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Diana Monteiro, Edwin Taylor, David Mckenzie, Francisco Rantin, Ana Kalinin. Interactive effects of mercury exposure and hypoxia on ECG patterns in two Neotropical freshwater fish species: Matrinxã, *Brycon amazonicus* and traíra, *Hoplias malabaricus*. *Ecotoxicology*, 2020, 29 (4), pp.375-388. <10.1007/s10646-020-02186-4>. <hal-03097394>

HAL Id: hal-03097394

<https://hal.science/hal-03097394v1>

Submitted on 23 Nov 2022

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**Interactive effects of mercury exposure and hypoxia on ECG patterns in two
Neotropical freshwater fish species: Matrinxã, *Brycon amazonicus* and traíra, *Hoplias
malabaricus***

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Abstract

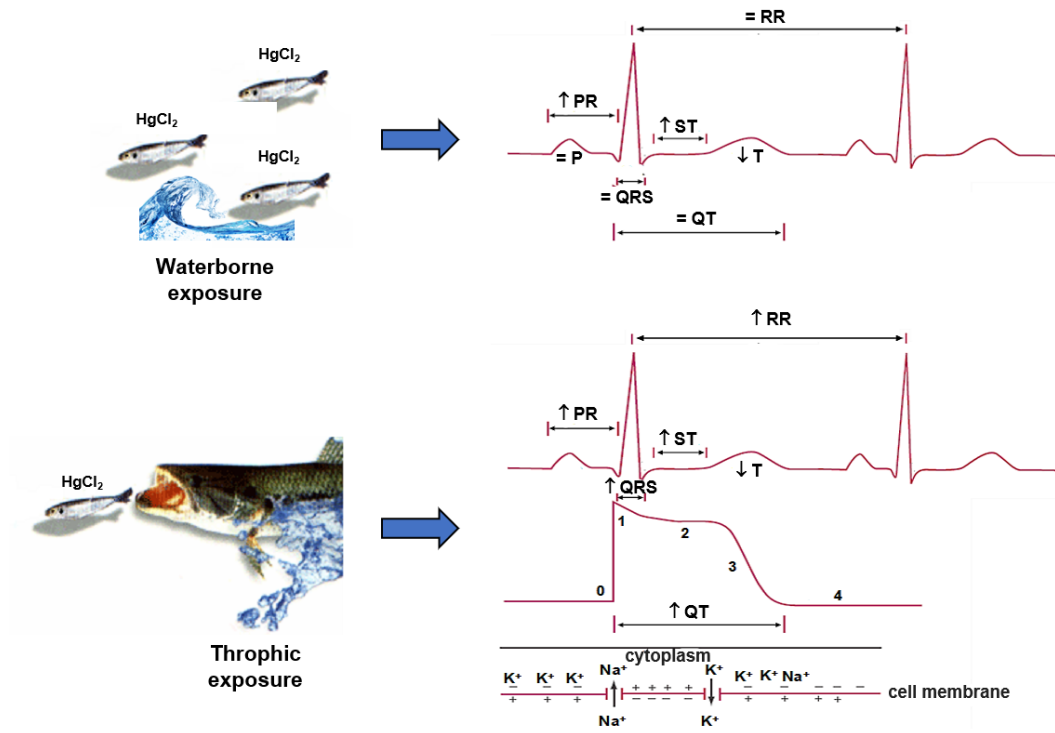
Hypoxia and mercury contamination often co-occur in tropical freshwater ecosystems, but the interactive effects of these two stressors on fish populations are poorly known. The effects of mercury (Hg) on recorded changes in the detailed form of the electrocardiogram (ECG) during exposure to progressive hypoxia were investigated in two Neotropical freshwater fish species, matrinxã, *Brycon amazonicus* and traíra, *Hoplias malabaricus*. Matrinxã were exposed to a sublethal concentration of 0.1 mg L⁻¹ of HgCl₂ in water for 96 h. Traíra were exposed to dietary doses of Hg by being fed over a period of 30 days with juvenile matrinxãs previously exposed to HgCl₂, resulting in a dose of 0.45 mg of total Hg per fish, each 96 h. Both species showed a bradycardia in progressive hypoxia but Hg exposure impaired cardiac electrical excitability in both fish species, leading to first-degree atrioventricular block, profound extension of the ventricular action potential (AP) plateau, and the development of cardiac arrhythmias and anomalies (atrial fibrillation, extra systoles, negative QRS complex, T wave inversion), especially in hypoxia below O₂ partial pressures (PO₂) of 40 mmHg. Moreover, the chronic dietary Hg exposure induced intense bradycardia in normoxia in traíra, lengthening of ventricular AP duration coupled with prolonged QRS intervals. This indicates slower ventricular AP conduction during ventricular depolarization. Overall, the data indicate that acute waterborne and chronic trophic inorganic mercury exposures, at sub lethal doses, cause damages in electrical stability and rhythm of the heart beat, leading to amyocardial dysfunction which is further intensified during hypoxia. These changes could lead to impaired cardiac output, with consequences for swimming ability, foraging capacity, and hence growth and/or reproductive performance.

Key-words: cardiac electrical excitability, heavy metal, ECG, bradycardia, action potential, *Brycon amazonicus*, *Hoplias malabaricus*.

49 **Highlights**

- 50 Hg exposures impaired cardiac electrical excitability in two tropical freshwater fish species.
- 51 Hg exposures intensified hypoxic bradycardia.
- 52 Hg caused profound extension of the ventricular action potential plateau.
- 53 Hg exposures triggered first-degree atrioventricular block.

54 Graphical Abstract



1. Introduction

The freshwater aquatic environments inhabited by fishes, particularly in the tropics, are often highly variable in terms physical and chemical properties (Carpenter et al., 2011). Hypoxia is a phenomenon that occurs over large areas in aquatic systems worldwide and may affect resident animals, leading to population decline and changes in community composition due to elimination of sensitive species (Shang and Wu, 2004). Hypoxia can be common in tropical freshwater habitats and fishes possess a variety of regulatory mechanisms to respond to this environmental stressor. These include increased gill ventilation and an increase in functional gas exchange surface in the gills (Glass et al., 1991). In most species, hypoxia also provokes bradycardia, due to increased vagal tone on the heart (Taylor et al., 1977; Taylor, 1992; Rantin, 1993). Relatively little is known about how responses to hypoxia might be influenced by other concurrent changes in water quality, such as increasing pollutant concentrations.

