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## Settleable atmospheric particulate matter affects the swimming performance and aerobic metabolic rate of Nile tilapia (*Oreochromis niloticus*)

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1 **Settleable atmospheric particulate matter affects the swimming performance and**  
2 **aerobic metabolic rate of Nile tilapia (*Oreochromis niloticus*)**

3

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32 **Abstract**

33 Atmospheric particulate matter (APM) produced by the steel industry is a complex  
34 mixture of particles that includes a wide variety of metals and metallic nanoparticles.  
35 These particles settle out onto areas surrounding the industries. There is evidence that this  
36 ‘settleable’ APM (SePM) may cause air-to-water cross-contamination with significant  
37 effects on aquatic biota. Recent investigations have reported sublethal impacts on gill  
38 structure and blood oxygen-carrying capacity of fishes, which raises the hypothesis that  
39 there will be consequences for gas exchange capacity and ability to support aerobic  
40 activities. Therefore, we investigated the effects of an environmentally relevant level of  
41 SePM contamination on swimming performance and associated aerobic metabolic rates  
42 in Nile tilapia, *Oreochromis niloticus*. Short-term exposure (96h) to SePM reduced  
43 critical swimming speed, energetic efficiency of aerobic swimming, standard metabolic  
44 rate, maximum metabolic rate, and aerobic scope. The compromised swimming  
45 performance could have adverse ecological effects by limiting foraging ability, predator  
46 evasion, territorial protection, and migration. The impairments to aerobic capacity could  
47 also affect overall fish performance by influencing long-term energy balance and  
48 allocation to growth and reproduction. Thus, despite being sublethal, SePM  
49 contamination is considerably debilitating and, if its limiting effects are not compensated  
50 in the longer term, it could reduce the survival and fitness of fish populations.

51  
52 **Keywords:** iron processing; industry; metal/metalloid; nanoparticle; swimming  
53 challenge; physiological responses.  
54

55        **1. Introduction**

56            Small amounts of metals/metalloids occur naturally in the aquatic environment  
57 through biogeochemical cycles. However, industrial activity causes a continuous and  
58 persistent production of atmospheric particulate matter (APM), affecting land and water  
59 bodies in diverse ecosystems (Callén et al., 2009; Park and Kim, 2005; Salgado, 2003;  
60 Tsai and Cheng, 2004). APM is usually composed of a complex mixture of particles that  
61 vary in shape and size. Its final composition can be variable and contains emerging metal  
62 compounds with unpredictable new combinations of metals and metal nanoparticles  
63 (Nordberg et al., 2007; Salgado, 2003; Soriano et al., 2012).

64            Although, recent publication has revealed potential risk to fish biota (e.g., Soares  
65 et al., 2022 De Angelis et al., 2022), the presence of metallic particles from APM  
66 deposition (sedimentable APM - SePM) in water bodies has not been properly monitored.  
67 Several environmental regulatory agencies still do not consider air-water cross-  
68 contamination in their environmental monitoring programs (e.g., CONAMA, 2018,  
69 Environmental Protection Agency – EPA, and European Union and Canada). For  
70 example, estuarine waters close to the steel industrial complex in Vitoria Bay in the state  
71 of Espírito Santo, Brazil, receive constant deposition, of varying intensity, of metallic  
72 particles from SePM such as Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb ( $\mu\text{g}$   
73  $\cdot \text{L}^{-1}$ , Souza et al., 2021b, 2021a).

74            Previous studies in the environs of Vitoria city have found that SePM can affect  
75 the aquatic environment and cause bioaccumulation (Souza et al., 2013) and  
76 biomagnification processes through the food web (Santos et al., 2017; Souza et al., 2018).  
77 Environmentally relevant levels of SePM contamination have adverse effects on fishes,  
78 such as deleterious impacts on hematological status and innate immunity, and stress  
79 responses (*O. niloticus*, Soares et al., 2022). In particular, SePM had effects that would

80 compromise blood oxygen-carrying capacity, causing a reduction in hematocrit,  
81 erythrocyte numbers, and mean corpuscular hemoglobin concentration (Soares et al.,  
82 2022). Moreover, exposure to SePM also caused gill damage and ventilatory alterations  
83 in the same species (De Angelis et al., 2022). These findings indicate that SePM may  
84 cause limitations to physiological performance when aerobic metabolic demand  
85 increases, with consequences for activities such as swimming, feeding, reproducing, and  
86 diverse other components of population functioning.

