten fish populations since the consequences limits physiological performance. This
52 was the first investigation to evaluate the physiological responses of fish to hypoxia after
53 SePM contamination. We suggest that the present level of environmental SePM deserves
54 attention. The present results base the need for extensive dedicated studies on SePM
55 effects in aquatic fauna.

56

57 **Keywords:** atmospheric particulate matter; iron; industry; metal/metalloid;
58 nanoparticle; environmental risk; physiological responses.

59

60

61 **1. Introduction**

62 Recent studies have demonstrated that aquatic environments may be affected by
63 atmospheric particulate matter (APM) derived from industrial activities for steel
64 processing (Callén et al., 2009; Park and Kim, 2005; Tsai and Cheng, 2004). Steel
65 industries emit a complex mixture of solid and/or liquid particles into the atmosphere
66 which may vary in composition, shape and size, and includes metallic nanoparticles and
67 metallic compounds (Souza et al., 2021a). Estuarine waters in Vitória Bay, Espírito Santo
68 State - BR, are contaminated by a range of metallic particles (including Al, Cr, Mn, Fe,
69 Ni, Cu, Zn, As, Se, Ag, Cd, Hg, Pb) derived from settleable APM (SePM) (Souza et al,
70 2021b and 2021c). Isotopic analysis of carbon, nitrogen, lead, and strontium revealed that
71 these SePM contaminants are emitted by a nearby steel industry complex in the city of
72 Vitória (Souza et al., 2018b). Existing legislations in several countries do not consider air
73 to water cross-contamination in environmental monitoring programs (e.g. CONAMA
74 491/2018 - BRASIL, 2018; Environmental Protection Agency – EPA, European Union
75 and Canada). It is relevant the some studies effectively assess whether SePM
76 contamination poses a real threat to ecosystems health and if so, the magnitude of the
77 related risk.

78 Most studies on the effects of metals and metalloids in fishes have focused on
79 tissue bioaccumulation and on establishing lethal doses (Fernandes et al., 2008; Vicente-
80 Martorell et al., 2009; Demirak et al., 2006; Souza et al., 2013; Arantes et al., 2016), with
81 little known about potential sub-lethal functional impacts. The study of sub-lethal
82 contamination can provide valuable information on the potential ecological risks related
83 to SePM contamination. Such studies allow observing the development of functional
84 problems before they cause lethality or will enable the observation of functional damage
85 that affects the performance of individuals to cope with specific environmental

86 conditions, such as swimming, hypoxia tolerance, temperature acclimation, or others.
87 Functional impairment, even if developed at a sublethal level, can lead to population
88 damage by altering the use of the environment or by generating energy reallocation and
89 thus influencing growth, reproduction, and offspring size. However, the identification of
90 sublethal damages are the first step to establish the need of a broader investigation. We
91 argue that physiological measurements may be useful to reveal functional impairments,
92 since it provides direct measurements to assess how animals cope with relevant
93 environmental conditions.

94 Recent investigation observed that short-term SePM exposition affected Nile
95 tilapia, *Oreochromis niloticus*. SePM reduced blood oxygen-carrying capacity, exerted
96 immunotoxic effects and elicited humoral stress response (Soares et al., *submitted*). The
97 authors suggested that such effects would impair homeostasis maintenance and limit fish
98 performance. Therefore, SePM may limit fish capacity to cope with ecophysiological
99 challenges such as environmental hypoxia, temperature variations, or routine activities
100 such as swimming, foraging, courtship, or agonistic encounters.

101 In this investigation, we have analyzed the effect of short-term exposure (96 h) of
102 an environmentally relevant SePM concentration on the Nile tilapia response to hypoxia.
103 We accessed SePM impact on the cardiorespiratory and metabolic responses to
104 progressive hypoxia and on gills morphology. Tilapia is a globally widespread invasive
105 species, which is native from northern Africa and inhabits the SePM-contaminated
106 locations in the state of Espírito Santo, Brazil. Hypoxia is a common environmental
107 stressor in freshwater and estuarine environments and, therefore, effective physiological
108 adjustment will be significant for population survival and fitness.

109

110 **2. Materials and Methods**

111 2.1. *Settleable atmospheric particulate matter - SePM*

112 The SePM was collected as described in Souza and coworkers (2021a). Containers
113 placed on roofs at a height of 20 m, in proximity to the steel industrial complex in Vitória
114 city – Brazil (Ilha do Boi - 20°18'32"S, 40°16'33"O, 14 km from the Tubarão industrial
115 complex), collected between 5 and 8 g per m² each month, over an area of 5,000 m². The
116 SePM comprises a complex mixture of organic and inorganic (chiefly metallic)
117 substances (Souza et al., 2021c). Its composition may vary over time (Santos et al, 2017).
118 Therefore, in order to assure repeatability, SePM was sampled from pooled material
119 collected over a year. The SePM was aliquoted and stored at the Comparative
120 Zoophysiology and Biochemistry Lab (LZBC) in the Department of Physiological
121 Sciences at the Federal University of São Carlos (UFSCar, Brazil). SePM sampling was
122 stored as testimony for any further analyses and evaluations.

123 The pooled SePM was analyzed for its main components and particle size. We
124 used granulometry, muffle furnace and solubilization with HNO₃/H₂O₂ in a microwave
125 digester to access particle size (Gee and Or, 2002), proportion of organic materials
126 (Goldin, 1987; Suguio, 1973), and contents of inorganic materials (Environmental
127 Protection Agency – EPA, 3052 - USEPA, 1996). Aquarium water samples were
128 analyzed by mass spectrometry with inductively coupled plasma (Q-ICPMS, Agilent
129 7500 Series CX technology) equipped with an ASX-100 autosampler (CETAC-
130 technologies, Omaha, NE, USA), at the Brazilian Agricultural Research Corporation
131 (EMBRAPA, Brazil) and Food Science and Technology Institute of Córdoba (YCITAC
132 - Universidad Nacional de Cordoba, Argentina). The analyses of water samples are
133 reported here to attest the ecologically relevant level of contamination. Detailed analyses
134 and composition of the pooled SePM was previously published (Soares et al, *submitted*).

135

136 2.2. Fish supply and acclimation

137 Juveniles of *O. niloticus* (body mass, $M_B = 110 \pm 87\text{g}$) were obtained from a local
138 fish farm (Piscicultura Poletini, São Paulo State, Brazil). Exposure protocols and
139 experiments were therefore performed on animals raised in unpolluted conditions. Prior
140 to experimentation, fish were maintained for 30d in 1,000L holding tanks equipped with
141 a continuous supply of well-aerated and dechlorinated water, at $25 \pm 1^\circ\text{C}$, under natural
142 photoperiod (~12 h:12 h) and constant physicochemical parameters: pH 6.7–7.3, DO 6.0–
143 7.3 $\text{mg}\cdot\text{L}^{-1}$, hardness 48–53 $\text{mg}\cdot\text{L}^{-1}$ (as CaCO_3), alkalinity 40–43 $\text{mg}\cdot\text{L}^{-1}$ (as CaCO_3),
144 ammonium 0.1 $\text{mg}\cdot\text{L}^{-1}$; chloride 42 $\text{mg}\cdot\text{L}^{-1}$ and conductivity 110 $\mu\text{S}\cdot\text{cm}^{-1}$. During
145 acclimation, fish were fed *ad libitum* daily with commercial pellets (Supra[®] Acqua line
146 specific for tilapia: 5-8 mm; 12% moisture; 32% protein; 6% fat). Fish were fasted for
147 24h prior to contaminant exposure and all experimental procedures. These procedures
148 were approved by the Committee of Ethics in Animal Experimentation (CEUA-UFSCar).
149

150 2.3. Experimental Design

151 After laboratory acclimation, each fish was individually maintained for 96h in an
152 aquarium (200 L) containing either clean biofilter water (control group, $n = 10$) or water
153 exposed to SePM (SePM group, $n = 10$), with a fish:water volume ratio of 1g:1L (OECD
154 203, 2019). The SePM exposure dose ($1 \text{ g}\cdot\text{L}^{-1}$) refers to raw SePM mass and represents
155 an environmentally relevant contamination in the estuarine area close to Vitória city,
156 Espírito Santo State - BR, (Santos et al, 2017, IEMA, 2021). Water was continually
157 aerated and mixed by air stone and maintained at $25 \pm 1^\circ\text{C}$. The aquariums tanks were
158 shielded with black plastic to avoid visual disturbance, but light cycle was artificially
159 preserved. At 48h, 50% of the water was replaced to counter waste ammonia
160 accumulation, and to re-establish initial contaminant concentrations for the SePM group.

161 Water samples (5 mL) were collected before and after the replacement procedure, to
162 analyze composition. After 96h, each fish was instrumented and underwent exposure to
163 progressive hypoxia (P_wO_2 100 – 5%) in clean water, during which we measured oxygen
164 uptake. We also recorded cardiorespiratory parameters in normoxia and deep hypoxia to
165 assess the SePM impact on them (see below).

166 Each fish was subjected to the complete experimental sequence: SePM/blank
167 exposure for 96h; instrumentation and 24h recovery; progressive hypoxia protocol;
168 euthanasia, and dissection to sample gill tissue. After each experimental trial,
169 respirometer chamber and experimental tank were cleaned with uncontaminated biofilter
170 water. Background tests of oxygen uptake were performed during the process. Each fish
171 underwent the whole protocol. The topics below were organized according to data
172 description in results and discussion sections.