Of all contaminant metals mercury (Hg) is by far the most toxic, due to its persistence in water and sediments and its ability to bioaccumulate and biomagnify through aquatic food webs (Lacerda and Malm, 2008). Hg transfer and biomagnification have been demonstrated in both freshwater and marine food webs and can lead to hazardous concentrations in fishes and fish-eating wildlife (Lavoie et al., 2013). Although Hg contamination in aquatic ecosystems can derive both from processes, the main sources today are anthropogenic activities, such as agricultural materials, mining and smelting operations, chloralkali, pulp, and paper industries (Wang et al., 2004), among others. The environmental impact of Hg can be intensified by ecological disasters, a case in point being the collapse of a mining dams in the Brazilian state of Minas Gerais (Mariana on November, 05th 2015 and Brumadinho on January 28th 2019).

The aim of this study was to analyze the effects of sub-lethal doses of inorganic mercury (HgCl₂) on cardiac electrical activity (electrocardiogram, ECG) during hypoxia in two ecologically distinct Neotropical fish species, matrinxã *Brycon amazonicus* (Characidae)

and traíra *Hoplias malabaricus* (Erythrinidae). These species were exposed, respectively, to acute water borne HgCl₂ for 96 h or chronic dietary HgCl₂ for 30 days. The matrinxã is an omnivorous and a rheophilic species native to the Amazon basin that inhabits clear and well-oxygenated waters, frequently contaminated by Hg. The traíra is a widespread sedentary piscivorous species, which commonly inhabits tropical lakes.

According to Pollock et al. (2007), low O₂ rarely occurs in isolation in aquatic systems and it is of utmost importance to investigate interactions between hypoxia and contaminants in fishes. A previous study from our group demonstrated that waterborne and dietary Hg exposure decreased the plasticity of cardiorespiratory responses to hypoxia in these two fish species, reducing their ability to increase gill ventilation and respiratory frequency and, therefore, to maintain O₂ extraction and regulate their metabolic rate (Monteiro et al., 2013). In the present study, we used electrocardiograms (ECGs) to evaluate the effects of hypoxia and Hg exposures on electrical activity of the heart. ECG recordings provide information about cardiac rhythm, the duration of the ventricular action potential (AP), and the rate of impulse propagation through different chambers of the heart. The rate, rhythm and orderly spread of contraction depend on the electrical excitation of cardiomyocytes (Badr et al., 2016). The ECG can, therefore, reveal disturbances in cardiac function, in particular of impulse conduction (Vornanen, 2017). As a result, the utility of ECG monitoring in toxicological studies has gained traction because it is noninvasive means of obtaining a mechanistic, cause and effect, understanding of how animals might be affected by pollutants.

2. Material and Methods

2.1 Animals

Matrinxã (Wt = 154.5 ± 7.9 g), were obtained at the Águas Claras fish farm, Mococa, São Paulo State, Brazil. Traíra (Wt = 246.3 ± 7.5 g), were obtained at the Parque do Lago fish

farm, Dourado, São Paulo State, Brazil. Fishes were acclimated for 60 days prior to experimentation in 500 or 1000 L holding tanks provided with a continuous supply of well-aerated and dechlorinated water at a constant temperature (24 ± 1 °C). During this period, matrinxã were fed *ad libitum* with commercial fish pellets (40% of protein). Traíra will only accept live prey, therefore they were fed at a ratio of 2 % of biomass day⁻¹ (Rios et al., 2005) with small matrinxã (5-8 g).

2.2 Mercury exposures

For waterborne Hg exposure, *B. amazonicus* were divided into two experimental groups: control (Ctrl; n = 12) and Hg-exposed (wHg; n = 12), given ~20% of their LC50-96 h as previously established by Monteiro et al., 2010, namely 0.1 mg L⁻¹ of HgCl₂ (> 99.5 % purity, Sigma-Aldrich) for 96 h in static holding tanks. Concentrations of total mercury ranging from 1 to 230 µg L⁻¹ are usually detected in water bodies near industrialized areas (Hypolito et al., 2004; Alinnor, 2005; Bollen et al., 2008).

For trophic Hg exposure, *H. malabaricus* were divided into two treatment groups: control (Ct, n = 12) and Hg-exposed (tHg, n = 12), via contaminated food. The food supply consisted of four live juvenile *B. amazonicus* (5-8 g) each 96 h. The Ct received animals from clean water whereas tHg received animals exposed to 0.1 mg L⁻¹ of HgCl₂ for 96 h, as described above. Feeding rate was 2% of the total body weight per day, over 30 consecutive days. The total Hg concentration in the whole body of each living prey was 8.1 ± 0.9 mg kg⁻¹, analyzed by a Cold Vapor Atomic Absorption Spectrometry (USP, 2000). Considering the average weight of the prey, each traíra received about 0.45 mg of total Hg every 96 h. This dose was calculated to be similar to fish collected in Hg-impacted ecosystems (Alho and Vieira, 1997; Hylander et al., 2000).

The water and trophic contamination refers mainly to inorganic Hg, since no methyl mercury level was detected in the experimental water or in the matrinxã exposed to HgCl₂ and

used as prey for the traíra. Methyl mercury was analyzed by gas chromatography – mass spectrometry (GC-MS) according to the method of AOAC (1995) and the quantification limits were 20 ng L⁻¹ (water) and 1 µg kg⁻¹ (tissues).

All treatments and experimental designs are described in detail in previous studies from our laboratory (Monteiro et al., 2010; 2013).