87         This hypothesis can be investigated with measurements of swimming capacity and  
88 associated aerobic metabolism (McKenzie et al., 2007; Plaut, 2001; Priede, 1985).  
89 Swimming capacity is a key physiological variables; that have direct ecological relevance  
90 because they define fish capacity to forage, evade predators, protect territory, and migrate,  
91 among many other activities that can influence survival and fitness (McKenzie et al.,  
92 2007; Plaut, 2001; Priede, 1985). At the same time, the associated aerobic metabolism  
93 indicates the resting metabolic rate, the minimum and maximum aerobic capacity, and  
94 the respective aerobic scope. Fish activities are energetically located inside its aerobic  
95 scope. A scope alteration just changes the overall animal capacity to perform. Therefore,  
96 that is directly correlated to its ecology; since aerobic scope is considered a parameter of  
97 animal performance in relation to environmental conditions, especially related to  
98 instantaneous energy-demanding activities (Domenici et al., 2013; Lucas et al., 2021;  
99 Milinkovitch et al., 2012).

100         Swimming performance and elements of aerobic metabolism have long been  
101 known to be sensitive to environmental factors and stressors and have been used  
102 extensively as biomarkers of the toxicological effects of pollutants (Beamish, 1978; Brett,  
103 1967; Sprague, 1971).

104 We assessed the result of short-term (96h) exposure to an environmentally  
105 relevant level of SePM contamination on the swimming performance and aerobic  
106 metabolism of Nile tilapia. This tropical freshwater species provides a good model for  
107 ecotoxicology because its physiology is well-studied (Solis et al., 2007). It has been  
108 introduced into many tropical and sub-tropical countries across the globe, where it  
109 provides important fisheries (De Silva et al., 2004; Schofield et al., 2011), and it inhabits  
110 the SePM-contaminated sites in the Vitória Bay area (Gallão et al., 2019). Our approach  
111 allowed us to identify and assess sublethal effects of SePM on functional biomarkers that  
112 are relevant to animal performance. This allows projection of consequences for  
113 individuals and populations of an important food fish, with implications that are relevant  
114 both to environmental quality and human health.

115

## 116 **2. Materials and Methods**

### 117 *Settleable Atmospheric Particulate Matter - SePM*

118 SePM collection and analysis methods have been described previously (Soares et  
119 al., 2022). It is composed of a complex mixture of particles, especially emerging metal  
120 compounds with unpredictable new combinations of metals and metal nanoparticles; its  
121 composition varies in shape and size. (Nordberg et al., 2007; Salgado, 2003; Soriano et  
122 al., 2012). The SePM used in this experiment was collected at Ilha do Boi (20°18'32"S,  
123 40°16'33"O), near the industrial complex of Tubarão in Vitória City (5-8 g/m<sup>2</sup> of SePM  
124 each month over an area of 5,000 m<sup>2</sup>, Souza et al., 2021a). This location has an intensive  
125 influence of SePM emitted by this industrial complex, which is integrated by an iron  
126 pelleting industry and an iron and steel exportation harbor (Santos et al., 2017). The SePM  
127 presence in the water environment may be related to input from air and water run-off from  
128 hard-standing areas (McLusky and Elliott, 2004). Recently, isotopic analyses

129 demonstrated that the metals present in that SePM (emitted from the nearby industrial  
130 complex) were in fact the main source of aquatic contamination in those water bodies.  
131 (Souza et al., 2018). Industrial activity may vary seasonally, influencing SePM deposition  
132 (Santos et al., 2017; Souza et al., 2019). Therefore, we sampled the contaminant from a  
133 pool of raw SePM collected over a year in order to assure experimental repeatability (De  
134 Angelis et al., 2022; Soares et al., 2022).

135         Similar to previous experiments, we used  $1 \text{ g}\cdot\text{L}^{-1}$  of raw SePM mass to produce  
136 environmentally realistic contamination in the exposed group (De Angelis et al., 2022;  
137 Soares et al., 2022), this level of contamination is similar to that observed in estuarine  
138 waters close to Vitória Bay observed in environmental records (aquatic environment) and  
139 reported by the Institute of the Environment and Water Resources for the estuarine area  
140 near the city of Vitória, ES - Brazil (IEMA, 2021; Santos et al., 2017; Souza et al., 2013).  
141 Arrivabene et al. (2015) reported SePM in the estuarine waters of Santa Cruz and Vitória  
142 Bay, which is related to air-water cross-contamination from metallurgical activity. Souza  
143 et al. (2013) established chemical characterization of metallic contaminants in these  
144 estuarine waters and observed significant amounts of metals and metalloids from SePM  
145 in surface waters, interstitial water, and sediments (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se,  
146 Ag, Cd, Hg, and Pb -  $\mu\text{g}\cdot\text{L}^{-1}$ ). The raw SePM was diluted in the experimental water (see  
147 below). A sample of the SePM used for contamination was stored for any further analyses  
148 and evaluations. The Control group was not exposed to SePM.

