



## Poison in the nursery: Mercury contamination in the tadpole-rearing sites of an Amazonian frog

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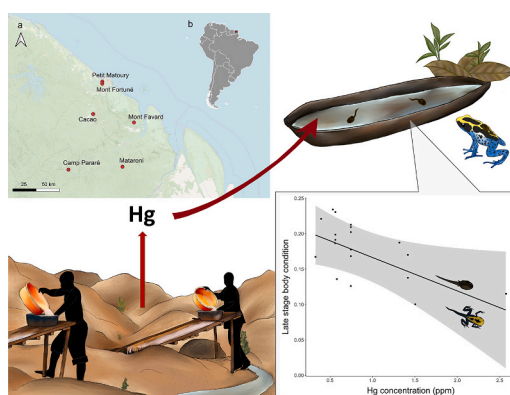
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### HIGHLIGHTS

- Hg pollution in phytotelmata and its effects on the species therein is unknown
- Hg concentrations were measured in pools used by the poison frog *Dendrobates tinctorius* for tadpole deposition
- Hg concentrations were relatively high and tended to increase in proximity to ASGM sites
- Tadpole body condition at later developmental stages is negatively correlated with Hg concentration

### GRAPHICAL ABSTRACT



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### ABSTRACT

Artisanal and small-scale gold mining (ASGM) has become a major threat for Neotropical forests. This technique for obtaining gold is a substantial driver of small-scale deforestation and the largest contributor of Hg emissions to both the atmosphere and freshwater systems globally. Previous studies have demonstrated the impacts of Hg accumulation on various aquatic ecosystems and organisms. However, its consequences in other, more discrete systems such as phytotelmata (water-holding plant structures), and the organisms therein, have so far gone unnoticed. Here, we show high concentrations of Hg (mean  $\pm$  SD:  $1.43 \pm 2.19$  ppm) in phytotelmata and other small pools, the aquatic microenvironments used by the Neotropical poison frog *Dendrobates tinctorius* as tadpole-rearing sites. In 17 % of the cases, we detected Hg concentrations above the severe effect level (SEL = 2 ppm) for

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freshwater sediments. Hg concentrations varied depending on pool characteristics and tended to increase in proximity to known ASGM sites. We did not find an effect of Hg concentration on the number of *D. tinctorius* tadpoles in a given pool. Tadpoles were found in pools with concentrations of up to 8.68 ppm, suggesting that *D. tinctorius* fathers do not avoid pools with high Hg levels for tadpole deposition. While further research is needed to determine the potential effects of Hg on tadpole development, we found an intriguing tendency for tadpoles in later developmental stages to have lower body condition when occurring in pools with higher Hg concentrations. Our findings provide evidence of relevant Hg concentrations in the terrestrial water systems used by phytotelm-breeding anurans, and highlight the need of further field and experimental studies investigating the implications of Hg contamination for tadpole development and behaviour and the overall conservation of Amazonian biodiversity.

## 1. Introduction

Tropical rainforests such as Amazonia are among the most biodiverse terrestrial ecosystems in the world (e.g., Grenyer et al., 2006), but also among the most threatened (Laurance et al., 2000). Many regions of the Amazon basin are characterised by naturally high levels of environmental mercury (Hg), mostly due to the geochemical characteristics of the soils and biological decay (Roulet et al., 1998; Fadini and Jardim, 2001). However, anthropogenic activities such as artisanal and small-scale gold mining (ASGM; Peterson and Heemskerk, 2001; Kalamandeen et al., 2018) have led to dramatic increases in Hg levels in the environment over the last century (Kumar et al., 2018). ASGM is commonly illegal and, because it tends to occur in remote places, it is often in -or next to- protected areas and biodiversity hotspots (Durán et al., 2013; Cuellar-Valencia et al., 2023; Palacios-Torres et al., 2018; Alvarez-Berrios and Aide, 2015; Bax et al., 2019), making it difficult to assess timely and directly the impacts on local wildlife. Nowadays, gold mining is the single largest contributor of Hg emissions to both the atmosphere and freshwater globally (UN Environment, 2019). However, other factors, such as industrial emissions, the historic application of mercury-containing pesticides and fertilisers in agriculture, and sewage irrigation, also contribute to Hg pollution (Mirlean et al., 2008; Chen et al., 2013; Bavec et al., 2014; Schneider, 2021; Fisher et al., 2023).