2.3 *In vivo* experimentation: surgery and ECG recording

After waterborne and trophic exposures, fishes were anaesthetized by immersion in a solution of benzocaine (0.1 g L⁻¹) then placed on a surgical tray with the gills irrigated by a flow of water containing the anaesthetic (0.05 g L⁻¹). Two ECG electrodes were placed in a ventral position, the first (positive) between the gills and the heart, and the second (negative) in a ventral position close to the pelvic fins. After surgery, fishes were placed in the experimental chamber and left undisturbed to recover for 12 h in normoxic water (140 mmHg) at 25°C (temperature for all experiments). A reference electrode was placed in the water of the experimental chamber. This preparation allowed electrocardiographic (ECG) recordings of the first diagonal branch (D1) lead. The ECG was obtained by coupling the electrode set to an amplifier (ML 136 Animal Bio Amp, AD Instruments) which was connected to a data acquisition system PowerLab (Powerlab 8/30, AD Instruments, Castle Hill, NSW, Australia). A 3 Hz-40 Hz band pass filter was used to remove muscle movement and power line artifacts. This computer-based ECG system permits detailed analysis of wave durations (P, T, and QRS complex), intervals (RR, QT, and PR), and segment (ST). The PR and QT intervals are equivalent, respectively, to the interval from onset of atrium depolarization and ventricle depolarization to total repolarization (i.e., the atrial AP duration - A_{APD} ; and ventricular AP duration - V_{APD}). The ST segment represents the plateau of the ventricular AP.

After recovery from surgery, normoxic ($PO_2 = 140$ mmHg or 7.3 mg L^{-1} of dissolved O_2) control values were recorded and, subsequently, the PO_2 was gradually reduced to 120, 100, 80, 60, 40, and 20 mmHg. Each tension was maintained for 45 min with recordings collected over the last 15 min. Normoxia was maintained by bubbling the water with compressed atmospheric air while hypoxic levels were created by dispersing pure N_2 into the water of the experimental tank. The O_2 tension of water was controlled by O_2 electrodes (FAC001- O_2) connected to a FAC-204A O_2 Analyzer (FAC, São Carlos, SP, Brazil). All experiments were conducted at 25°C .

2.4 Statistical analysis

Results are presented as means \pm S.E.M. Differences between means at a 5% ($P < 0.05$) level were considered significant. Data from control and Hg exposed groups were tested for statistically significant differences using the Student t-test. The Kolmogorov-Smirnov method was applied to evaluate normality of the samples and the F-test was applied for homogeneity of variances (GraphPad InStat, GraphPad Software, USA). Analysis of variance one-way Anova complemented by Dunnett's multiple comparison test (GraphPad InStat version 3.00, GraphPad Software, USA) was used to check the possible existence of significant variations between the values obtained at different O_2 tensions for the same experimental group. Two-way analysis of variance Anova complemented by Tukey's multiple comparison test (SigmaStat version 2.0 Jandel SigmaStat Statistical Software, USA) was used to determine interactions between water/diet Hg concentrations and water O_2 levels on ECG responses.

3. Results

3.1 Effects of acute exposure to waterborne $HgCl_2$ in *matrinxã*

Figure 1 shows the effects of graded hypoxia on ECG parameters of matrinxã in both experimental groups (wCt and wHg).

Fig 1

The Ct group maintained a constant P wave duration (38.6 ± 0.6 ms) at all O₂ tensions, from normoxia to deep hypoxia. For the wHg group, the P wave duration was kept constant (38.2 ± 0.4 ms) from normoxia down to a PO₂ of 80 mmHg, below which it was higher (nearly 30 %) than the Ct at O₂ tensions from 60 to 20 mmHg. No significant differences were observed in the QRS complex duration, either for gradual hypoxia or between Ct and wHg groups. Conversely, the durations of T wave were significantly lower (26 to 38 %) than the Ct ones in the PO₂ range between 140 and 20 mmHg. Furthermore, the T wave duration of the Ct group was maintained constant until 40 mmHg (169.1 ± 6.3 ms) and increased significantly in the O₂ tension of 20 mmHg (247.1 ± 1.8 ms). The same tendency was observed for the wHg group, but T wave duration values increased significantly below 60 mmHg, reaching maximum values at 20 mmHg (175.5 ± 7.9 ms).

The RR intervals of both experimental groups remained nearly constant (1125.3 ± 80.1 ms) until a PO₂ of 60 mmHg. Below this O₂ tension, RR intervals increased significantly and progressively in both groups, reaching maximum values at 20 mmHg (Ct: 2759.3 ± 349.5 ms, and wHg: 5996.7 ± 715.0 ms). This indicates the typical reflex bradycardia in response to hypoxia for teleost fishes. The P wave durations of the wHg group were significantly higher than those of the Ct group at PO₂'s between 40 and 20 mmHg (48 and 117 %, respectively).

The Ct and wHg groups maintained a constant PR interval from normoxia to deep hypoxic conditions (74.2 ± 2.2 ms and 89.1 ± 3.1 ms, respectively). However, PR durations of the wHg group were significantly higher (~ 20 %) than the Ct ones at all O₂ tensions.

The ST segment was maintained constant in the Ct group at all O₂ tensions, whereas a significant increase in ST segment occurred in the wHg group at 20 mmHg. However, the

values of ST segment of the wHg group were significantly higher (25 to 54 %) than those of the Ct group at O₂ tensions from 140 to 20 mmHg.

Table 1 shows the effects of graded hypoxia on the QT interval or ventricular AP durations of *B. amazonicus*. For the wHg group, V_{APD} values increased significantly during deep hypoxia, while this tendency was not observed in the Ct group. QT interval was significantly higher (~20%) in the wHg group than in the Ct group, but only at a PO₂ of 20 mmHg.

The two-way Anova analysis indicated a significant interaction effect between HgCl₂ exposure and water O₂ tensions just for RR interval, indicating that the Hg exposure changed the predictable pattern of response of heart rate (f_H) of the matrix in hypoxia, making the reflex bradycardia more profound. No interactive effects were found for the other parameters evaluated.

The electrocardiograms representing the characteristic waveforms during normoxia and hypoxia for *B. amazonicus* are presented in Figure 2. These reveal a prolongation of the PR interval and ST segment with concomitant reduction of T wave duration/amplitude after acute waterborne Hg exposure. Moreover, in severe hypoxia, about 30% of fish from Ct group showed some ventricular extrasystoles (approximately 2 per min), whereas 80% of fish from wHg group exhibited these (3-4 per min). Arrhythmias, such as atrial fibrillation, were also observed predominantly in wHg group during gradual hypoxia, suggesting myocardial ischemia.