149         We collected water samples from the experimental aquaria for quantitative and  
150 qualitative metal analyses. We used mass spectrometry with inductively coupled plasma  
151 (Q-ICPMS, Agilent 7500 Series CX technology) equipped with an Awe SX-100  
152 autosampler (CETAC-technologies, Omaha, NE, USA) at the Food Science and

153 Technology Institute of Córdoba (YCITAC - Universidad Nacional de Córdoba,  
154 Argentina).

155

### 156 *Fish supply and acclimation*

157 Juveniles of *O. niloticus* were provided by the Polettini fish farm (Mogi-Mirim –  
158 SP, Brazil) and transported to the Department of Physiological Sciences at UFSCar (São  
159 Carlos – SP), where they were acclimated in 1,000 L holding tanks during 30 d. Each  
160 tank had a continuous supply of dechlorinated, well-aerated water ( $25 \pm 1^\circ\text{C}$ ; DO 6.0–7.3  
161  $\text{mg.L}^{-1}$ ; hardness 48–53  $\text{mg.L}^{-1}$ ; pH 6.7–7.3, (as  $\text{CaCO}_3$ ); alkalinity 40–43  $\text{mg.L}^{-1}$  as  
162  $\text{CaCO}_3$ ; chloride 42  $\text{mg.L}^{-1}$ ; ammonium 0.1  $\text{mg.L}^{-1}$ ; and conductivity 110  $\mu\text{S.cm}^{-1}$ ). Fish  
163 remained under natural photoperiod (~12 h:12 h) and were fed *ad libitum* with specific  
164 commercial pellets: Ø 5-8 mm, 6 % fat, 32 % protein and 12 % moisture (Supra<sup>®</sup> Acqua  
165 line) during acclimation period. Food supply was withdrawn 24h before contamination  
166 protocol and experiments. The UFSCar Committee of Ethics in Animal Experimentation  
167 (CEUA-UFSCar) analyzed and approved all procedures.

168

### 169 *Experimental Design*

170 After acclimation, 20 fish (body mass,  $M_B = 180 \pm 98\text{g}$ , length =  $17.9 \pm 0.17\text{ cm}$ ,  
171 width =  $1.9 \pm 0.02\text{ cm}$ , and height =  $6.7 \pm 0.09\text{ cm}$ ) were divided into two groups of  
172 similar mass, which underwent the exposure protocol. For 96h, each fish was put in an  
173 individual aquarium (200 L) with clean water (Control, n = 10) or water with SePM  
174 (SePM, n = 10). Each aquarium was shielded with a black plastic sheet to avoid visual  
175 disturbance and respected the recommended OECD fish:water volume ratio (1g:1L,  
176 (OECD 203, 2019). After 48h, 50% of aquarium water was replaced with the relevant  
177 water. After 96 h, each fish underwent an incremental critical swimming speed ( $U_{crit}$ )



178 protocol with concomitant respirometry (Brett 1964), in a Steffensen-type acrylic swim-  
179 tunnel respirometer as described by McKenzie et al. (2017), see details below.

180 In the present protocol, fish swam in clean, biofiltered, and aerated water, having  
181 acclimated to the tunnel for 24h. Therefore, this would allow the SePM group to partially  
182 recover from exposure effects, if that were possible. To further investigate the potential  
183 for fish to recover from the effects of SePM, a second swimming trial was performed at  
184 96 h after the first, with all animals held in uncontaminated water with the same individual  
185 aquarium exposure protocol (Rec group). This Rec test was only applied to the SePM  
186 group. It has previously been demonstrated that the swimming performance of Nile tilapia  
187 is constant over two sequential 96h periods in clean water (McKenzie et al., 2017).