173

174 *2.4. Morphological analyses*

175 After hypoxia, each fish was euthanized by benzocaine overdose ($1 \text{ g}\cdot\text{L}^{-1}$)
176 followed by medullary section. Samples of the second gill arch in both groups (Control
177 and SePM, $n = 5$) were carefully dissected out and fixed in Bouin (24 h), washed with
178 water for 24 hours, dehydrated in alcohol (70 – 95%), and embedded in historesin (Leica,
179 Germany). Histological longitudinal sections ($3 \mu\text{m}$ thickness - Micron HM 360) were
180 stained with toluidine blue. Gill tissues were analyzed by light microscopy (Olympus
181 BX51, Denmark and Motic Image Plus 2.0 software, China) and quantified in 4 random
182 microscope fields/section ($n = 20$) for each animal. Gill morphometry was examined
183 (Image J Pro-plus 6.0 software, United States), such as filament epithelial thickness,
184 distance between lamellae, and lamellar height. The level of histopathology was
185 calculated using a histopathological index (HI) (Poleksic; Mitrovic-Tutundzic, 1994):

186 (1) $10^0 \sum I + 10^1 \sum II + 10^2 \sum III$, where:

187 PI , PII , and $PIII$ are the total number of alterations in each stage; 10^0 , 10^1 , and 10^2
188 are integrated factors according to damage severity. The average HI value was classified
189 into four categories: 1-10, normal organ function; 11-20, from mild to moderate organ
190 damage; 21-50, from moderate to severe damage; +100, irreparable to the tissue.

191

192 2.5. Responses to hypoxia

193 The exposure protocol was followed by anesthesia and instrumentation for
194 cardiorespiratory recording. After instrumentation each fish was placed in a polyethylene
195 respirometer (5L chamber) that was immersed in a polyethylene tank (75 L). Fish were
196 recovered for 24h in clean normoxic water (PwO_2 of 100%), at $25 \pm 1^\circ\text{C}$.

197 The protocol was designed to record metabolic rate in normoxic water and during
198 progressive hypoxia ($PwO_2 = 70, 50, 30, 20, 10$ and 5%), which was developed at
199 acclimation temperature, $25 \pm 1^\circ\text{C}$ in clean water. The normoxic and each hypoxic level
200 were controlled by bubbling either compressed atmospheric air or N_2 in the water. Each
201 level of PwO_2 was maintained during 30 minutes. Water oxygen saturations (% of
202 atmospheric O_2) were measured using optodes (PreSens OXY-4 mini probes) and
203 associated software. The 100% saturation was set by calibrating the electrode using fully
204 aerated water.

205 The rates of oxygen uptake ($\dot{M}O_2$ - $\text{mMol}O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) in each tension were
206 calculated using intermittent stopped-flow respirometry (measure time – 6 minutes, flush
207 time – 4 minutes, see McKenzie et al., 2012 for general protocol) with the optode in the
208 respirometer and associated PreSens software. The $\dot{M}O_2$ was calculated for the periods of
209 intermittent stopped flow (Rosewarne et al. 2016) as follows:

210 (1) $\dot{M}O_2 = [\beta O_2 \cdot V_{RE} \cdot W_0^{-1} \cdot (\frac{\delta pO_2}{\delta t})]$

211 Where βO_2 is the coefficient for O_2 solubility in water ($\text{mg of } O_2 \cdot \text{L}^{-1}$); V_{RE} , the
212 respirometer volume (L); W_0^{-1} , the animal body mass (Kg); and $(\frac{\delta pO_2}{\delta t})$ represents O_2
213 decline over time ($\% \text{ of } O_2 \cdot \text{h}^{-1}$).

214 $\dot{M}O_2$ was analyzed and plotted at each level of progressive hypoxia. Routine
215 metabolic rate (RMR) was estimated as the mean rate of O_2 consumption at rest in
216 normoxia ($PwO_2 = 100 \%$); RMR estimates the aerobic energy expended for minimal
217 activity at rest (Krogh, 1914). O_2 crit values were evaluated for each experimental group
218 by regression (SigmaPlot 12.5, Systat Software Inc) according to Bilberg and coworkers
219 (2010).

220

221 *2.6. Instrumentation and cardiorespiratory recording*

222 As a reference for the physiological adjustments to deal with hypoxia, the
223 cardiorespiratory parameters were recorded during normoxia ($PwO_2 = 100\%$) and deep
224 hypoxia ($PwO_2 = 5 \%$). The instrumentation followed SePM/blank exposure. For that,
225 each fish was anaesthetized by immersion in 0.1% benzocaine in aerated water until loss
226 of equilibrium and spontaneous ventilatory activity. Fish were transferred to an operating
227 table where the gills were continuously irrigated with aerated anesthetic (0.05%
228 benzocaine). A polyethylene catheter (PE 60) was placed dorsally in the buccal cavity
229 and a second catheter (PE 60) was implanted in the distal portion of the opercular
230 cleithrum. Two needle type electrocardiogram (ECG) electrodes were implanted
231 ventrally: one laid between the gills and the heart, and the other close to the pelvic fins.
232 The reference electrode remained in the water of the experimental chamber.

233 Following recovery, the buccal catheter was connected to a pressure transducer
234 (MLT0380/D - ADInstruments) to amplify and continuously record buccal pressure
235 signals (Bridge Amp/Powerlab - ADInstruments) and derive instantaneous respiratory

236 frequency (f_R , on Labchart - ADInstruments). Inspired water (PiO_2) was sampled from
237 the respirometry chamber and expired water (PeO_2) was sampled continuously from the
238 opercular catheter. The ECG leads were connected to an Animal bioAmp/Powerlab
239 (ADInstruments), to amplify and continuously record ECG, to calculate heart rate (f_H).

240 Gill ventilation volume ($\dot{V}_G - LH_2O \cdot kg^{-1} \cdot min^{-1}$) was calculated using the Fick
241 principle:

242 (2) $\dot{V}_G = [\dot{M}O_2 / (C_iO_2 - C_eO_2)]$, where:

243 C_iO_2 represents the inspired O_2 concentration (the average oxygen level in the
244 respirometer chamber); and C_eO_2 represents the expired O_2 concentration (the average
245 oxygen level recorded in the water from operculum cannula – Cech and Brauner, 2011).

246 The mean respiratory frequency ($f_R - breaths \cdot min^{-1}$) was measured from the
247 buccal pressure variation during 1 minute. Ventilatory tidal volume ($V_T - L \cdot kg^{-1} \cdot breath^{-1}$)
248 was calculated as the ratio between gill ventilation and the respiratory frequency

249 (3) $V_T = \dot{V}_G / f_R$

250 Oxygen extraction from the ventilatory current was calculated as:

251 (4) $EO_2 = (PiO_2 - PeO_2) / PiO_2 \cdot 100$

252

253 *2.7. Statistical analysis*

254 All data were analyzed for normality (Shapiro-Wilk). Differences in morphologic
255 parameters between experimental groups (Control and SePM) were analyzed by Student's
256 T-test. Mean values of each cardiorespiratory variable were compared between groups
257 and O_2 tensions (normoxia and hypoxia) using TwoWay ANOVA and Student-Newman-
258 Keuls (SNK). Tests were considered significant at the 95% level of confidence ($P < 0.05$).

259 Data are presented as means \pm SE. All data were analyzed with R v.4.1.1 (R Core Team
260 2021).

261

262 3. Results

263 *SePM contamination*

264 Contamination with raw SePM resulted in the presence of a wide variety of metal
265 species in the water. Metal concentrations in the contaminated water were mainly
266 constituted by iron ores and aluminum, plus titanium and cerium (Table 1). Despite that,
267 in our experiment, 96h exposure to environmentally relevant concentration of SePM did
268 not result in fish death.

269 **Table 1. Metal concentrations ($\mu\text{g} \cdot \text{L}^{-1}$) in the experiment**
270 **water in Control (clean water) and SePM (exposed) groups**
271

Metal	Control	SePM
B	<LOD	<LOQ
Al	<LOQ	645.82 \pm 1.11
Ti	<LOQ	114.66 \pm 2.81
V	1.05 \pm 0.02	4.36 \pm 0.007
Cr	<LOQ	1.51 \pm 0.02
Mn	<LOD	44.12 \pm 0.31
Fe-56	<LOD	627.44 \pm 5.72
Ni	<LOD	0.50 \pm 0.005
Cu	4.73 \pm 0.07	18.79 \pm 0.08
Zn	7.12 \pm 0.05	35.73 \pm 0.09
As	<LOD	<LOD
Se	<LOD	<LOD
Rb	10.10 \pm 0.08	10.49 \pm 0.07
Sr	22.12 \pm 0.31	26.95 \pm 0.15
Y	<LOD	1.51 \pm 0.01
Zr	<LOQ	1.65 \pm 0.05
Nb	<LOD	<LOQ
Ag	0.22 \pm 0.004	0.19 \pm 0.004
Cd	7.18 \pm 0.16	<LOD
Sn	<LOD	<LOD
Ba	0.57 \pm 0.01	0.33 \pm 0.001
La	<LOD	1.64 \pm 0.02
Ce	<LOD	3.57 \pm 0.02
Ta	<LOD	<LOD
W	0.10 \pm 0.01	<LOQ
Hg-201	<LOD	<LOD
H-202	<LOD	<LOD