Hg is an environmental contaminant of global concern due to its toxicity and the risks it poses to wildlife and human health (Ashe, 2012; Rice et al., 2014; Gibb and O'Leary, 2014; Alvarez-Berrios et al., 2016; WHO (World Health Organization), 2017; Markham and Sangermano, 2018). After being released to the air, land or water, and transformed by microorganisms into methylmercury (MeHg), its most toxic form, Hg can bioaccumulate in both aquatic and terrestrial organisms and biomagnify across the food webs (Kidd et al., 2012; Mason et al., 1996). Furthermore, gaseous Hg released in the atmosphere (for example, due to ASGM activities) can travel long distances before being deposited on surfaces and plants, or returned to the terrestrial landscape through dry or wet deposition (Mélières et al., 2003; Zhou et al., 2021; Gerson et al., 2022).

To date, assessments of Hg interactions with tropical ecosystems and biodiversity lag behind those conducted in temperate and polar areas (but see Cuellar-Valencia et al., 2023; de Oliveira Drummond et al., 2022; Palacios-Torres et al., 2018; Soe et al., 2022; as examples of studies done in the tropics). Moreover, most studies carried out to date have focused on Hg dynamics and impacts in freshwater (rivers: Webb et al., 2015; Vieira et al., 2018 and lakes: Brito et al., 2017; Gomes et al., 2020) and estuarine systems (Mol et al., 2001; Vezzzone et al., 2023), while implications in other more discrete, inland aquatic systems remain unexplored. Phytotelmata, for example, are small bodies of water contained in vegetation structures (e.g. leaf axils, fallen bracts, holes in trunks and roots), which provide essential habitat for the breeding, feeding, refuge and development of a wide range of species (Varga, 1928; Fincke, 1999; Kitching, 2000; Lehtinen et al., 2004), including many insects (Orr, 1994; Kitching, 2000; Campos and Fernández, 2011), and amphibians (e.g., Kam et al., 1996; Schiesari et al., 2003; Lehtinen et al., 2004; Heying, 2004). Because these small pools are directly formed by rainwater and accumulate decomposing leaf litter from the

surrounding canopy (Kitching, 2000), it could be expected that they accumulate high concentrations of Hg over time, particularly in the context of ASGM (Malm et al., 1998; Mélières et al., 2003; Gerson et al., 2022). Furthermore, differences in the physico-chemical properties among phytotelmata, as well as in their morphology and durability, could influence the amount of Hg, its chemical form and, ultimately, its toxicity and absorption. For example, acidic pH conditions, warmer water temperatures, high dissolved organic carbon, and low dissolved oxygen and electrical conductivity, have been shown to increase Hg methylation (i.e. MeHg) in water and sediments in some environments (Mauro et al., 1999; Bank et al., 2007; Jardine et al., 2013; Brito et al., 2017).

Neotropical poison frogs of the family Dendrobatidae are, in most part, dependent on phytotelmata as tadpole-rearing sites (Summers and McKeon, 2004; Carvajal-Castro et al., 2021) due to their elaborate parental care behaviour. Generally, one of the parents takes care of the terrestrial clutches and, upon hatching, transports tadpoles on its back to the bodies of water, such as phytotelmata, where offspring will remain confined until metamorphosis (Weygoldt, 1987; Summers and McKeon, 2004). Selecting a good tadpole-rearing site is determinant for tadpole survival, and some poison frog species are known to base such decisions on chemical cues, either for detecting (and avoiding) potentially cannibalistic conspecifics or predators (Schulte et al., 2011; Schulte and Lötters, 2014) or for finding new deposition sites in the forest (Serrano-Rojas and Pašukonis, 2021; Peignier et al., 2023). Because tadpoles are omnivores, largely feeding on detritus and larvae of insects and frogs (Summers and McKeon, 2004; Grant et al., 2006; Rojas, 2014), have a thin and highly permeable skin (Rowe et al., 2003; Quaranta et al., 2009), and need to process water for respiration, they can be particularly exposed to, and affected by, both waterborne and dietary contaminants (Burger and Snodgrass, 2001; Unrine et al., 2007; Rissoli et al., 2016). Thus, Hg may pose a considerable threat to tadpoles of this and other species developing in phytotelmata if adults cannot detect and avoid Hg contaminated water bodies (e.g., via chemical cues) for oviposition or tadpole deposition. Previous studies in subtropical and temperate zones have reported high concentrations of Hg bioaccumulated in amphibian larvae through environmental exposure and diet (Byrne et al., 1975; Unrine et al., 2007; Bank et al., 2007), and negative effects of Hg on development (i.e. smaller body size, delayed metamorphosis, higher prevalence of malformations) and survival (Unrine et al., 2004; Shi et al., 2018).