Fig 2

3.2 Effects of chronic exposure to trophic HgCl₂ in traíra

The effects of graded hypoxia on the ECG of the tCt and tHg groups are shown in Figure 3.

Fig 3

236

237 The two groups exhibited no statistically significant differences in P duration (Ct: 95.5
 238 ± 1.8 ms, and tHg: 104.8 ± 1.5 ms), QRS complex (Ct: 82.9 ± 0.8 ms, and tHg: 98.2 ± 2.7
 239 ms), T wave duration (Ct: 283.2 ± 5.9 ms, and tHg: 208.9 ± 10.3 ms), or ST segment (Ct:
 240 308.9 ± 5.8 ms, and tHg: 365.1 ± 2.5 ms) during hypoxia. However, at all PO₂, trophic Hg
 241 exposure induced significant increases in the duration of P wave (14 - 27 %), QRS complex
 242 (34 - 75%), and ST segment (33 – 52%), with concomitant reduction of T wave duration (36 -
 243 49%), in comparison to the controls.

244 The Ct group maintained a constant RR interval from normoxia down to 40 mmHg
 245 (1341.2 ± 22.9 ms), below which these values increased markedly, reaching maximum values
 246 at 20mmHg (2052.1 ± 197.1 ms). On the other hand, RR intervals of the tHg group (1797.8 \pm
 247 43.5 ms) increased progressively and significantly above a PO₂ of 80 mmHg, reaching
 248 maximum values at 20 mmHg (2796.8 ± 230.1 ms). Furthermore, the duration of RR intervals
 249 for the tHg group were significantly higher (36 - 74%) than the tCt at all PO₂.

250 The PR interval was maintained constant until 20 mmHg in the Ct group (161.4 ± 6.4
 251 ms) and tHg group (189.1 ± 6.6 ms). PR duration for the tHg group was significantly higher
 252 (12 to 23%) than the Ct group at PO₂ between 140 and 20 mmHg.

253 The effects PO₂ changes on the QT (V_{APD}) of *H. malabaricus* are presented in Table 2.
 254 For both experimental groups, V_{APD} values did not change during hypoxia. Nevertheless,
 255 group tHg presented significantly higher V_{APD} values (approximately 20%) at all O₂ tensions
 256 (140 to 20 mmHg).

257 The two-way Anova revealed no significant interactions among the factors Hg levels
 258 and water O₂ tensions, for any measured variable. Thus, Hg exposure and hypoxia
 259 independently affected the pattern of response of the traíra ECG.

260 Examples of recording of curves of ECG in Ct and tHg groups in normoxia and
 261 hypoxia are shown in Fig. 4. As can be seen from this figure, chronic and trophic Hg exposure

induced increases in the RR interval, P-wave duration, QRS complex durations, with prolongation of the PR interval and ST segment. Altered ventricular repolarization was indicated by a flattening and, in severe hypoxia, position inversion of the T wave, predominantly in the tHg group. In addition, approximately 85% of the fish exposed to Hg displayed ECG with abnormal features, including atrial fibrillation and a negative QRS complex.

Fig 4

4. Discussion

Bradycardia has been characterized for many fish species when exposed to acute hypoxia, including in matrinxã and traíra (Monteiro et al., 2013; Kalinin et al., 1993). In most species the cardiac frequency remains constant with moderate decreases of PO₂, whereas bradycardia (increase in RR intervals) develops with severe hypoxia (Glass et al., 1991; Taylor, 1992). Generally, the development of bradycardia in fish coincides with ECG waveform changes, such as alterations in T wave duration and amplitude, suggesting a relationship between cardiac dysfunction and hypoxic bradycardia (Glass et al., 1992; Rantin et al., 1995).

In our previous study, we demonstrated an effect of Hg on electrocardiographic recordings and *ex vivo* myocardial contractility under normoxic conditions (Monteiro et al., 2017). As a novel aspect, this study reports Hg-induced changes in ECG waveforms from two tropical fish species facing a hypoxic challenge. The present data indicate that Hg exposures, *via* water or food, intensified hypoxia-induced myocardial anomalies, causing severe cardiac dysfunction and reduction in f_H . Similar data on impairment of cardiac function is available following exposure of fish to other toxic metals. Trout apparently died from heart failure following exposure to lethal levels of copper in freshwater (Wilson and Taylor, 1993) and exposure to sublethal copper reduced swimming speed due to the indirect effects of ammonia

288 accumulation on depolarization of muscle that was accompanied by changes in f_H (Beaumont
289 et al., 2000; Taylor et al., 1996).

290 In a fish ECG, the P wave represents the atrium depolarization, QRS complex
291 indicates the ventricular depolarization, and the T wave corresponds to the repolarization of
292 the ventricle (Rantin et al., 1995; Liu et al., 2016). Both PR interval and the ST segment were
293 increased at all O₂ levels, after waterborne and trophic Hg exposures. In the heart of *B.*
294 *amazonicus*, acute Hg exposure also intensified the duration of the RR interval, P wave, and
295 ST segment during deep hypoxia. On the other hand, these changes in the heart of *H.*
296 *malabaricus* were observed at all O₂ tensions, from normoxia to severe hypoxia. Separating
297 direct and indirect effects of toxic metals on cardiac function in intact fish is difficult. This
298 was achieved in the present study by trophic exposure to Hg, thus bypassing any direct
299 impacts of hg on the gills. Trophic exposure to Hg caused an overall dysfunctional effect on
300 the heart. The main effects of Hg were on the time-dependent processes such as the QRS
301 complex, T wave and AP durations. The RR, PR, and QT intervals and ST segment were
302 increased at all O₂ levels as were the duration of P and QRS waves, though the duration of the
303 T wave was reduced at all O₂ levels. Subsequent hypoxia caused a significant increase in RR
304 interval, marking a hypoxic bradycardia that was intensified in the presence of Hg.