Pb	<LOQ	14.79 ± 0.18
Bi	<LOD	<LOD

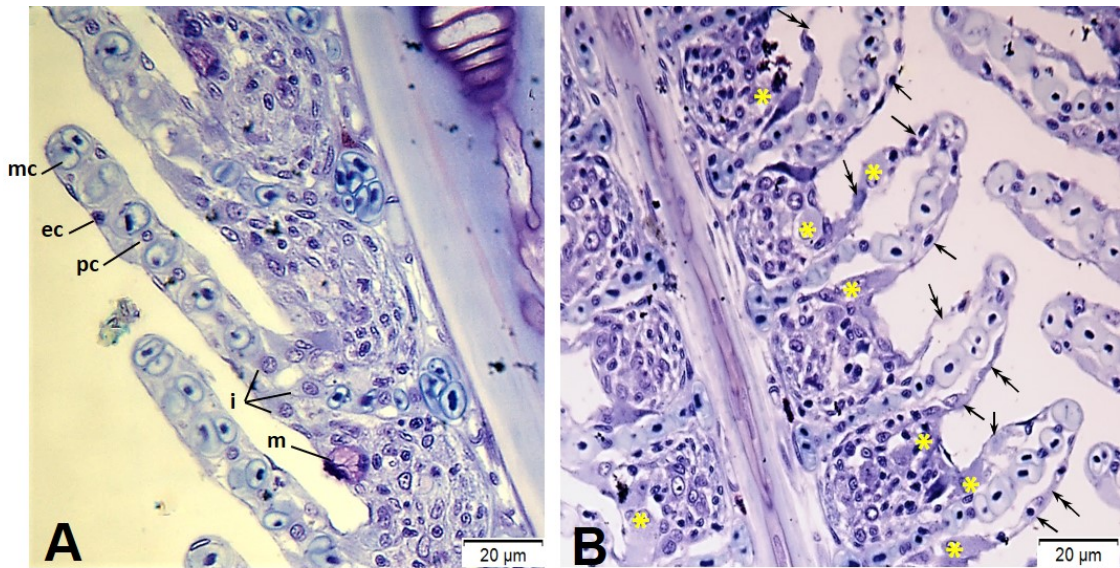
272 **Values are mean ± SEM. LOD: limit of detection. LOQ: limit of**
273 **quantification. Limit of detection (LOD, µg· L⁻¹): B 0.05, Mn 0.51,**
274 **Fe 13.0, Ni 0.22, As 0.23, Se 0.52, Y 0.0, Nb 0.0, Sn 0.02, La**
275 **0.01, Ce 0.0, Ta 0.02, Hg-201 0.06, Hg-202 0.04, and Bi 0.02.**
276 **Limit of quantification (LOQ, µg· L⁻¹): B 0.15, Al 12.8, Ti**
277 **0.22, Cr 0.16, Zr 0.06, Nb 0.02, W 0.02, and Pb 0.0.**
278

279 *SePM affected gill structure*

280 In Control group, it is possible to observed the normal aspect of gill epithelium
281 (Figure 1A) with epithelial cells (ec), ionocytes (i), marginal channel (mc), and mucous
282 cells (m). The mean epithelial thickness was 43.69 ± 0.69 µm in the filament (Figure 2A).
283 The distance between lamellae mean 26.49 ± 1.41 µm (Figure 2B), and lamellar height,
284 106.59 ± 0.46 µm (Figure 2C).

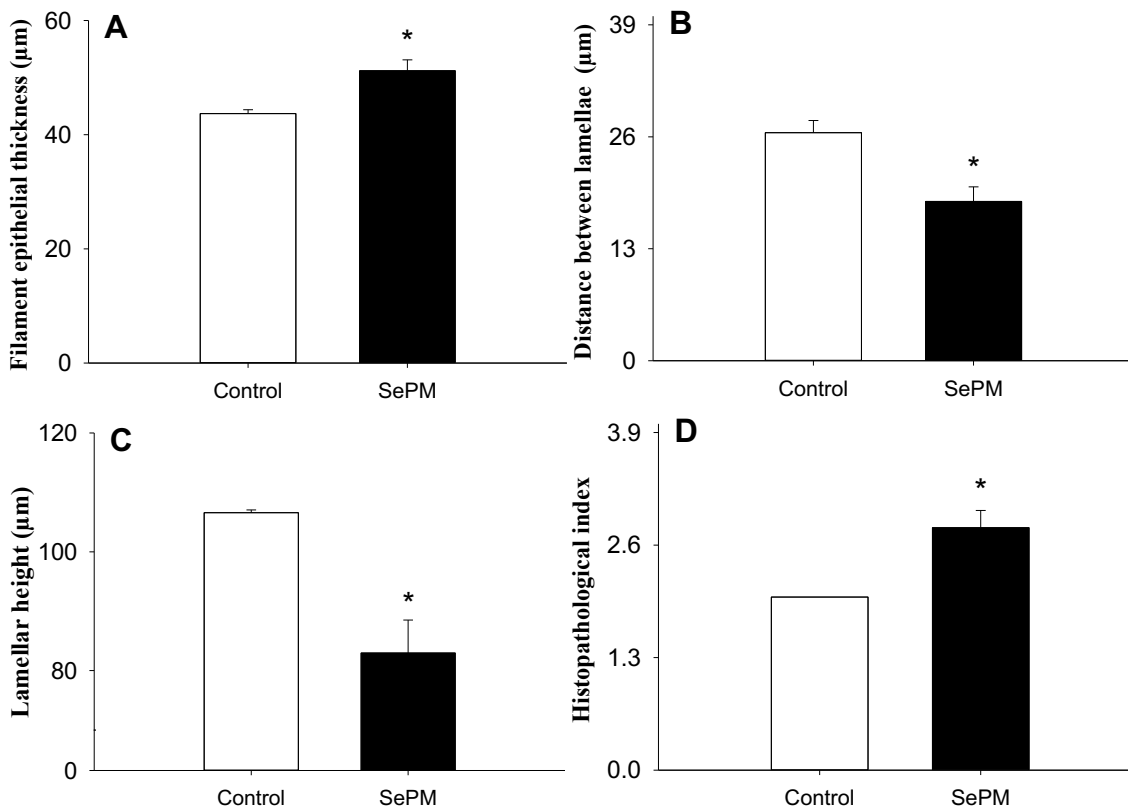
285 SePM contamination affected gill tissue and histopathological alterations were
286 observed. The most frequent alterations were epithelial cells hypertrophy, epithelial
287 displacement, and ionocytes proliferation (Figure 1B). The contamination resulted in
288 15 % increase in filament epithelial thickness (51.20 ± 1.91 µm, p = 0.003 - Figure 2A);
289 30 % reduction in distance between lamellae (18.50 ± 1.69 µm, p = 0.001 - Figure 2B);
290 and a 22 % reduction in lamellar height (82.97 ± 5.55 µm, p = 0.001 - Figure 2C). The
291 observed alterations resulted in higher histopathological index (Figure 2D).

292



293
 294 **Figure 1. Morphology of Nile tilapia gills. (A) normal gill structure with their respective**
 295 **structures; (B) single arrow indicates hypertrophy of epithelial cells, double arrow**
 296 **indicates epithelial displacement, and asterisk indicates proliferation of ionocytes.**
 297 **Abbreviations: pc = pillar cells, mc = marginal channel, ec = epithelial cell, m = mucous**
 298 **cell, and i = ionocyte. Scale bar = 20μm.**

299



300

301

302

303

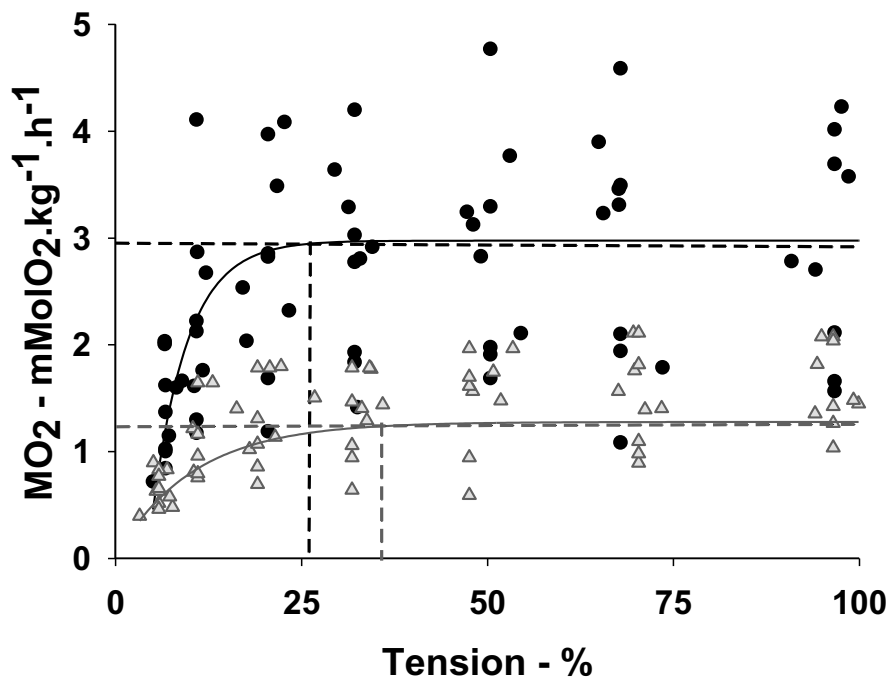
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305

Figure 2. Gill morphometric variables: filament epithelial thickness (A), distance between lamellae (B), lamellar height (C) and histopathological index (D) in Nile tilapia (*Oreochromis niloticus*) exposed to settleable atmospheric particulate matter (SePM) for 96 h. Bars represent means ± standard error. * denotes mean difference (Student's t-test, P < 0.05).