Here, we measured the concentrations of Hg found in different types of phytotelmata (hereafter referred to as "pools") over six different locations in French Guiana, a French Overseas Department with high prevalence of ASGM (Boudou et al., 2006; Grimaldi et al., 2015). We covered a gradient from pristine/protected forest to disturbed, semi-urbanised areas, at variable distances from known ASGM sites, to assess whether pools potentially used as tadpole-rearing sites contained Hg and, if so, in what concentrations. We focused on pools that could be used by the dyeing poison frog, *Dendrobates tinctorius*, as males of this species use phytotelmata with a wide range of physical and chemical properties for tadpole deposition (Fouilloux et al., 2021). *Dendrobates tinctorius* parents can also use available water-filled plastic containers (e.g., Rojas, 2015) to deposit their tadpoles. Thus, we also sampled



artificial pools in this study. Because ASGM activities use Hg to facilitate gold extraction, we expected to find higher Hg concentrations in closer proximity to ASGM sites. Furthermore, because phytotelmata in tree holes, both in live and fallen trees, are generally more stable over time than other plant structures such as fallen palm bracts, they can accumulate higher amounts of rainwater and litterfall; thus, we predict this type of phytotelmata to have the highest Hg concentrations. Finally, we also recorded the presence/absence, number and size of *D. tinctorius* tadpoles in each of the pools sampled to investigate (1) potential parental avoidance of pools with high Hg concentrations, as seen in other anuran species which avoid herbicide polluted pools for oviposition (e.g. Takahashi, 2007; Vonesh and Buck, 2007); and (2) the potential impact of Hg on larval body condition. If parents are able to detect Hg in the water, we predict pools with higher Hg concentrations to hold fewer (or no) tadpoles than pools with low concentrations or no Hg. Moreover, if Hg negatively affects tadpole growth and development, as previously shown in some temperate species (e.g. Unrine et al., 2004), we predict pools with high Hg levels to harbour tadpoles with poorer body condition than those in pools with little or no Hg.

## 2. Material and methods

### 2.1. Study species

*Dendrobates tinctorius* (Fig. 1a) is a rainforest dweller endemic to the

Eastern Guiana Shield (French Guiana, Suriname and Northwestern Brazil), where it can be found on elevated ridges or plateaus up to 600 m above sea level (Noonan and Gaucher, 2006). They are diurnal, and characterised by a highly variable bright colouration coupled with the possession of skin toxins (Rojas and Endler, 2013; Lawrence and Rojas et al., 2019) and an elaborate male parental care. Their clutches are laid terrestrially, on the leaf litter or within hollow fallen logs or tree roots, and taken care of by the male until tadpoles hatch (Rojas and Pašukonis, 2019). Upon hatching, males transport the tadpoles on their back to bodies of water contained in the vegetation (i.e., phytotelmata; Rojas, 2014, 2015; Fouilloux et al., 2021). Once deposited, tadpoles will remain in these pools (Fig. 1b-d) selected by their father until metamorphosis, which is reached after 2–3 months (Rojas and Pašukonis, 2019). *D. tinctorius* was declared a protected species in French Guiana in 2020 (Decree No. TREL2032100A from November 19th 2020).

### 2.2. Study areas and field sampling

From January to March 2023, we collected a mix of water and organic matter samples from pools in six different study sites in French Guiana. These sites are known to hold *Dendrobates tinctorius* populations, and are found under different degrees and types of disturbance, and at variable distances from the closest ASGM site (see Table 1). In each study site, we searched for pools along transects between 10 and 15 km in length, targeting suitable microhabitats such as fallen trees and trees



**Fig. 1.** A male *Dendrobates tinctorius* carrying two tadpoles on his back (a) and the diversity of sampled pool types used as tadpole-rearing sites. Pools used include phytotelmata formed in palm bracts (b), trunk/root holes (c) and fallen trees (d), as well as artificial pools formed in anthropogenic litter (e).