305 The PR interval is equivalent to the interval from onset of atrium depolarization to
306 total repolarization (*i.e.*, the atrial AP duration A_{APD}). PR elongation, also known as first-
307 degree atrioventricular block (AV-block), happens when conduction through the AV node is
308 slowed. Thus, Hg induced an AV-block, impairing transmission of the the AP from atrium to
309 the ventricle at all O₂ tensions. Cardiac contractile rhythmicity in the fish heart is determined
310 by specialized pacemaker cells located in the sinoatrial (SA) node (Hassinen et al., 2017).
311 These Hg-induced abnormalities may be due to the ability of the metal to inhibit the inward
312 Na⁺ (Visentin et al., 1990), Na⁺/K⁺- ATPase activity (Anner et al., 1992), and sarcoplasmic
313 reticulum (SR) function (Hechtenberg et al., 1991; Monteiro et al., 2017).

After Hg exposures, there was a remarkable prolongation of the plateau (ST segment) of ventricular AP, with concomitant reduction of ventricular repolarization time (T wave duration), in all O₂ tensions. The AP plateau represents the cardiac systole duration, which changes with f_H to allow both adequate time for ventricular filling and a force of contraction that sustains a steady cardiac output (Tikkanen et al., 2017). A prolongation of the ventricular AP duration can be important to maintain the cardiac tissue perfusion, mainly during bradycardia that occurs when hypoxia is prolonged and/or after dietary exposure to Hg.

According to Driedzic and Gesser (1994), a prolonged AP probably indicates a prolonged time with open Ca²⁺ channels and should also favor a possible Ca²⁺ influx via the Na⁺-Ca²⁺ exchange at positive membrane potentials. Patch-clamp experiments demonstrated that inorganic Hg amplified the voltage-dependent Ca²⁺ current through L-type channels (Rossi et al., 1993). Reactive oxygen species (ROS) can also regulate L-type Ca²⁺ channel function by direct redox modification of cysteines on the channel protein. Exposing guinea-pig ventricular myocyte to ROS enhanced Ca²⁺ influx through the L-type Ca²⁺ channel (Hool, 2007). Our previous studies found that the same exposures to the same doses of Hg increased ROS generation and antioxidant enzyme activities in the heart of these fish species, causing oxidative stress, evidenced by significant increases in lipid and protein oxidation (Monteiro et al., 2010; 2013).

On the other hand, Hg exposures reduced ventricular repolarization time (T wave duration) probably due its effects on activation of the membrane K⁺ conductance. According to Jungwirth et al. (1991) Hg²⁺ increases cell membrane K⁺ selectivity, and decreases cell membrane resistance, enhancing the K⁺ conductance of the cell membrane in cultured kidney cells. Abnormalities in ventricular repolarization were also revealed by a flattening and/or position inversion of T wave, predominantly in Hg exposed groups during severe hypoxia. Alterations of the T wave (e.g., increase in amplitude and area, inversion, and biphasic aspect) indicate acute ischemia with insufficient O₂ supply to the myocardium, leading to drastic

changes in the direction of ventricular repolarization (Rantin et al., 1995). Mittal et al. (2012) demonstrated that sustained hypoxia induced K^+ channel current inhibition by increased ROS production in pulmonary artery smooth muscle cells. ROS are generated by mitochondria during hypoxia in cardiomyocytes (Duranteau et al., 1998).

The prolonged P wave duration (atrial depolarization) in matrinxa below 60 mmHg and in traíra at all O_2 tensions indicates a slowing of conduction velocity in the atrium by conduction delay or blockage. Conduction delay happens when the propagating impulse fails to conduct, and gap junction coupling plays a pivotal role in sustaining the impulse propagation (Gaztañaga et al., 2012). Connexins are ubiquitous channel forming gap junction channels that directly connect cardiomyocytes and conductive tissues to synchronize the atrial and ventricular contraction/relaxation cycles (Leybaert et al., 2017). It is known that Hg reduce functional electric coupling mediated by connexin 43 gap junction channels (Schirrmacher et al., 1998).

Hypoxia evokes a reflex bradycardia mediated by the cholinergic fibers of the cardiac vagus, which allows the blood to stay in the gill lamellae longer to promote gas exchange when PO_2 of the water falls (Satchell, 1991). Tikkanen et al. (2017) studied the effects of sustained anoxia on cardiac electrical excitability in crucian carp (*Carassius carassius*) and demonstrated that the oxygen shortage induced a strong bradycardic reflex and remarkable lengthening of ventricular AP duration (V_{APD}), which is equivalent to the interval from onset of ventricle depolarization to total repolarization (QT interval). In *H. malabaricus*, Hg exposure also induced bradycardia in normoxia and anticipated the reflex bradycardia in response to hypoxia, besides V_{APD} prolongation. These results suggest that Hg exposure deteriorated cardiac electrical behavior that would challenge the survival of this species under hypoxic conditions. These findings are aggravated by the prolonged QRS intervals, which indicate slower ventricular conduction of AP during depolarization. QRS interval widening has been associated with low gap junction conductance and/or reduced sodium channel

activity (Posnack et al., 2014). In mammals, exposure to inorganic Hg affects the cardiovascular systems, triggering significant prolongations of the PR, QRS and QT intervals, which are associated with increased risk of arrhythmias and cardiac failure (Wildemann et al., 2015; Peregrina-Chávez et al., 2018).

Abnormalities in the generation or conduction of electrical impulses has been associated with arrhythmias. Several types of cardiac arrhythmias and anomalies (extra systoles, atrial fibrillation, negative QRS complex) appeared in the current study after Hg exposure, in both fish species in deep hypoxia. That is, mercury exposures disturbed the coordinated electrical activation of the heart, which is crucial for the maintenance of a regular f_H and contractility, which might cause myocardial dysfunction.

Negative effects of Hg on *ex vivo* cardiac contractility have been reported in these two species of fish, in a study measuring myocardial isometric twitch force development, contraction/relaxation dynamics and cardiac pumping capacity of ventricular muscle strips (Monteiro et al., 2017). According to Vornanen (2017), the pumping function of fish heart depends on the electrical excitability of cardiac myocytes, to maintain the excitability at a level that ensures steady cardiac contractility under various physiological and environmental stressors. Consequently, electrical excitability is crucial for the adjustment of cardiac performance to attend physiological demands, especially concerning physiological adaptations to metal contamination.