188

#### 189 *Swimming Performance*

190 After exposure protocol, each fish was put into a bucket which was flushed with  
191 clean biofiltered water for 5 min. After that, the fish was transferred to the respirometry  
192 chamber of the swim tunnel (volume 13.4 L) and left to recover for 24h in aerated clean  
193 water at  $25 \pm 1^\circ\text{C}$ . During recovery, they were kept swimming at 1 body length  $\cdot \text{s}^{-1}$  (BL $\cdot\text{s}^{-1}$ )  
194 <sup>1</sup>). In that gentle speed, fish could maintain body position using some pectoral sculling  
195 and gentle tail beats (see McKenzie et al., 2003). The anterior portion of the swim  
196 chamber was covered with opaque black plastic sheeting to avoid visual disturbance of  
197 the fish, which spontaneously occupied the covered area.

198 The swimming trial was based on the protocol of McKenzie et al. (2017). Each  
199 fish underwent increasing levels of swimming speed. Each level increased 1 BL $\cdot\text{s}^{-1}$  in  
200 swimming speed and had 30 min duration. After reaching 4 BL $\cdot\text{s}^{-1}$ , increments were  
201 reduced to 0.5 BL $\cdot\text{s}^{-1}$  until exhaustion (collapsing against the back of the swim chamber,  
202 McKenzie et al., 2017).

203 Swimming speeds were corrected for the blocking effect of each fish (Bell and  
204 Terhune, 1970). The second swimming trial (Rec group) was held with the same  
205 characteristics as the first. The  $U_{crit}$  ( $BL \cdot s^{-1}$ ) was calculated by adding the velocity of the  
206 most recently completed swimming step to the product of the velocity incremental  
207 increase and the time proportion of swimming until fatigue at the last current velocity, as  
208 described by Brett (1964):

$$209 \quad (1) U_{crit} = U_s + (U_{ss} \cdot T_{ss})$$

210 where  $U_s$  is the last completed velocity of the swimming step,  $U_{ss}$  is the incremental  
211 increase in velocity, and  $T_{ss}$  is the time proportion of swimming until fatigue at the last  
212 current velocity.

213

#### 214 *Swimming respirometry*

215 During swimming, oxygen uptake was measured by intermittent closed  
216 respirometry in two cycles of 15 min at each speed (measuring time – 9 minutes, waiting  
217 time – 1 minute, and flushing time – 5 minutes). An optical oxygen probe and the  
218 associated software (Fibox, GmbH and Oxyview, Pre-sens) allowed for continuous  
219 recordings of the oxygen tension in the respirometry chamber. The oxygen uptake rate  
220 was calculated at each swimming speed based on the gradient of decline in  $O_2$  during the  
221 closure period, at a sampling rate of once each 15 s. The rate of oxygen uptake ( $\dot{M}O_2$  -  
222  $mMolO_2 \cdot kg^{-1} \cdot h^{-1}$ ) was then calculated as

$$223 \quad (2) \dot{M}O_2 = [\beta_{O_2} \cdot V_{RE} \cdot W_0^{-1} \cdot (\frac{\delta p_{O_2}}{\delta t})]$$

224 where  $\beta_{O_2}$  denotes the coefficient for  $O_2$  solubility in water ( $mg$  of  $O_2 \cdot L^{-1}$ );  $V_{RE}$ , the  
225 chamber volume (L);  $W_0^{-1}$ , fish body mass (Kg); and  $(\frac{\delta p_{O_2}}{\delta t})$  indicates the  $O_2$  reduction  
226 over time (% of  $O_2 \cdot h^{-1}$ ). After each swimming protocol, blank tests were performed to

227 measure rates of background microbial oxygen uptake, then the respirometer was  
228 thoroughly washed with clean water.

229 For each swim test, a least-squares exponential regression was applied to the  
230 relationship between swimming speed and the  $\dot{M}O_2$  measurements. Extrapolation back  
231 to the y-intercept, a notional swimming speed of zero, was employed to estimate standard  
232 metabolic rate (SMR), the minimum rate of ATP use required to sustain life, in the  
233 absence of voluntary muscular movements and digestion/absorption of nutrients (Chabot  
234 et al., 2016). Maximum metabolic rate (MMR) was measured as the highest  
235  $\dot{M}O_2$  achieved during the swimming trail, which is assumed to represent the maximum  
236 rate of oxygen uptake that an animal can achieve to create ATP aerobically (Norin and  
237 Clark, 2016). Aerobic metabolic scope (AS) was measured as:

$$238 \quad (3) \text{ AS} = \text{MMR} - \text{SMR}$$

239 which estimates the maximum capacity to supply oxygen to sustain metabolic activities  
240 beyond SMR, activities such as digestion, locomotion, growth, reproduction, and so on  
241 (Fry, 1971). We also assessed the net metabolic cost of swimming (COS -  $\text{mMolO}_2 \cdot \text{kg}^{-1}$   
242  $\cdot \text{h}^{-1}$  at a sustained speed of  $2 \text{ BL} \cdot \text{s}^{-1}$  as:

$$243 \quad (4) \text{ COS} = \dot{M}O_2 (\text{at } 2 \text{ BL} \cdot \text{s}^{-1}) - \text{SMR}$$

244 an estimate of the metabolic cost of activity at low velocity (Allen et al., 2021).

245 The swimming efficiency (Eswim -  $\mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ ) was evaluated as  
246 described by McKenzie et al. (2017):

$$247 \quad (5) \text{ Eswim} = \text{COS} \cdot 2^{-1} (\text{speed of } 2 \text{ BL} \cdot \text{s}^{-1})$$

248 Finally, we analyzed the maximum energetic efficiency of swimming  
249 performance ( $E_{\text{max}}$  -  $\mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ ), a variable of significant ecological relevance  
250 (Ohlberger et al., 2006), was calculated based on Kunz et al. (2018):

$$251 \quad (6) E_{\text{max}} = \text{MMR} \cdot U_{\text{crit}}^{-1}$$

252

253 *Statistical analyses*

254 All data were analyzed for normality and homogeneity (Shapiro-Wilk and  
255 Levene). Differences between experimental groups (Control and SePM) were analyzed  
256 by Student's T-test. Recovery was tested by comparing the mean values of SePM (first  
257 swimming test) and Rec (second swimming test) with paired Student's T-test. All data  
258 were analyzed with R v.4.1.1 (R Core Team 2021). We assumed the significance level of  
259 95% ( $P < 0.05$ ). The relevant data are presented as means  $\pm$  SE.

260

261 **3. Results**

262 *Settleable Atmospheric Particulate Matter*

263 SePM contamination introduced several metal elements into the water. The main  
264 bioavailable components were iron and aluminum, followed by titanium and cerium  
265 (Table 1). Fe and Al represented composed approximately 97% of the total metals  
266 available in the contaminated water. However, despite their high concentration, both  
267 metals were below the reference LC50 for fishes (Fe - 1.46-1.71  $\text{mg} \cdot \text{L}^{-1}$  and Al - 0.095-  
268 235  $\text{mg} \cdot \text{L}^{-1}$  -Kennedy, 2011). Additionally, these metals were below the upper limit (Fe,  
269 0.3-5.0; Al, 0.1-0.2  $\text{mg} \cdot \text{L}^{-1}$ ) indicated for protected freshwater bodies (for drinking  
270 supply, aquatic communities, aquaculture, and fishing, CONAMA, 2005).

271

272 *SePM affected swimming performance and aerobic metabolic rates*

273 The Control group had a mean Ucrit of  $5.68 \pm 0.21 \text{ BL} \cdot \text{s}^{-1}$  at 25°C. This  
274 swimming performance was associated with maintenance of significant respiratory  
275 support for activity (Figure 1). SMR was  $4.49 \pm 0.32 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  in the Control  
276 group. Activity increased metabolic rate so that MMR was 3.2 times higher at  $14.58 \pm$

277  $0.82 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , such that Control AS was  $10.09 \pm 0.61 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$   
278 (Figure 2). The Control group had COS at  $3.21 \pm 0.51 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , Eswim of  $1.58$   
279  $\pm 0.25 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$ , while Emax was  $2.75 \pm 0.12 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$  (Figure  
280 3).

281 The SePM group showed impaired performance with a Ucrit of  $4.15 \pm 0.26 \text{ BL} \cdot$   
282  $\text{s}^{-1}$ , 27% lower than the Control fish ( $p < 0.001$  - Figure 1). This reduction in performance  
283 was associated with effects of SePM on respiratory metabolism. SePM contamination  
284 reduced SMR by 26 % ( $3.32 \pm 0.17 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ,  $p = 0.001$  - Figure 2A) and MMR  
285 by 27 % ( $10.51 \pm 0.78 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ,  $p = 0.002$  - Figure 2B) when compared to the  
286 Control group. Additionally, SePM contamination decreased AS by 29 % compared to  
287 the control group ( $7.19 \pm 0.77 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ,  $p = 0.003$  - Figure 2C).