**Table 1**

Overview of the study sites, the type of surrounding habitat, the distance to the closest ASGM site and the number of pools sampled.

| Location      | Surrounding habitat | Distance to closest ASGM site (km) | Number of sampled pools |
|---------------|---------------------|------------------------------------|-------------------------|
| Petit Matoury | Urban               | 32                                 | 13                      |
| Mont Fortuné  | Urban               | 31                                 | 28                      |
| Cacao         | Agricultural        | 1.6                                | 16                      |
| Mataroni      | Logging roads       | 16                                 | 14                      |
| Mont Favard   | Natural Reserve     | 27                                 | 13                      |
| Camp Pararé   | Natural Reserve     | 15                                 | 14                      |

with buttresses or hole structures. When a pool was found, we recorded its type according to the following three categories for phytotelmata: holes in fallen trees; holes in trunks and roots of living trees; and fallen palm bracts. In addition, we also included pools formed in rocky substrates and artificial plastic or metallic containers, as *D. tinctorius* tadpoles can be found in both types. Artificial pools were found in large numbers in Petit Matoury and Cacao, and only a small random subset was sampled. We obtained the distance to the closest ASGM site for each study area from the French 'Unité Spécialisée Nature', National Office for Forests (ONF).

In addition, we noted each pool's morphological and physico-chemical characteristics, and its GPS position. Morphological parameters included height from the ground to the pool edge, largest width and length parallel to the water surface, and water depth. Based on these measurements, we calculated the surface area per water volume ratio (SA/V) of each pool using the formula for a semi-ellipsoid. Physico-chemical parameters included water pH, alkalinity (KH), and water temperature. Water temperature and pH were recorded with a pH metre (pH 20, Apera Instruments®), and KH was measured using aquarium water testing strips (JBL EasyTest).

For each pool, we collected a 4 ml mixture of water and sedimentary organic matter with a sterile syringe. Tadpoles use sedimentary organic matter for feeding and hiding, and may be therefore exposed to Hg concentrations from both water and organic matter. Further, we recorded the presence/absence of tadpoles and, when present, attempted to assess the total number of tadpoles and noted the species. If *D. tinctorius* tadpoles were found in the pool, we used a net or plastic spoons to catch five individuals representing the observed variation in developmental stage. Each captured tadpole was weighed to the nearest 0.01 g and photographed over graph paper to measure its length afterwards. We also noted each tadpole's developmental stage sensu Gosner (1960), and classified it into three categories (early pre-metamorphosis: 25–29; late pre-metamorphosis: 30–35; and pro-metamorphosis/metamorphic climax: 36–45) to account for its nonlinear nature. This means that larval development time to change from e.g. stage 26 to 27 is not equivalent to that from stage 42 to 43. Documenting tadpole developmental stages also allowed us to account for differences in exposure times to Hg in the pool. All tadpoles were returned to the same pool where they were caught immediately after. Capture and sample collection were done under a permit from the Direction Régionale des Territoires et de la Mer de Guyane (R03–2022-12-28-00004) after assessment by the CSRPN, the regional scientific committee. Procedures employed at Nouragues Research Station were also approved by the Reserve's scientific committee.

### 2.3. Mercury analyses

Total Hg (THg) was quantified in 400 µL of water and sedimentary organic matter using a Direct Mercury Analyser (DMA-80, atomic absorption spectrometer, Milestone). The method was validated by the

analysis of certified reference material (CRM) TORT-3 (Lobster hepatopancreas from the National Research Council of Canada (NRCC); certified Hg concentration  $0.292 \pm 0.022$  ppm dw) at the beginning and the end of analytical cycle and after every 10 samples. Measured values for TORT-3 were  $0.298 \pm 0.007$  ppm dw ( $n = 15$ ). Blanks were included at the beginning of each analytical run and the limit of detection of the DMA-80 was 0.001 ng Hg. Mercury concentrations are presented in ppm.