Oveall, the results highlighted the negative impacts of environmental factors, like Hg contamination and hypoxia, on rhythm of the cardiac pacemaker, rate of impulse propagation, duration of ventricular AP, and genesis of cardiac arrhythmias. These alterations in electrical cardiac conduction could lead to pathophysiological outcomes, most noticeably an increase in hypoxia sensitivity. They could also cause sub-lethal impairments of cardiac output, swimming performance, capacity to forage and capture prey, with consequences for growth and/or reproductive rates.

Environmental stressors, like metal contamination, might elevate maintenance costs, leading to energy deficiency (Sokolova and Lannig, 2008). Furthermore, understanding the interactive effects of low levels of dissolved O₂ and sublethal exposures to contaminants are crucial for understanding how hypoxia may affect communities of organisms (Pollock et al., 2007). Hg-induced changes in cardiac electrophysiology could have severe implications for fishes living in hypoxic tropical waters. The present results together with the earlier data lead to the conclusion that acute waterborne and chronic trophic inorganic Hg exposures can both cause impairments in electrical stability and rhythm of the heart beat, leading to amyocardial dysfunction that is further intensified in hypoxia. Knowledge of interactions among multiple potential stressors is crucial to understanding of how populations are impacted and predicting the consequences of simultaneous exposure to environmental stressors. Moreover, cardiac electrical activity might be a useful ecotoxicological endpoint biomarkers for biological monitoring.

Acknowledgments

The authors are thankful to Águas Claras (Mococa-SP) and Parque do Lago (Dourado-SP) fish farms, which provided the fishes.

Ethics in Animal Experimentation

This study was performed with the approval of the Committee of Ethics in Animal Experimentation (CEUA - approval 04/2007) and Committee of Environmental Ethics (CEA - approval 003/2006) of the Federal University of São Carlos.

Funding Information

This study was supported by São Paulo State Research Foundation (FAPESP, Proc. #06/50772-6).

418

419 **Conflict of interest**

420 The authors declare that they have no conflict of interest.

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Table 1. Effects of graded hypoxia on the QT intervals or ventricular action potential durations (V_{APD}) for *B. amazonicus* from groups Ct and wHg (n = 12). Values are mean \pm SEM. Bold values indicate a significant difference in relation to the normoxic values, while asterisks indicate significant difference in relation to the control group ($p < 0.05$).

PO ₂ (mmHg)	QT interval (V_{APD} - ms)	
	Ct	wHg
140	373.9 \pm 9.4	373.5 \pm 14.9
120	361.7 \pm 10.4	373.8 \pm 21.6
100	358.3 \pm 11.8	371.3 \pm 22.7
80	364.9 \pm 15.3	384.9 \pm 23.3
60	379.9 \pm 16.5	394.2 \pm 25.4
40	402.1 \pm 12.3	431.8 \pm 9.0
20	422.4 \pm 23.7	506.3 \pm 17.3*

Table 2. Effects of graded hypoxia on the QT intervals or ventricular action potential durations (V_{APD}) for *H. malabaricus* from groups Ct and tHg (n = 12). Values are mean \pm SEM. Bold values indicate a significant difference in relation to the normoxic values (140 mmHg), while asterisks indicate significant difference in relation to the control group ($p < 0.05$).

PO ₂ (mmHg)	QT interval (V_{APD} - ms)	
	Ct	tHg
140	587.7 \pm 28.0	704.1 \pm 38.6 *
120	579.3 \pm 28.0	707.9 \pm 34.5 *
100	578.8 \pm 28.6	690.3 \pm 37.4 *
80	570.1 \pm 19.7	709.9 \pm 32.8 *
60	563.2 \pm 23.5	724.4 \pm 33.4 *
40	572.4 \pm 20.3	698.7 \pm 29.1 *
20	584.8 \pm 24.9	706.4 \pm 34.1 *

Figure captions

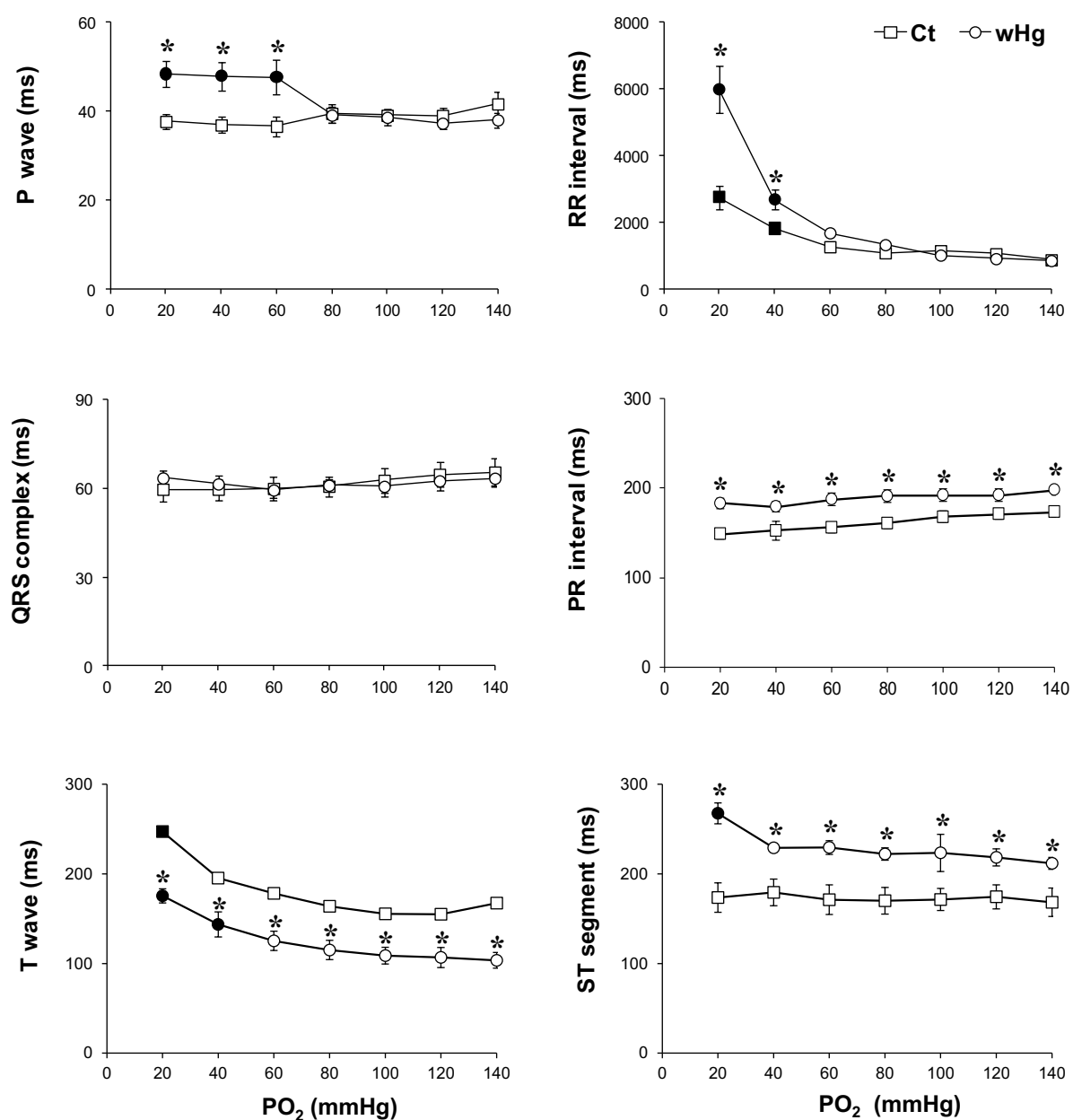
Figure 1. ECG parameters of matrinxã, *B. amazonicus*. Effects of decreasing O₂ tensions of inspired water (PO₂) on the P wave duration, RR interval, QRS complex duration, PR interval, T wave duration, and ST segment of matrinxãs from control (Hg-free water) or Hg exposed (via water = wHg) groups. Data correspond to mean \pm standard error of the mean (SEM); n = 12. Closed symbols denote a significant difference in relation to the normoxic values obtained at 140 mmHg (P < 0.05). Significant differences in comparison to the control group are indicated by * (P < 0.05).