306 **Physiology:**

307 Resting normoxic RMR was not different between experimental groups.
308 Regardless of treatment, $\dot{M}O_2$ was maintained nearly constant until O_2 tension was
309 reduced to about 25-35%. In both groups, it was possible to identify an O_{2crit} , and below
310 that, $\dot{M}O_2$ was reduced progressively with oxygen saturation (Figure 3). The ranges of
311 oxyregulation were different. SePM exposure affected the capacity to deal with hypoxia,
312 and O_{2crit} was 31% higher in the SePM group than in Control group (34% and 26%,
313 respectively - Figure 3).



314 **Figure 3. Oxygen uptake (MO_2 - $mmolO_2 \cdot kg^{-1} \cdot h^{-1}$) and O_{2crit} estimation in**
315 **Nile tilapia, *Oreochromis niloticus*, in progressive hypoxia. Symbols represent**
316 **Control (black circle) and SePM (grey triangles) groups. Horizontal dashed**
317 **lines indicate routine metabolic rate in each group. Continuous lines indicate**
318 **the fitted regression of the metabolic alteration during progressive hypoxia.**
319 **Vertical dashed lines indicate the critical oxygen (O_{2crit}) in each group (26%**
320 **and 34% in Control and SePM group, respectively).**
321

322
323 SePM contamination resulted in 45% decrease in total gill ventilation in normoxia
324 (Control $\dot{V}_G = 36.69 \pm 4.88$ and SePM $\dot{V}_G = 20.19 \pm 1.32$ $LH_2O \cdot kg^{-1}$, $p = 0.004$ - Fig
325 4A). Nevertheless, f_R was 12% higher in the SePM group, even in normoxia, increasing

326 it from 59.64 ± 2.21 to 66.81 ± 2.95 breaths \cdot min⁻¹ ($p = 0.022$ - Fig 4B). Despite those
327 alterations, the ventilatory tidal volumes were similar between groups in normoxia ($\dot{V}_T =$
328 0.84 ± 0.16 and 0.74 ± 0.24 LH₂O \cdot Kg⁻¹ \cdot breaths min⁻¹, $p = 0.126$, Fig 4C).

329 The reduction in oxygen availability induced an increase in the ventilatory effort,
330 and therefore, \dot{V}_G , f_R , and \dot{V}_T increased in both groups. However, SePM-contaminated
331 fish had a limited rise in gill ventilation, -21% lower than Control fish (Control $\dot{V}_G =$
332 344.62 ± 40.33 and SePM $\dot{V}_G = 272.89 \pm 25.40$ LH₂O \cdot kg⁻¹, $p < 0.001$ - Fig 4A).
333 Nevertheless, under hypoxia, contaminated fish were capable of increasing f_R as much as
334 Control fish (Control $f_R = 71.64 \pm 3.00$ and SePM $f_R = 71.44 \pm 4.28$, $p = 0.491$ - Fig 4B).
335 SePM exposition tend to reduce \dot{V}_T in hypoxia (Control $\dot{V}_T = 6.28 \pm 1.74$ and SePM \dot{V}_T
336 $= 4.03 \pm 0.57$ LH₂O \cdot Kg⁻¹ breaths min⁻¹, $p = 0.781$ - Fig 4C). However, \dot{V}_T variability
337 was high and no difference we observed between experimental groups ($p = 0.781$).

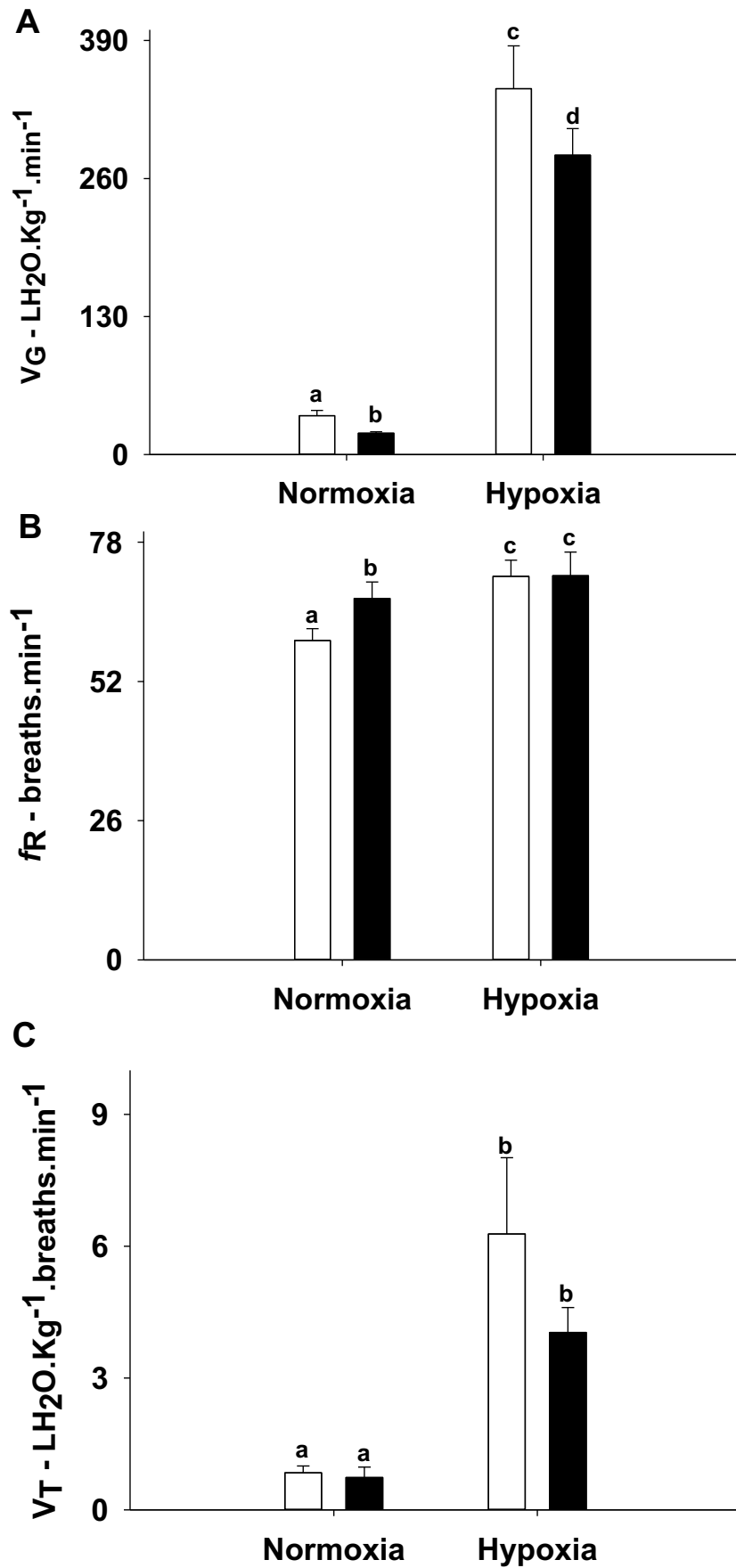
338 Oxygen extraction was 32% higher in SePM group than in Control in normoxia
339 (Control $EO_2 = 57.71 \pm 3.79$ and SePM $EO_2 = 76.40 \pm 3.25$ %, $p = 0.001$ - Fig 5A). Both
340 groups had EO_2 reduced after the ventilatory increase in deep hypoxia, down to $40.10 \pm$
341 3.18 and 46.64 ± 4.61 %, respectively ($p < 0.001$, Fig 5A). However, the EO_2 in SePM
342 group was 16 % higher than Control in deep hypoxia ($p = 0.001$ - Fig 5A). Heart rate was
343 13% reduced in normoxic SePM fish (Control $f_H = 39.22 \pm 1.99$ and SePM $f_H = 34.00 \pm$
344 1.47 bpm, $p = 0.021$ - Fig 5C). Both groups developed the same level of hypoxic
345 bradycardia (Control $f_H = 22.35 \pm 1.67$ and SePM $f_H = 24.21 \pm 1.53$ bpm, $p = 0.253$ - Fig
346 5C).