### 2.4. Statistical analyses

We checked the normality of our data using a Shapiro-Wilks test, and log-transformed Hg concentration data to achieve normality of residuals. We used a linear mixed-effect model with a Gaussian distribution to assess the relationship between measured Hg concentration (response variable), distance to the closest known ASGM site, pool type, height, pH and SA/V (explanatory variables). We included study site as a random intercept to account for unmeasured differences between locations. We excluded KH and water temperature from our analysis since its variation between pools was very low and independent from the type of pool and the study site (see Table S1).

To investigate the effect of Hg concentration on the probability of presence of *D. tinctorius* tadpoles and tadpoles from all species recorded, we used generalised linear mixed-effects models (GLMM) with a binomial error distribution. In addition to Hg concentration, we included the pool type and pool traits (i.e. SA/V, height and pH) as fixed effects, and study site as random effect. For the analysis with only *D. tinctorius*, we also included the number of tadpoles present in the water body as a fixed effect, as it may influence parental care decisions (Rojas, 2014). Further, we studied the effect of Hg on the total number of *D. tinctorius* tadpoles per pool using a GLMM with a Poisson distribution, including Hg concentration, pool type and pool traits as fixed effects, and study site as random effect. We repeated this last analysis for all tadpole species together.

We calculated the body condition of tadpoles using the scaled mass index (Peig and Green, 2009, 2010). For that, we used tadpole body length measured from the tip of the snout to the most posterior point of the body, excluding their tail to avoid errors due to tail injury. To analyse the effect of Hg concentration on tadpole body condition, we used a linear mixed-effects model with a Gaussian distribution. We included Hg concentration, number of tadpoles in the pool and pool traits as fixed effects, and pool ID nested in study site as a random effect to control for non-independence of the data. In addition, because body mass and SVL vary with Gosner stage as tadpoles approach metamorphic climax, we included stage categories and the interaction with Hg in our model to control for possible differences in body condition due to developmental stage rather than Hg levels.

For all analyses, we used the variance inflation factor to identify multicollinearity between independent variables in the different models and removed correlated variables when required ( $VIF > 3$ ; Zuur et al., 2010). We used model selection based on Akaike's Information Criterion for small sample sizes (AICc) (Burnham et al., 2011), using the R package 'MuMIn' (Barton and Barton, 2020), selecting the model with the lowest AICc value. If two or more of the highest ranking models were within delta AIC < 4, we performed model averaging (Symonds and Moussalli, 2011). Parameters that included zero within their 95 % CI were considered uninformative (Arnold, 2010). We accepted statistical significance at  $p < 0.05$  and trends at  $p < 0.1$ . All statistical analyses were performed using the software R version 4.1.2 (R development Core Team 2022).

## 3. Results and discussion

Total Hg levels found in the water and sediments of all pools in this study (including rock and artificial pools) were relatively high, ranging from 0.007 to 13.040 ppm (mean  $\pm$  SD:  $1.43 \pm 2.19$  ppm), being highest

in Cacao and lowest in Mont Fortuné (Table 2). The concentrations found in this study are comparable to those reported in polluted water bodies near mining sites (Jiang et al., 2006; Soe et al., 2022) or abandoned chlor-alkali plants (Degetto et al., 1997; Song et al., 2018) elsewhere. One sample was under the detection limit. An outlier value of 172.06 ppm belonging to an artificial pool (a rusty paint barrel) was removed from the main analysis, as we were unable to determine whether this high value could be attributed to a measurement error or to leftovers of the previous content therein. In 17 % of our samples, Hg concentrations were above the severe effect level (SEL = 2 ppm) for freshwater sediments reported by the US National Oceanographic and Atmospheric Administration (Buchman, 2008). Above this level, sediments are considered heavily polluted and adverse effects are expected to occur in the majority of sediment-dwelling organisms.

Pools in locations closer to known ASGM sites tended to accumulate higher Hg concentrations (Table 3, Fig. 2a). Among the pools sampled, those in Cacao, which is only 1.6 km from the closest known ASGM site, had the highest concentrations of Hg. All other locations were ≥ 15 km from the nearest known ASGM. These findings suggest a potential link between ASGM activities and Hg pollution in pools. Another possible explanation for the high Hg concentrations found in Cacao could be the (historic) use of Hg-containing fungicides or phosphorus fertilisers with Hg impurities in the agricultural fields that dominate the landscape (Mortvedt, 1995; Turull et al., 2018; Tang et al., 2018). Although Hg-containing fungicides have been banned in the EU since 2017 (<https://minamataconvention.org>), they were commonly used during the second half of the 20th century and can remain in the soil for long periods of time (Murphy and Aucott, 1999; Horowitz et al., 2014; Ali et al., 2019; Schneider, 2021). Pools in Mont Fortuné, in contrast, had the lowest values of Hg. This could be explained by the fact that, despite being surrounded by urban areas, this location is not directly adjacent to agricultural fields or close to illegal ASGM activities, which often take place in remote locations (Durán et al., 2013; Rahm et al., 2015).