288 SePM did not change the energetics of sustained swimming at low velocity, with  
289 values of COS of  $3.72 \pm 0.31 \text{ mMolO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  ( $p = 0.333$  - Figure 3A) and Eswim of  
290  $2.09 \pm 0.23 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$  ( $p = 0.102$  - Figure 3B) that were similar to the Control  
291 group. However, SePM contamination did alter Emax, which was 11% lower in the SePM  
292 group compared to the Control, at  $2.46 \pm 0.07 \text{ } \mu\text{molO}_2 \cdot \text{Kg}^{-1} \cdot \text{BL}^{-1}$  ( $p = 0.011$  - Figure  
293 3C).

294

### 295 *Second swimming trial*

296 The second swimming trial provided no evidence of recovery in swimming  
297 performance or metabolic rates (Table 2). Swimming performance was 18% further  
298 reduced in the Rec group, with a Ucrit of  $3.41 \pm 0.17$  (Table 2). All other variables  
299 remained stable when compared to the first swimming trial. Although all 10 fish in the  
300 SePM group survived the 96h contamination and the first swim test, 3 of these fish did

301 not survive the additional 96h recovery period before the second swim test. All fish that  
302 underwent the second swim test survived to the end of the experimental protocol.

303

## 304 **Discussion**

305 Our experimental approach used a functional test to address the effects of  
306 sublethal levels of contamination on a widely-studied fish species. An environmentally  
307 relevant concentration of SePM limited the swimming performance of *O. niloticus*, an  
308 effect that was associated with substantial alterations in respiratory metabolism. This is  
309 clear evidence of effects on functional integrity, an impairment that indicates significant  
310 limitations on respiratory metabolism that may reduce fish fitness due to direct and  
311 indirect impacts on mobility and energy supply.

312

### 313 *SePM and general metal toxicology*

314 Our exposure protocol used an environmentally relevant level of SePM  
315 contamination and was able to replicate the presence and concentration of bioavailable  
316 metals as reported in Santa Cruz and Vitoria Bay (IEMA, 2021; Souza et al., 2021a; Souza  
317 et al., 2021; Soares et al., 2022; De Angelis et al., 2022). The analysis of SePM confirmed  
318 that the contamination is highly complex in metal composition. The observed range of  
319 metals corroborates the results of previous studies (Galvão et al., 2022; Soares et al.,  
320 2022; Souza et al., 2021a; Souza et al., 2021). Metals have a broad range of impacts on  
321 fish physiology, including oxidative stress, genotoxicity, hematological alterations,  
322 histopathological alterations in many organs, and problems in diverse physiological  
323 systems (Lushchak, 2011; Pyle et al., 2002; Taylor et al., 1996; Giles, 1984). Moreover,  
324 previous analyses also described the presence of different-sized particles, which may vary

325 in shape and composition. Furthermore, part of these are in nanometric scale (Salgado,  
326 2003; Santos et al., 2017; Souza et al., 2021b).

327 Therefore, the bioavailable metals in experimental water comprise a complex and  
328 unpredictable contaminant due to the range of different elements, possible combinations  
329 of metallic/metalloid components, and the presence of metallic nanoparticles (Salgado,  
330 2003; Souza et al., 2018; 2021a; 2021b; Soares et al., 2022). This makes it difficult to  
331 identify the main components responsible for functional alterations observed in tilapia.  
332 Presently, using raw SePM in experimental protocols is the only way to replicate such  
333 environmental contamination and study its impacts (Soares et al., 2022; De Angelis et al.,  
334 2022). Although the precise mechanisms underlying the SePM effect have yet to be  
335 revealed, the observed limits to performance are of obvious interest since the level of  
336 individual metals was below their lethal concentration (Kennedy, 2011) and under the  
337 maximum limits set by the Brazilian regulatory agency (CONAMA, 2005). It seems that  
338 we are dealing with an emerging problem that might be highly ecologically relevant.

339

#### 340 *SePM exposure limits swimming performance*

341 Short-term swimming performance tests have generated valuable data to evaluate  
342 fish health after physiological challenges, such as infections (Tierney and Farrell, 2004)  
343 and sublethal toxicant exposures (Beaumont et al., 1995; McKenzie et al., 2007).  
344 Swimming performance has been used to identify physiological damage caused by  
345 diverse dissolved metals in salmonids, such as copper (Waiwood and Beamish, 1978),  
346 zinc (Alsop et al., 1999), and nickel (Pane et al., 2004). This was the first investigation  
347 that used fish swimming performance to evaluate the effect of SePM.