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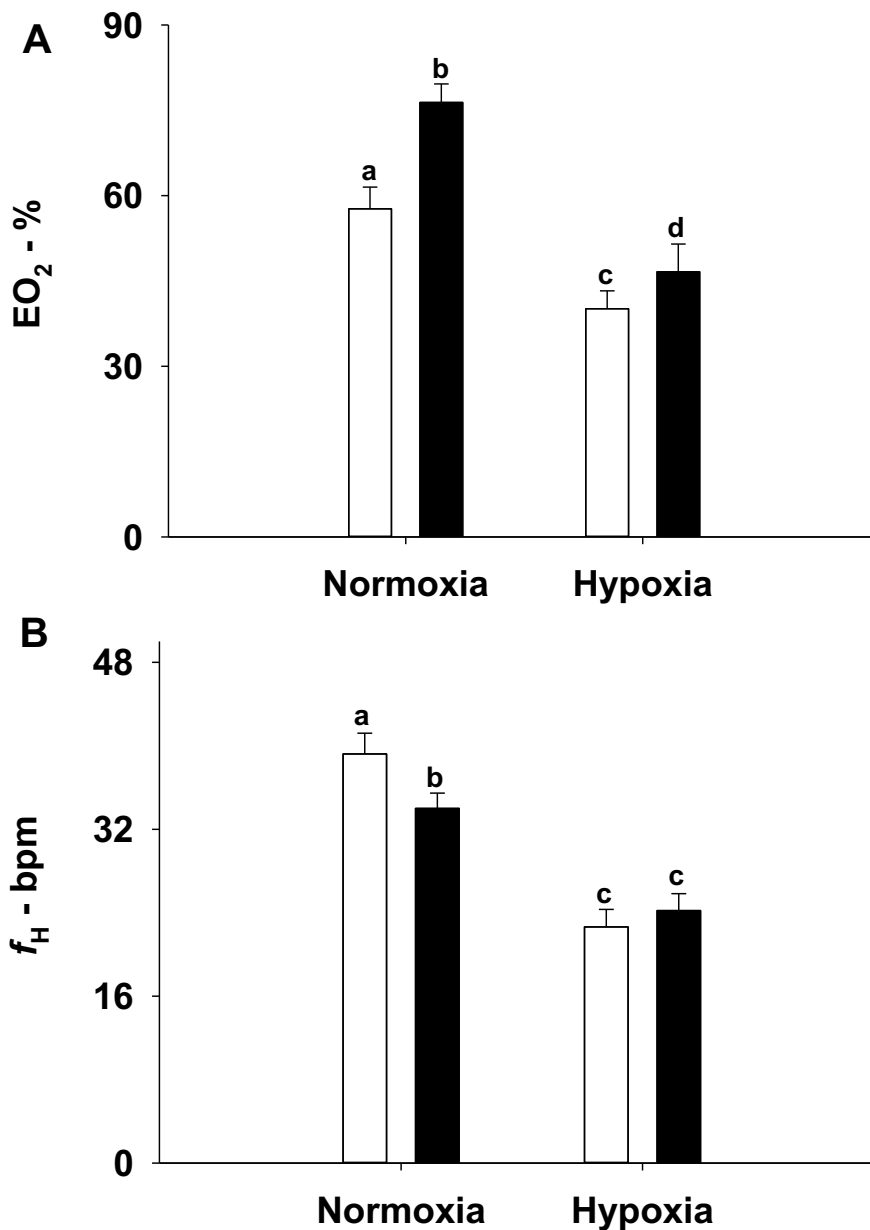
354

355

Figure 4. Ventilatory variables of Nile tilapia, *Oreochromis niloticus*, in normoxia and deep hypoxia. Bars represent mean values \pm standard error in Control (white bar) and SePM

356 (black bar) groups. Panels: A. Gill ventilation, $\dot{V}_G - \text{LH}_2\text{O} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; B. Respiratory
 357 frequency, $f_R - \text{breaths} \cdot \text{min}^{-1}$; C. Ventilatory tidal volume, $V_T - \text{LH}_2\text{O} \cdot \text{kg}^{-1} \cdot \text{breaths} \cdot \text{min}^{-1}$.
 358 Different letters denote different means. Two-Way ANOVA and SNK, $P < 0.05$, $n = 10$.

359



360

361

362 **Figure 5. Oxygen extraction (EO₂) and heart rate (f_H) values of Nile tilapia, *Oreochromis***
 363 ***niloticus*, in normoxia and deep hypoxia. Bars represent mean values ± standard error in**
 364 **Control (white bar) and SePM (black bar) groups. Panels: A. Oxygen extraction, EO₂ - %;**
 365 **B. Heart rate, f_H – bpm. Different letters denote different means. Two-Way ANOVA and**
 366 **SNK, $P < 0.05$, $n = 10$.**

367

368

369 **4. Discussion**

370 *Settleable Atmospheric Particulate Matter - SePM*

371 In this study, we observed that an ecologically relevant concentration of SePM
372 caused functional impacts in fish. SePM is composed by a highly complex mixture of
373 different-sized particles varying in shape and composition. The particles are composed
374 by both organic and inorganic materials with the latter including diverse metallic elements
375 (Souza et al., 2021b, 2021a, Soares et al., *submitted*). It is hard to predict the final effect
376 of such a complex mixture or even identify the main component causing the observed
377 alterations, even considering the knowledge regarding individual metal contamination.
378 The difficulty is caused by the combination of multiple factors: the number of metallic
379 species, the multiple possible combinations among metallic/metalloid elements, and the
380 fact that some metals are present at nanometric scale (Salgado, 2003). At the moment, the
381 only way to replicate that contamination and assess its effect is by using raw SePM
382 samples. In order to standardize our evaluation of the toxic effects of SePM, we have
383 followed a previously used methodology (Soares et al., *submitted*). We used samples from
384 pooled SePM (collected over a year), and SePM concentration that mimics the level of
385 water contamination ($1 \text{ g}\cdot\text{L}^{-1}$ of the raw SePM) reported for the aquatic environment near
386 Vitoria city ES-Brazil (Institute of Environment and Hydric Resources – IEMA, 2021;
387 Souza et al., 2021b, 2021a).

388 Recent metal identification in natural water bodies of Santa Cruz and Vitória Bay
389 (ES-Brazil) demonstrated a variable but sustained presence of SePM deposited by air-to-
390 water cross-contamination (Souza et al., 2018b, 2019). The APM production by the local
391 industrial complex may vary over time; however, it is constant delivered (IBEF, 2011;
392 Santos, et al., 2017). Most part of the SePM mass was composed by Fe and Al, yet their
393 values in water were within LC_{50} for each metal (Fe, 1.46-1.71 and Al, 0.095-235 mg L^{-1}

394 ¹, Kennedy, 2011). They were below the maximum limits established by the Brazilian
395 regulatory agency CONAMA (357/2005 – Fe, 0.3-5.0; Al, 0.1-0.2 mg. L⁻¹) for Brazilian
396 freshwater bodies of classes 1 to 3, referred to as ‘protected for aquatic communities,
397 aquaculture, fishing, and supply’.

398

399 *SePM affects fish gills and ventilation*

400 There was 100% fish survival following 96h of exposure to SePM, confirming
401 that the experimental protocol was sublethal. However, the SePM exposition led to an
402 early but evident process of gill damage, which started a significant reduction in the
403 performance to cope with hypoxia.

404 Gills are commonly the first target of diverse water pollutants due to its
405 characteristic contact with the external environment (Perry and Laurent, 1993). Gill
406 damage usually leads to relevant harm in fish physiology since it would affect diverse
407 functional systems and might inflict severe long-term impact. Metals have been reported
408 to cause alterations on mucous cells, chloride cells (Mallatt, 1985), and epithelial edema
409 (Sola et al., 1995; Campbell et al., 1999). Both dissolved metals and metallic
410 nanoparticles have also been reported to cause gills damage (Shaw, 2011), affecting
411 diverse functions such as respiratory functions (Ni – Pyle et al., 2002; Cu – Wilson and
412 Taylor, 1993), acid–base balance, ammonia excretion (Cu – Wilson and Taylor, 1993;
413 Taylor et al., 1996), and osmoregulation (Cd – Mccarty; Houston, 1975; Giles, 1984).

414 The gill alterations observed in the present experiment are early indicatives of
415 histopathology, such as the reduction of lamellar structure (distance and height) and may
416 work as a mechanism to increase the tissue barrier. The gill alterations typically
417 associated with metal contamination have generally been described as both transitory and
418 permanent. Epithelial lifting, hyperplasia, hypertrophy of the epithelial cells, and partial

419 fusion of some secondary lamellae are considered transitory alterations, serving as a
420 mechanism to increase the barrier against the entrance of contaminants (Hadi and Alwan,
421 2012). While other alterations such as necrosis, lamellar rupture, and shedding of gill
422 epithelium are considered permanent injuries (Abdel-Khalek, et al. 2016).

423 Although this first response seems to help avoid the progression of contamination,
424 it also results in limiting oxygen uptake since they cause the increase of the diffusion
425 distance and the reduction of the respiratory area (Wilson and Taylor, 1993; Abdel-
426 Khalek, et al. 2016). Filament thickening together with epithelial hypertrophy and
427 displacement may reflect an inflammatory reaction due to tissue lesions. Such symptoms
428 are likely to impact O₂ uptake. A detailed description of that effect in the water-blood
429 barrier and its duration are beyond the scope of our study and should be considered in a
430 dedicated investigation.

431 The observed alterations in gill tissue also complement the results previously
432 observed by Soares and coworkers (*submitted*), who reported stress response and
433 hematocrit reduction after SePM. Stress is likely to follow tissue lesion, mainly if pain is
434 associated with the process. Besides, we can assume that hematocrit reduction would be
435 an expected result of the combination of several small bleeding points in damaged gill
436 tissue and hyperventilation due to the O₂ uptake impairment.

437 Resting normoxic-tilapia had no alteration in the overall oxygen uptake after 96h
438 in SePM-contaminated water. However, SePM induced an increase in f_R and EO_2 and f_H
439 decrease. Those alterations were present even at rest in normoxia and suggest a
440 cardiorespiratory compensation for a limitation in oxygen uptake, since they are the
441 typical alteration observed in hypoxia response. Soares and coworkers (*submitted*) have
442 reported that the same level of SePM contamination also reduced tilapia hematocrit (-
443 12%), hemoglobin (-26%), and red blood cell counts (-38%). This reduction in oxygen-

444 carrying capacity likely generated the due hypoxemia and caused the increase in
445 ventilatory drive (Leite et al., 2007; Zeraik et al., 2013).