Deeper pools in relation to their surface area, as well as pools closer to the ground, had higher concentrations of Hg (Table 3, Fig. 2b-c).

**Table 3**

Effect size, standard error (SE), lower (LCI) and upper (UCI) 95 % confidence intervals, and P-values of explanatory variables for the analysis of Hg concentrations in pools. We performed model averaging of best models ( $\Delta AICc < 4$ ) to estimate the effect size of each variable. Informative parameters are presented in bold (95 % confidence intervals do not overlap with zero).

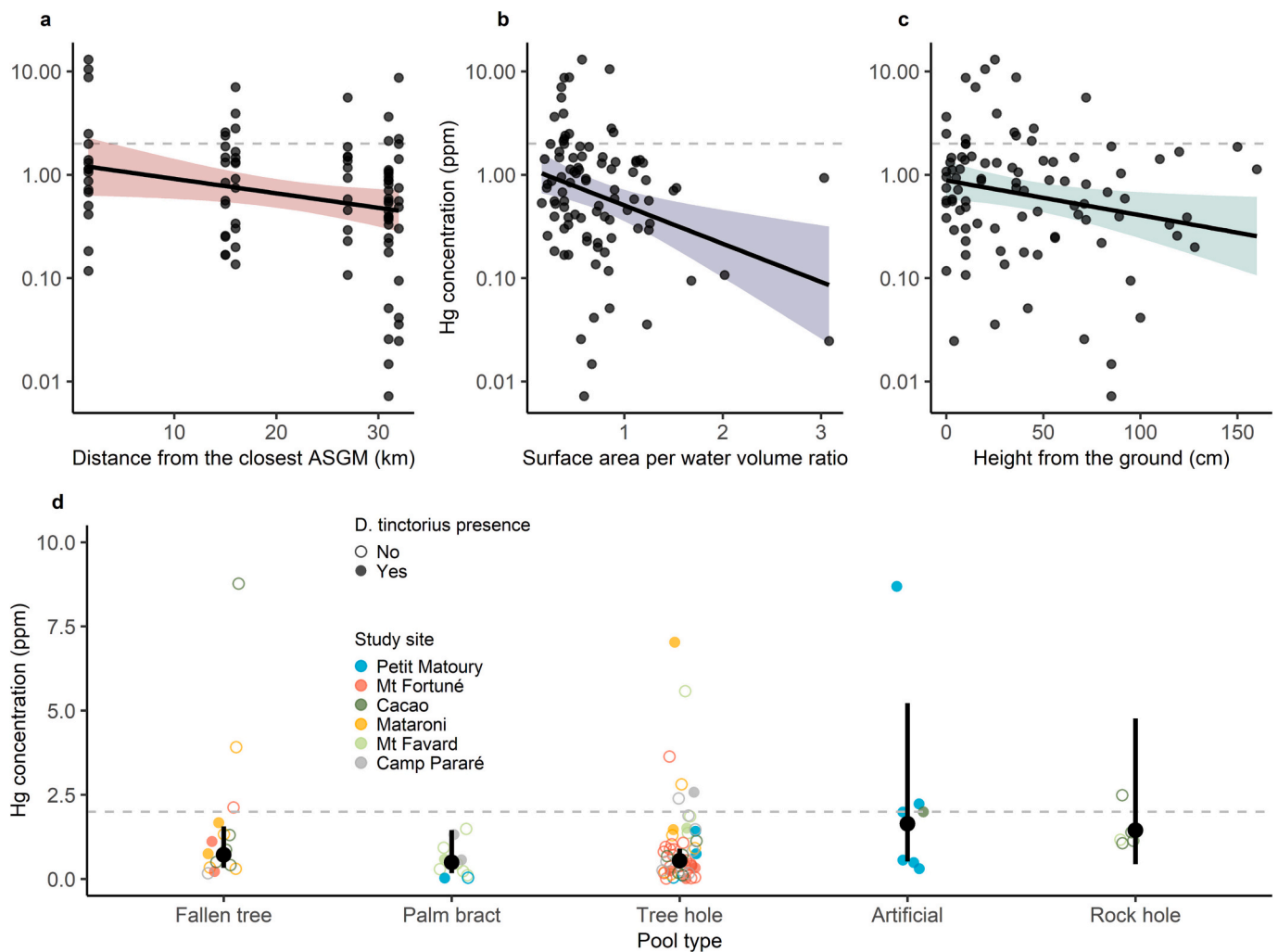
| Variable                       | Estimate     | SE          | LCI          | UCI          | P-value     |
|--------------------------------|--------------|-------------|--------------|--------------|-------------|
| Intercept                      | -0.33        | 0.34        | -1.00        | 0.35         | 0.34        |
| <b>Pool height</b>             | <b>-0.29</b> | <b>0.14</b> | <b>-0.57</b> | <b>-0.01</b> | <b>0.04</b> |
| <b>SA/V</b>                    | <b>-0.40</b> | <b>0.16</b> | <b>-0.72</b> | <b>-0.09</b> | <b>0.01</b> |
| Distance from the closest ASGM | -0.37        | 0.21        | -0.78        | 0.05         | 0.09        |
| Pool type Palm bract           | -1.07        | 0.71        | -2.48        | 0.33         | 0.13        |
| Pool type Tree hole            | -0.27        | 0.40        | -1.05        | 0.52         | 0.51        |
| Pool type Artificial           | 0.78         | 0.74        | -0.68        | 2.24         | 0.30        |
| Pool type Rock hole            | 0.59         | 0.66        | -0.72        | 1.89         | 0.38        |
| pH                             | -0.10        | 0.14        | -0.37        | 0.18         | 0.49        |