348 Previous experiments using a similar swimming protocol at the same temperature  
349 in Nile tilapia reported Ucrit values similar to our Control group (McKenzie et al., 2017),

350 confirming the consistent quality of the fish population used and the utility of the  
351 swimming trials. Consequently, the reduced values of Ucrit and Emax in SePM-  
352 contaminated fish identify clear impairment of swimming performance and metabolic  
353 effectiveness.

354 It is parsimonious to attribute the Ucrit reduction to the associated changes in  
355 aerobic metabolism. Functional cardiorespiratory integrity is necessary to support a high  
356 level of aerobic swimming (McKenzie, 2011). Therefore, a break in the integrity of  
357 mechanisms for oxygen uptake and delivery may account for the reduction in swimming  
358 capacity. The observed decrease in Emax supports such an argument.

359 Our experimental approach allowed us to reveal that SePM limited the ability to  
360 swim, which has obvious ecological relevance. Swimming relates to the capacity to  
361 perform essential behavioral tasks for interactions with the environment, such as  
362 maintaining a position against currents, foraging, migrating, reproducing, and many  
363 others. The evidence that swimming capacity was limited by impaired metabolic support  
364 is also relevant because it indicates that other aerobically demanding activities would be  
365 similarly impacted.

366

### 367 *SePM exposure affected aerobic metabolism*

368 Metabolic measures provide a significant description of energy mobilization in  
369 individual animals (Rosewarne et al., 2016), which is considered a direct reflection of the  
370 functional integrity of an individual and its potential to operate in nature (Rice, 1990).  
371 The assessment of aerobic profile has been reported as ecologically relevant as a potential  
372 bioindicator of metabolic performance, growth rate, and survival (Killen et al., 2010) as  
373 they bring a unifying currency of animal performance (Wikelski and Ricklefs, 2001). The  
374 swimming performance protocol allowed for calculating robust metabolic indexes



375 regarding the fish metabolic profile and revealed aspects of the energy used for basal  
376 maintenance (SMR) and the relative cost of the activity under low (COS, Eswim) and  
377 high demand reaching up its limit and the related increase scope (MMR, AS, and Emax).

378 Control tilapia had a metabolic profile very similar to previous reports for the  
379 species. For instance, using a similar swimming technique at the same temperature,  
380 McKenzie et al. (2017) reported similar mean values for SMR, MMR, and AS  
381 (respectively,  $4.1 \pm 0.5$ ,  $16.8 \pm 1.3$ ,  $12.6 \pm 0.1 \text{ mMolO}_2 \cdot \text{Kg}^{-1} \cdot \text{h}^{-1}$ ) in the same strain of  
382 Nile tilapia.

383 The 96h exposure to SePM clearly limited both the total energy used and the  
384 efficiency of the use of this energy. The exposed fish had reduced standard metabolic  
385 rates. Although they were capable of matching the aerobic demands of low levels of  
386 sustained swimming (COS and Eswim), SePM-contaminated fish could not achieve the  
387 same metabolic rate to support high swimming speeds. Therefore, the overall aerobic  
388 metabolic phenotype was affected, with a reduction in basal metabolic processes and an  
389 impaired ability to meet the increased metabolic rate required to support intense activity  
390 such as swimming at high speeds.

391 Xenobiotics are reported to cause apparently conflicting metabolic effects, such  
392 as: metabolic limitations and metabolic masking (Hammer, 1995). The first condition  
393 would impair both metabolite supply and removal. Therefore, it would limit the SMR and  
394 would have an additional contribution to the reduction in the energetic efficiency of  
395 swimming and the critical speed. Additionally, it would reduce the maximal energy  
396 production capacity and aerobic scope. Even at sublethal level, metals have been reported  
397 to induce alterations with an ultimate metabolic consequence and long-term effects such  
398 as reduction in development, growth, and reproduction (Lucas et al., 2021). For instance,  
399 a low level of Cu and Cd was observed to reduce the metabolic rate in the freshwater fish

400 species *Pimephales promelas* (Pistole et al., 2008). Zn also reduced the metabolic activity  
401 in *Rhamdia quelen* (Leitemperger et al., 2019).