446 The combination of higher f_R with both reduced f_H and oxygen-carrying capacity
447 is intriguing. If f_H and O_2 carrying capacity are lower, fish might have a higher stroke
448 volume to maintain routine oxygen uptake and oxygen supply to tissues (Wang and Hicks,
449 2002; Wells, 2009). Stroke volume and pulse pressure increase are associated to hypoxic
450 bradycardia and they are assumed to enhance lamellar perfusion to increase the effective
451 area for gas exchange (Farrell, 2007; Reid et al., 2006). Such alterations may open poorly
452 perfused vascular spaces in the gill lamellae (Randall, 1982), and also recruit unperfused
453 lamellae (Farrell et al., 1980). That mechanism would take account for the observed
454 higher EO_2 in SePM-contaminated fish. Furthermore, a higher arteriovenous O_2
455 difference could also contribute to the elevated EO_2 with decreased f_H . The combination
456 among higher f_R and EO_2 with lower f_H indicates that alterations might be working in the
457 present case, even when the fish is resting in normoxia.

458 Another relevant aspect is that the expected ventilatory adjustment to support a
459 higher ventilatory drive is \dot{V}_G increasing (Fernandes and Rantin, 1989). The fact that f_R
460 was increased while \dot{V}_G was reduced is a functional evidence of gill damage. In such a
461 case, increasing \dot{V}_T would have limiting advantages since breathing area would be
462 reduced. Therefore, the hyperventilation of the healthy areas is required, and f_R increasing
463 would reflect that mechanism.

464

465 *SePM limits the performance to cope with hypoxia*

466 An impaired respiratory surface is likely to require higher ventilatory work and
467 blood perfusion to satisfy tissue demands for oxygen, particularly during environmental
468 hypoxia. Since SePM exposure affected routine ventilation, it also impacted the

469 ventilatory strategy to cope with hypoxia. The Control fish evoked the expected
470 cardiorespiratory alteration in hypoxia. The capacity to perform those adjustments is
471 responsible to maintain $\dot{M}O_2$ stable until O_{2crit} . Therefore, a limited capacity is expected
472 to reduce the oxyregulation area in the progressive hypoxia trial.

473 Tilapia is a very hypoxia-resistant species, previous reports of O_{2crit} vary between
474 16 and 30 mmHg (0.84 to 1.6 $mgO_2 \cdot L^{-1}$ – Fernandes and Rantin, 1987; 1989, and Martins
475 et al., 2011). The variation in estimated O_{2crit} may reflect differences in animal size,
476 temperature, hypoxia protocol, and calculation methods (Rogers et al. 2016).
477 Nonetheless, our data demonstrate that SePM changed tilapia capacity to cope with
478 hypoxia. We observed that SePM-contaminated fish had higher O_{2crit} , which rise from 26
479 to 34% (1.84 to 2.76 $mgO_2 \cdot L^{-1}$), reflects the problem.

480 Environmental hypoxia is a common, daily naturally occurring phenomenon in
481 freshwater ecosystems (Richards, 2009). Therefore, it is a severe and unavoidable
482 physiological challenge that can exert strong selective effects on aquatic organisms
483 (Graham, 1997). Hypoxic events may vary in frequency and severity according to factors
484 such as photosynthesis, water column depth, salinity, temperature, tide, and organic
485 matter (Nezlin et al. 2009). The higher O_{2crit} of the SePM-contaminated fish means a
486 reduced scope for activity when the oxygen level is low in environment (Vitoria Bay -
487 Claireaux and Chabot, 2016). This could have knock-on effects on many activities that
488 underpin fish population dynamics, notably space use, foraging and feeding, intraspecific
489 interactions, and so forth. The observed effect is especially relevant if we consider the
490 short-term contamination used in our protocol. The progression in tissue damage might
491 provide long-term gill alteration changing fish physiology or affect energy allocation due
492 to the respiratory cost of ventilation.

493

494 *SePM may affect energy allocation*

495 SePM exposure increased f_R . Since such compensation was associate to
496 maintenance of resting metabolism, the energy allocation might be different. We can
497 safely assume that a higher portion of resting energy might be allocated to support
498 breathing in SePM-contaminated fish. Modulating f_R to meet oxygen demands is
499 energetically expensive (Rantin et al. 1992). The cost of water ventilation has been
500 experimentally estimated and may vary from 2 to 50% according to fish species and their
501 morphophysiological characteristics (Shelton, 1970; Boehlert et al., 1991). In tilapia,
502 ventilation has been estimated to consume about 3% of routine aerobic metabolism, even
503 under normoxic conditions. In hypoxia, this can rise to 10% of total aerobic energy use
504 (Fernandes and Rantin, 1994) therefore accounting for significant energy loss.

505 A permanently higher ventilation cost in normoxia and its increasing during
506 hypoxia events potentially have an important long-term impact on energy usage for body
507 maintenance and also, to support high demand challenges, such as growth, reproduction,
508 post-prandial metabolic increment, swimming, and others. We can predict that unless
509 other relevant functional compensations restore ventilatory capacity in fish under chronic
510 contamination, this will potentially affect animal fitness and the stability of the population
511 in the natural environment. We suggest that despite sublethal, the level of contamination
512 tested is not innocuous.

513

514 *Final considerations*

515 This was the first investigation to evaluate the physiological responses of fish to
516 hypoxia after of air-to-water cross-contamination by SePM from the iron ore processing
517 industry. We observed that after 96h, SePM exposure caused significant alterations on gill
518 tissue, leading to changes in cardiorespiratory variables, which caused a direct impact on

519 the efficiency to deal with hypoxia. That limitation might extend to other challenges such
520 as swimming, foraging, species interaction, and others. Therefore, SePM exposure,
521 although not leading to immediate death, can potentially impact fish populations. The
522 current result substantiates the need for extensive dedicated studies on the effects of SePM
523 on aquatic fauna.

524

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530

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539

540 **References**

- 541 Abdel-Khalek, A.A., Badran, S.R., Marie, M.A.S., 2016. Toxicity evaluation of copper oxide
542 bulk and nanoparticles in Nile tilapia, *Oreochromis niloticus*, using hematological,
543 bioaccumulation and histological biomarkers. *Fish Physiol. Biochem.* 42, 1225–1236.
544 <https://doi.org/10.1007/s10695-016-0212-8>.
- 545 Ahmad, M. K., Islam, S., Rahman, M. S., Haque, M. R., and Islam, M.M., 2010. Heavy Metals
546 in Water , Sediment and Some Fishes of. *Int. J. Environ. Resarch* 4, 321–332.

- 547 Arantes, F.P., Savassi, L.A., Santos, H.B., Gomes, M.V.T., Bazzoli, N., 2016. Bioaccumulation
548 of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a
549 large commercially valuable catfish species from Brazil. *An. Acad. Bras. Cienc.* 88, 137–
550 147. <https://doi.org/10.1590/0001-3765201620140434>
- 551 Arrivabene, H.P., Souza, I. da C., C6, W.L.O., Conti, M.M., Wunderlin, D.A., Milanez, C.R.D.,
552 2015. Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and
553 bioaccumulation of three mangrove plant species in Brazil. *Chemosphere* 127, 27–34.
554 <https://doi.org/10.1016/j.chemosphere.2015.01.011>
- 555 Begum, A., Harikrishna, S., Khan, I., 2009. Analysis of heavy metals in water, sediments and fish
556 samples of Madivala lakes of Bangalore, Karnataka.pdf. *Int. J. ChemTech Res.* 1, 245–249.
- 557 Bilberg, K., Malte, H., Wang, T., Baatrup, E., 2010. Silver nanoparticles and silver nitrate cause
558 respiratory stress in Eurasian perch (*Perca fluviatilis*) 96, 159–165.
559 <https://doi.org/10.1016/j.aquatox.2009.10.019>
- 560 BRASIL. 2005. Conama Resolu73o7n n 357, Di3rio Oficial da Uni73o7n, 18 de mar73o de 2005.
561 Available in: <http://www.mma.gov.br/port/conama/res/res05/res35705.pdf>
- 562 BRASIL. 2018. Conama Resolu73o7n n 491, Di3rio Oficial da Uni73o7n, 19 de novembro de 2018.
563 Available in: <http://www2.mma.gov.br/port/conama/legiabre.cfm?cod>
- 564 Burggren, W.; Mendez-Sanchez, J.F.; Bautista, G.M.; Pe73a, E.; Garc3a, R.M.; Gonz3lez, C.A.A.,
565 2019. Developmental changes in oxygen consumption and hypoxia tolerance in the heat and
566 hypoxia-adapted tabasco line of the Nile tilapia *Oreochromis niloticus*, with a survey of the
567 metabolic literature for the genus *Oreochromis*. *J Fish Biol* 94, 732–744.
- 568 Call3n, M.S., de la Cruz, M.T., L3pez, J.M., Navarro, M. V., Mastral, A.M., 2009. Comparison
569 of receptor models for source apportionment of the PM10 in Zaragoza (Spain).
570 *Chemosphere* 76, 1120–1129. <https://doi.org/10.1016/j.chemosphere.2009.04.015>
- 571 Campbell P.G.S., 1995. Interactions between trace metals and aquatic organisms: a critique of the
572 free-ion activity model. In: Tessier A, Turner DR, editors. *Metal speciation and*
573 *bioavailability in aquatic systems*. New York, USA: Wiley 45-102.
- 574 Cech, J., Brauner, C., 2011. Techniques in Whole Animal Respiratory Physiology, *Encyclopedia*
575 *of Fish Physiology: From Genome to Environment*. Elsevier Inc.
576 <https://doi.org/10.1016/B978-0-1237-4553-8.00128-3>
- 577 Claireaux, G., Chabot, D., 2016. Responses by fishes to environmental hypoxia: integration
578 through Fry's concept of aerobic metabolic scope. *J. Fish Biol.* 88 (1), 232–251.
- 579 Dang Z, Lock R.A.C, Flik G., Wendelaar bonga S.E., 1999. Metallothionein response in gills of
580 *Oreochromis mossambicus* exposed to copper in fresh water. *Am J Physiol* 277, 320–331
- 581 Demirak, A., Yilmaz, F., Levent Tuna, A., Ozdemir, N., 2006. Heavy metals in water, sediment
582 and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. *Chemosphere* 63,
583 1451–1458. <https://doi.org/10.1016/j.chemosphere.2005.09.033>
- 584 Farag, A.M., Nimick, D.A., Kimball, B.A., Church, S.E., Harper, D.D., Brumbaugh, W.G., 2007.
585 Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish
586 in the boulder river watershed, Montana, and the role of colloids in metal uptake. *Arch.*
587 *Environ. Contam. Toxicol.* 52, 397–409. <https://doi.org/10.1007/s00244-005-0021-z>
- 588 Farrell, A. P., Sobin, S. S., Randall, D. J. and Crosby, S. (1980). Intralamellar blood flow patterns
589 in fish gills. *Am. J. Physiol.* 239, R428R436.
- 590 Farrell, A. P. Tribute to P. L. Lutz: a message from the heart - why hypoxic bradycardia in fishes?
591 *Journal of Experimental Biology* 210, 1715–1725 (2007).
- 592 Fernandes, C., Font3inhas-Fernandes, A., Cabral, D., Salgado, M.A., 2008. Heavy metals in
593 water, sediment and tissues of *Liza saliens* from Esmoriz-Paramos lagoon, Portugal.