Figure 2. Electrocardiograms of *B. amazonicus*. A and C: control (Hg-free water), B and D: Hg exposed groups (via water = wHg) during normóxia (A and B) and hypoxia (C and D). Atrial and ventricular depolarization and repolarization are represented on each ECG recordings as a series of waves: the P wave followed by the QRS complex and the T wave. In (D), arrowheads represent atrial fibrillation and arrow shows ventricular extra systoles.

Figure 3. ECG parameters of traíra, *H. malabaricus*. Effects of decreasing O₂ tensions of inspired water (PO₂) on the P wave duration, RR interval, QRS complex duration, PR interval, T wave duration, and ST segment of traíras from control (Hg-free diet) or Hg exposed (trophic exposure = tHg) groups. Data correspond to mean \pm standard error of the mean (SEM); n = 12. Closed symbols denote a significant difference in relation to the normoxic values obtained at 140 mmHg (P < 0.05). Significant differences in comparison to the control group are indicated by * (P < 0.05).

Figure 4. Electrocardiograms of *H. malabaricus*. A and C: control (Hg-free diet), B and D: Hg exposed (trophic exposure = tHg) during normóxia (A and B) and hypoxia (C and D).

601 Atrial and ventricular depolarization and repolarization are represented on each ECG
602 recordings as a series of waves: the P wave followed by the QRS complex and the T wave. In
603 (D), arrowheads represent atrial fibrillation. Note altered ventricular repolarization displayed
604 as flattened, inverted, or biphasic T wave as well an altered direction of the ventricular
605 depolarization as indicated by negative QRS complex.

606 **Fig 1**

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609 1.5 column fitting image.