402 Previous experiments reported that SePM contamination has a deleterious effect  
403 on both capacities for oxygen uptake and delivery in the tilapia (De Angelis et al., 2022;  
404 Soares et al., 2022). That was also associated with a compensatory ventilatory increase  
405 and ended up reducing the tolerance to hypoxia (De Angelis et al., 2022). Such effects  
406 are coherent with the current findings of reduced swimming performance and alterations  
407 in aerobic metabolism. The same authors reported that tilapia could sustain its routine  
408 metabolic rate (RMR) after a similar SePM contamination (De Angelis et al., 2022). We  
409 suggest that the combination of these factors (maintenance of RMR while reducing SMR  
410 with high ventilation cost) indicates problems with energy allocation. A larger part of the  
411 total energy would be used for ventilation, while other processes potentially have limited  
412 energy support. That interpretation corroborates the observed energetic limitation of  
413 swimming. A higher ventilatory effort is expected to increase the energy used for active  
414 ventilation at each swimming level (Freadman, 1981). Therefore, such an energy  
415 reallocation was revealed as inefficient energy use (i.e., low  $E_{max}$ ), lower MMR and AS  
416 when the fish were forced to increase their metabolism for swimming.

417 Additionally, AS has been proposed as an index of animal performance in relation  
418 to environmental conditions, since it is related to instantaneous energy-demanding  
419 activities and may demonstrate the physiological consequences of several environmental  
420 constraints such as temperature increase, hypoxia, and pollutants (Domenici et al., 2013;  
421 Lucas et al., 2021; Milinkovitch et al., 2012). Therefore, the observed reduction in AS on  
422 exposure to SePM may restrain a range of relevant energetically demanding activities  
423 such as growth, reproduction, and offspring size (Claireaux and Lefrançois, 2007).

424 Furthermore, the capacity to support increased energy demand levels is also  
425 supposed to impact recovery after exhausting activity. Part of the SePM group (30%) died  
426 during the additional recovery period after the first swimming trial. This may be related  
427 to SePM negative effects previously reported; SePM caused significant damage to the gill  
428 structure, aerobic capacity, oxygen-carrying capacity of the blood, and energy allocation  
429 in Nile tilapia (De Angelis et al., 2022; Soares et al., 2022), impairing O<sub>2</sub> uptake from the  
430 water, the metabolism and swimming performance as observed in the present experiment.  
431 MMR can be associated with intrinsic and extrinsic factors (Norin and Clark, 2016). Thus,  
432 the maximum swimming activity may cause a transitory debilitation in every vertebrate  
433 since it depletes cellular energy reserves and pH alteration due to lactic acid produced,  
434 and others. It also causes ionic unbalance in fish associated with the increased ventilatory  
435 effort. Post-activity recovery might cause long-term demands for an aerobic effort to  
436 sustain the biochemical effort to recover osmolarity, pH, and energetic balance.  
437 Apparently, SePM-contaminated fish was unable to recover as fast as control fish.  
438 Therefore, these casualties reinforce the apparent severity of this type of pollution and  
439 suggest that if such limitations are not compensated in the long term, SePM will impact  
440 a fish's overall use of energy. Consequently, it may have effects on development, growth,  
441 reproduction, post-prandial metabolic increment, and any other metabolically demanding  
442 activities (McKenzie et al., 2007; Killen et al., 2010; Plaut, 2001; Priede, 1985; Rice,  
443 1990) with potential consequent reductions in the overall fitness of the fish population  
444 and ecological impact.

445

#### 446 *Final Conclusions*

447 An environmentally relevant concentration level of SePM significantly limited the  
448 swimming performance of *O. niloticus* and caused significant reductions in metabolic

449 indexes, SMR, MMR, Emax, and AS. Although exposure to environmentally relevant  
450 levels of SePM did not lead to immediate death, it did induce significant limitations on  
451 physiological performance. Such a combination between swimming and metabolic  
452 limitations may affect activities that support fish population dynamics. SePM has the  
453 potential to affect multiple levels of fish performance, and therefore, it is still a rather  
454 unexplored toxic component that may lead to a growing and silent threat to aquatic biota.  
455 It does require further study to understand toxicity thresholds, the mechanisms of toxicity  
456 to several organisms, and forms of risk assessment. Future work should also address the  
457 effect of long-term contamination by SePM over a range of natural physicochemical  
458 variations such as salinity and temperature.

459

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