- 594 Environ. Monit. Assess. 136, 267–275. <https://doi.org/10.1007/s10661-007-9682-6>
- 595 Ferandes, M.N.; Rantin, F.T., 1989. Respiratory responses of *Oreochromis niloticus* (Pisces,
596 Cichlidae) to environmental hypoxia under different thermal conditions. J. Fish Biol. 35,
597 509–519.
- 598 Fernandes, M.N., Rantin, F.T., 1994. Relationships between oxygen availability and metabolic
599 cost of breathing in Nile tilapia (*Oreochromis niloticus*): aquacultural consequences.
600 Aquaculture 127, 339–346. [https://doi.org/10.1016/0044-8486\(94\)90236-4](https://doi.org/10.1016/0044-8486(94)90236-4)
- 601 Giacomini, M., Bryant, H.J., Val, A.L., Schulte, P.M., Wood, C.M., 2019. The osmorepiratory
602 compromise: Physiological responses and tolerance to hypoxia are affected by salinity
603 acclimation in the euryhaline Atlantic killifish (*Fundulus heteroclitus*). J. Exp. Biol. 222.
604 <https://doi.org/10.1242/jeb.206599>
- 605 Giles M.A., 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo*
606 *gairdneri*) during chronic exposure to cadmium. Can J Fish Aquat Sci 1678–85.
- 607 Goldin, A., 1987. Communications in Soil Science and Plant Analysis Reassessing the use of
608 loss - on - ignition for estimating organic matter content in noncalcareous soils. Commun.
609 Soil Sci. Plant Anal. 18, 1111–1116. <https://doi.org/doi/10.1080/00103628709367886>
- 610 Graham, J. B. *Graham Air-Breathing Fishes-Evolution Diversity and Adaptation*. (Academic
611 Press, 1997).
- 612 Hochachka, P. W., Buck, L. T., Doll, C. J. and Land, S. C. 1996. Unifying theory of hypoxia
613 tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack.
614 Proc. Natl. Acad. Sci. USA 93, 9493-9498. doi:10.1073/ pnas.93.18.9493
- 615 IBEF Instituto Brasileiro de Executivos de Finanças – IBEF. <<http://ibefes.ning.com/page/ibefes-faz-visita-tecnica>. (2001).
- 617 Idrus, F.A., Basri, M.M., Rahim, K.A.A., Rahim, N.S.A., Chong, M.D., 2018. Concentrations of
618 Cadmium, Copper, and Zinc in *Macrobrachium rosenbergii* (Giant Freshwater Prawn) from
619 Natural Environment. Bull. Environ. Contam. Toxicol. 100, 350–355.
620 <https://doi.org/10.1007/s00128-018-2270-3>
- 621 Kemp, W.M., Testa, J.M., Conley, D.J., Gilbert, D., Hagy, J.D., 2009. Temporal responses of
622 coastal hypoxia to nutrient loading and physical controls. Biogeosciences 6 (12), 2985–
623 3008.
- 624 Krogh, A., 1914. The quantitative relation between temperature and standard metabolism in
625 animals. Internationale Zeitschrift für Physikalisch-Chemische Biologie 1, 491–508.
- 626 Leite, C.A.C., Florindo, L.H., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2007. Gill
627 chemoreceptors and cardio-respiratory reflexes in the neotropical teleost pacu, *Piaractus*
628 *mesopotamicus*. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 193,
629 1001–1011. <https://doi.org/10.1007/s00359-007-0257-3>
- 630 Li J., Quabius E.S., Wendelaar bonga S.E., Flik G., Lock R.A.C., 1998. Effects of water-borne
631 copper on branchial chloride cells and Na⁺/K⁺ -ATPase activities in Mozambique tilapia
632 (*Oreochromis mossambicus*). Aquat Toxicol 1-11.
- 633 Mallatt J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical
634 review. Can J Fish Aquat Sci 630–48.
- 635 Martins, N.D. et al., 2011. Microcystin-LR: How it affects the cardio-respiratory responses to
636 hypoxia in Nile tilapia, *Oreochromis niloticus*. Chemosphere. 154-159.
- 637 Mccarty L.S., Houston A.H., 1975. Effects of exposure to sublethal levels of cadmium upon
638 water-electrolyte status in the goldfish (*Carassius auratus*). J Fish Biol 11–9.

639 McKenzie, D.J., Blasco, F.R., Belão, T.C., Killen, S.S., Martins, N.D., Taylor, E.W., Rantin, F.T.,
640 2017. Physiological determinants of individual variation in sensitivity to an
641 organophosphate pesticide in Nile tilapia *Oreochromis niloticus*. *Aquat. Toxicol.* 189, 108–
642 114. <https://doi.org/10.1016/j.aquatox.2017.06.001>

643 Mckenzie, D.J., Steffensen, J.F., Taylor, E.W., Abe, A.S., 2012. The contribution of air breathing
644 to aerobic scope and exercise performance in the banded knifefish *Gymnotus carapo* L .
645 1323–1330. <https://doi.org/10.1242/jeb.064543>

646 Nezlin, N.P., Kamer, K, Hyde, J, Stein, E.D., 2009. Dissolved oxygen dynamics in a eutrophic
647 estuary, Upper Newport Bay, Califórnia. *Estuarine, Coastal and Shelf Science.* 82, 139–151

648 OECD Guideline for testing of chemicals. 2019. Test N203: Fish, Acute Toxicity Testing Test.

649 Park, S.S., Kim, Y.J., 2005. Source contributions to fine particulate matter in an urban
650 atmosphere. *Chemosphere* 59, 217–226. <https://doi.org/10.1016/j.chemosphere.2004.11.001>

651 Perry S.F.; Laurent P., 1993. Environmental effects on fish gill structure and function. In: Rankin
652 J.C. & Jensen F.B. (ed.), *Fish Ecophysiology*. Chapman and Hall, London. 231-264.

653 Pyle G.G., SWANSON S.M., LEHMKUHL D.M., 2002. The influence of water hardness, pH,
654 and suspended solids on nickel toxicity to larval fathead minnows (*Pimephales promelas*).
655 *Water Air Soil Pollut* 215–26.

656 Rantin, F. T. et al., 1993. Cardio-respiratory responses in two ecologically distinct erythrinids
657 (*Hoplias malabaricus* and *Hoplias lacerdae*) exposed to graded environmental
658 hypoxia. *Environmental biology of fishes.* 93-97.

659 Rantin, F.T., Kalinin, A.L., Glass, M.L., Fernandes, M.N., 1992. Respiratory responses to
660 hypoxia in relation to mode of life of two erythrinid species (*Hoplias malabaricus* and
661 *Hoplias lacerdae*). *J. Fish Biol.* 41, 805–812. [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8649.1992.tb02708.x)
662 [8649.1992.tb02708.x](https://doi.org/10.1111/j.1095-8649.1992.tb02708.x)

663 R Core Team 2021. R: a language and environment for statistical computing. R Foundation for
664 Statistical Computing. Vienna, Austria

665 Rashed, M.N., 2001. Monitoring of environmental heavy metals in fish from nasser lake. *Environ.*
666 *Int.* 27, 27–33. [https://doi.org/10.1016/S0160-4120\(01\)00050-2](https://doi.org/10.1016/S0160-4120(01)00050-2)

667 Reid, S. G., Sundin, L. and Milson, W. K. 2006. The cardiorespiratory system in tropical fishes:
668 structure, function, and control. In *The Physiology of Tropical Fishes*, Vol. 21, *Fish*
669 *Physiology* (ed. A. L. Val, V. M. F. Almeida-Val and D. J. Randall), pp. 225-275. San Diego:
670 Elsevier.

671 Richards, J.G., 2009. Metabolic and molecular responses of fish to hypoxia. In. “Hypoxia”. 27,
672 444-480. Elsevier.

673 Rogers, N.J., Urbina, M.A., Reardon, E.E., Mckenzie, D.J. Wilson, R.W. 2016. A new analysis
674 of hypoxia tolerance in fishes using a database of critical oxygen level (Pcrit). *Conservation*
675 *Physiology.* 4, 1-19.