610 **Fig 2**

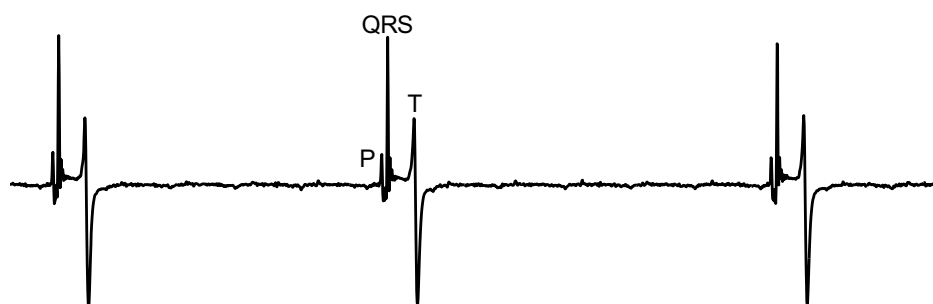
(A) *Control - normoxia (140 mmHg)*



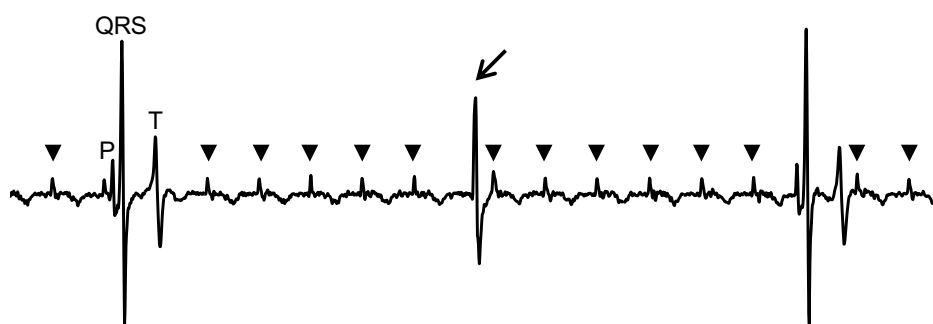
(B) *wHg - normoxia (140 mmHg)*



(C) *Control - hypoxia (20 mmHg)*



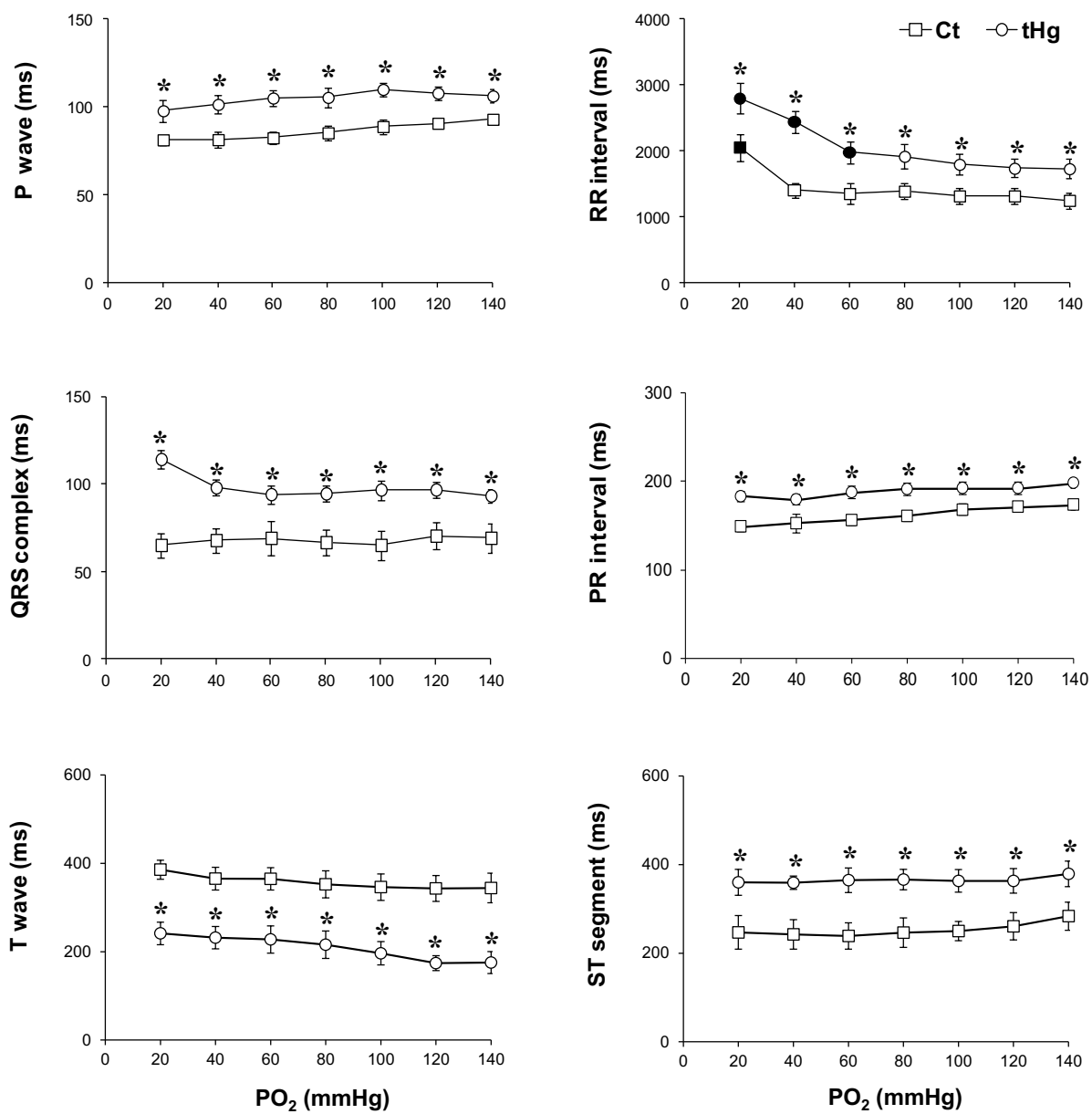
(D) *wHg - hypoxia (20 mmHg)*



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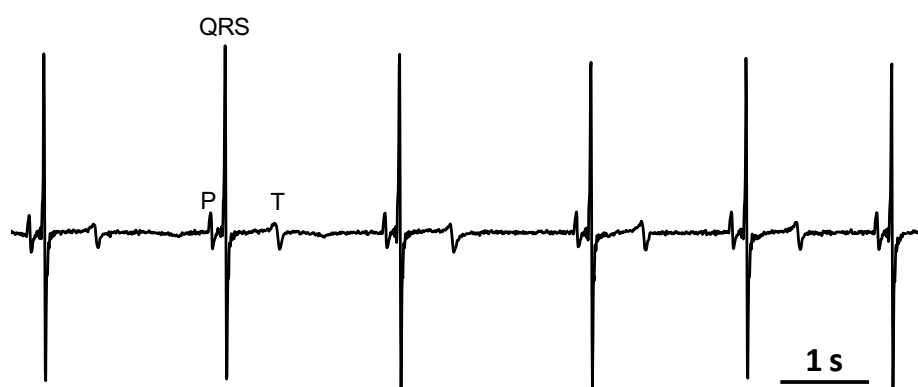
612 1.5 column fitting image.

Fig 3



618 **Fig 4**

(A) *Control - normoxia (140 mmHg)*



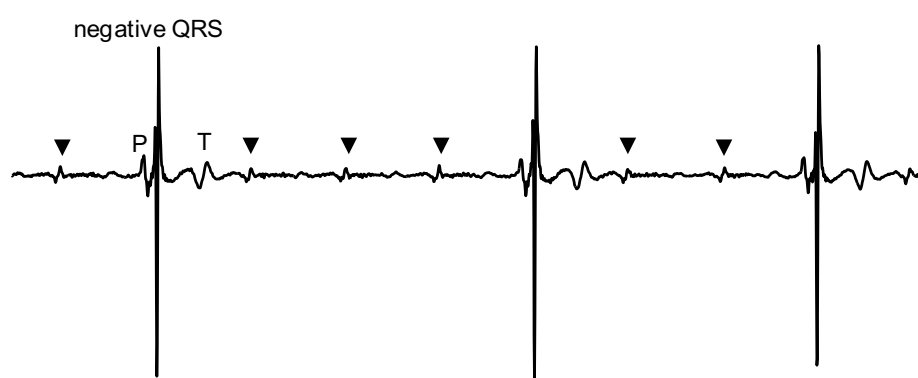
(B) *tHg - normoxia (140 mmHg)*



(C) *Control - hypoxia (20 mmHg)*



(D) *tHg - hypoxia (20 mmHg)*



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620 1.5 column fitting image.