676 Rosewarne, P.J., Wilson, J.M., Svendsen, J.C., 2016. Measuring maximum and standard
677 metabolic rates using intermittent-flow respirometry: A student laboratory investigation of
678 aerobic metabolic scope and environmental hypoxia in aquatic breathers. *J. Fish Biol.* 88,
679 265–283. <https://doi.org/10.1111/jfb.12795>

680 Salgado, P.E.T., 2003. Informações gerais e ecotoxicológicas de material particulado, Série
681 *Cadernos de Referência Ambiental* 14.

682 Santos, J.M., Reis, N.C., Galvão, E.S., Silveira, A., Goulart, E.V., Lima, A.T., 2017. Source
683 apportionment of settleable particles in an impacted urban and industrialized region in
684 Brazil. *Environ. Sci. Pollut. Res.* 24, 22026–22039. [https://doi.org/10.1007/s11356-017-](https://doi.org/10.1007/s11356-017-9677-y)
685 [9677-y](https://doi.org/10.1007/s11356-017-9677-y)

- 686 Shaw, B.J., Handy, R.D., 2011. Physiological effects of nanoparticles on fish: A comparison of
687 nanometals versus metal ions. *Environ. Int.* 37, 1083–1097.
688 <https://doi.org/10.1016/j.envint.2011.03.009>
- 689 Shelton, G., 1970. The regulation of breathing in W.S.Hoar and D.J. Randall, editors. *Fish*
690 *Physiology*. 293-359.
- 691 Sola F, Isaia J, Masoni A. 1995. Effects of copper on gill structure and transport function in the
692 rainbow trout *Oncorhynchus mykiss*. *J Appl Toxicol*.
- 693 Sollid, J., De Angelis, P., Gundersen, K., Nilsson, G.E., 2003. Hypoxia induces adaptive and
694 reversible gross morphological changes in crucian carp gills. *J. Exp. Biol.* 206, 3667–3673
- 695 Sollid, J., Nilsson, E., 2006. Plasticity of respiratory structures — Adaptive remodeling of fish
696 gills induced by ambient oxygen and temperature & 154, 241–251.
697 <https://doi.org/10.1016/j.resp.2006.02.006>
- 698 Souza, I. da C., Arrivabene, H.P., Craig, C.A., Midwood, A.J., Thornton, B., Matsumoto, S.T.,
699 Elliott, M., Wunderlin, D.A., Monferrán, M. V., Fernandes, M.N., 2018. Interrogating
700 pollution sources in a mangrove food web using multiple stable isotopes. *Sci. Total*
701 *Environ.* 640–641, 501–511. <https://doi.org/10.1016/j.scitotenv.2018.05.302>
- 702 Souza, I.C., Duarte, I.D., Pimentel, N.Q., Rocha, L.D., Morozesk, M., Bonomo, M.M., Azevedo,
703 V.C., Pereira, C.D.S., Monferrán, M. V., Milanez, C.R.D., Matsumoto, S.T., Wunderlin,
704 D.A., Fernandes, M.N., 2013. Matching metal pollution with bioavailability,
705 bioaccumulation and biomarkers response in fish (*Centropomus parallelus*) resident in
706 neotropical estuaries. *Environ. Pollut.* 180, 136–144.
707 <https://doi.org/10.1016/j.envpol.2013.05.017>
- 708 Souza, I. da C., Morozesk, M., Mansano, A.S., Mendes, V.A.S., Azevedo, V.C., Matsumoto, S.T.,
709 Elliott, M., Monferrán, M. V., Wunderlin, D.A., Fernandes, M.N., 2021c. Atmospheric
710 particulate matter from an industrial area as a source of metal nanoparticle contamination in
711 aquatic ecosystems. *Sci. Total Environ.* 753, 141976.
712 <https://doi.org/10.1016/j.scitotenv.2020.141976>
- 713 Souza, I. da C., Arrivabene, H.P., Azevedo, V.C., Duarte, I.D., Rocha, L.D., Matsumoto, S.T.,
714 Franco, A., Elliott, M., Wunderlin, D.A., Monferrán, M. V., Fernandes, M.N., 2021a.
715 Different trophodynamics between two proximate estuaries with differing degrees of
716 pollution. *Sci. Total Environ.* 770, 144651. <https://doi.org/10.1016/j.scitotenv.2020.144651>
- 717 Souza, I. da C., Morozesk, M., Azevedo, V.C., Mendes, V.A.S., Duarte, I.D., Rocha, L.D.,
718 Matsumoto, S.T., Elliott, M., Baroni, M. V., Wunderlin, D.A., Monferrán, M. V., Fernandes,
719 M.N., 2021b. Trophic transfer of emerging metallic contaminants in a neotropical mangrove
720 ecosystem food web. *J. Hazard. Mater.* 408. <https://doi.org/10.1016/j.jhazmat.2020.124424>
- 721 Suguio, K., 1973. *Introdução à sedimentologia*. São Paulo, SP: USP.
- 722 Taylor E.W., Beaumont M.W., Butler P.J., Mair J., Mujallid M.S.I., 1996. Lethal and sub-lethal
723 effects of copper upon fish: a role for ammonia toxicity? In: Taylor EW, editor. *Toxicology*
724 *of aquatic pollution: physiological, cellular and molecular approaches*. Society for
725 *Experimental Biology Seminar series* Cambridge, UK: Cambridge University Press 86-113.
- 726 Tsai, Y.I., Cheng, M.T., 2004. Characterization of chemical species in atmospheric aerosols in a
727 metropolitan basin. *Chemosphere* 54, 1171–1181.
728 <https://doi.org/10.1016/j.chemosphere.2003.09.021>
- 729 USEPA, 1996. Method 3052: microwave assisted acid digestion of siliceous and organically
730 based matrices. USEPA (United States Environ. Prot. Agency) Washingt.

- 731 Vicente-Martorell, J.J., Galindo-Riaño, M.D., García-Vargas, M., Granado-Castro, M.D., 2009.
732 Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted
733 estuary. *J. Hazard. Mater.* 162, 823–836. <https://doi.org/10.1016/j.jhazmat.2008.05.106>
- 734 Wang, T.; Hicks, J. W., 2002. An integrative model to predict maximum O₂ uptake in animals
735 with central vascular shunts. *Zoology* 105, 45–53.
- 736 Wells, R.M., 2009. Blood-Gas Transport and Hemoglobin function: Adaptations for Functional
737 and environmental Hypoxia. In: Hypoxia. Fish Physiology Vol27. Ed: Richards J.G.; Farrell,
738 A.P.; Brauner, C.J. Elsevier. pp 255- 300.
- 739 Wilson, R.W. & Taylor, E.W. (1993). The physiological responses of freshwater rainbow trout
740 (*Oncorhynchus mykiss*) during acutely lethal copper exposure. *J. comp. Physiol.B*, **163**, 38-
741 47.
- 742 Yoann, T., Sainte, F., Jonathan, M., Denis, C., Arturo, A., 2019. Archimer Effects of of hypoxia
743 on metabolic functions in marine organisms: Observed patterns and modelling assumptions
744 within the context of Dynamic Energy Budget (DEB) theory 143, 231–242.
- 745 Zeraik, V., Belão, T., Florindo, L., Kalinin, A., Rantin, F., 2013. Branchial O₂ chemoreceptors in
746 Nile tilapia *Oreochromis niloticus*: Control of cardiorespiratory function in response to
747 hypoxia. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*
748 166(1), 17 - 25. <https://dx.doi.org/10.1016/j.cbpa.2013.04.024>.
- 749

Supplementary material

Table 1. Cardiorespiratory and metabolic variables of resting Nile tilapia, *Oreochromis niloticus*, in normoxia ($P_wO_2 = 100\%$) and deep hypoxia ($P_wO_2 = 5\%$), at 25°C, in control and SePM exposed (SePM) groups

		Mass (g)	$\dot{M}O_2$ (mMolO ₂ · kg ⁻¹ · h ⁻¹)	\dot{V}_G (mLH ₂ O · kg ⁻¹ · min ⁻¹)	f_R (breaths · min ⁻¹)	V_T (LH ₂ O · Kg ⁻¹ · min ⁻¹)	O ₂ crit (%)	EO ₂ (%)	f_H (bpm)
Control	Normoxia		2.82±0.32	36.69±4.88	59.64±2.21	0.84±0.16	26	57.71±3.79	39.22±1.99
	Deep Hypoxia	110 ± 87	1.34±0.15	356.41±38.57 [#]	71.64±3.00 [#]	6.28±1.74		40.10±3.18 [#]	22.35±1.69 [*]
SePM	Normoxia		3.20±0.24	20.19±1.32 [*]	66.81±2.95 [*]	0.74±0.24	34	76.40±3.25 [*]	34.00±1.47 [#]
	Deep Hypoxia		1.26±0.09	246.46±20.22 ^{**}	71.44±4.28 [#]	4.03±0.57		46.64±4.61 ^{**}	24.21±1.53 [#]

Values are mean ± SE. Refer to oxygen consumption ($\dot{M}O_2$), gill ventilation volume (\dot{V}_G), respiratory frequency (f_R), ventilatory tidal volume (V_T), oxygen extraction from the ventilatory current (EO₂), heart rate (f_H), and critical tension (O₂crit). Asterisk denotes difference between experimental groups and hashtag denotes difference between tensions. ANOVA two way and Student-Newman-Keuls' tests ($P < 0.05$).

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