Pools in rock holes (mean ± SD: 3.38 ± 4.76 ppm) and artificial pools (2.32 ± 2.92 ppm) generally showed the highest levels of Hg, followed by fallen trees (1.59 ± 2.21 ppm) and tree holes (1.20 ± 1.83 ppm). Palm bracts had the lowest Hg concentrations (0.55 ± 0.50 ppm) (Fig. 2d). After accounting for pool height and SA/V, pool type and pH were uninformative in explaining variation in Hg concentration (Table 3). However, pool type and pool characteristics (SA/V and pool height) were interrelated (Table S2; Fig. S1, S2), and it is possible that other pool characteristics also influenced Hg concentrations (e.g., durability, material of artificial pools) (Fouilloux et al., 2021). For example, shallow pools in relation to their volume (i.e. high SA/V),

**Table 2**

Number of pool types sampled by study site, mean and standard deviation of Hg concentrations (ppm), total number of *D. tinctorius* tadpoles observed and number of *D. tinctorius* tadpoles sampled per study site. Columns coloured in green correspond to phytotelmata.

| Study site    | Type of pool |                  |             |           |            | Mean ± SD Hg concentration (ppm) | Number of <i>D. tinctorius</i> tadpoles observed | Number of <i>D. tinctorius</i> tadpoles sampled |
|---------------|--------------|------------------|-------------|-----------|------------|----------------------------------|--|---|
|               | Palm bract   | Tree hole/ roots | Fallen tree | Rock hole | Artificial |                                  |  |   |
| Petit Matoury | 2            | 4                | 0           | 0         | 6          | 1.38 ± 2.42                      | 147  | 11  |
| Mt Fortuné    | 0            | 25               | 3           | 0         | 0          | 0.77 ± 0.87                      | 30   | 14  |
| Cacao         | 0            | 6                | 5           | 5         | 2          | 2.72 ± 3.96                      | 3  | 2   |
| Mataroni      | 0            | 7                | 6           | 0         | 0          | 1.70 ± 1.94                      | 15   | 11  |
| Mt Favard     | 7            | 4                | 0           | 1         | 0          | 1.30 ± 1.47                      | 16   | 8   |
| Camp Pararé   | 2            | 12               | 1           | 0         | 0          | 1.08 ± 0.87                      | 16   | 9   |



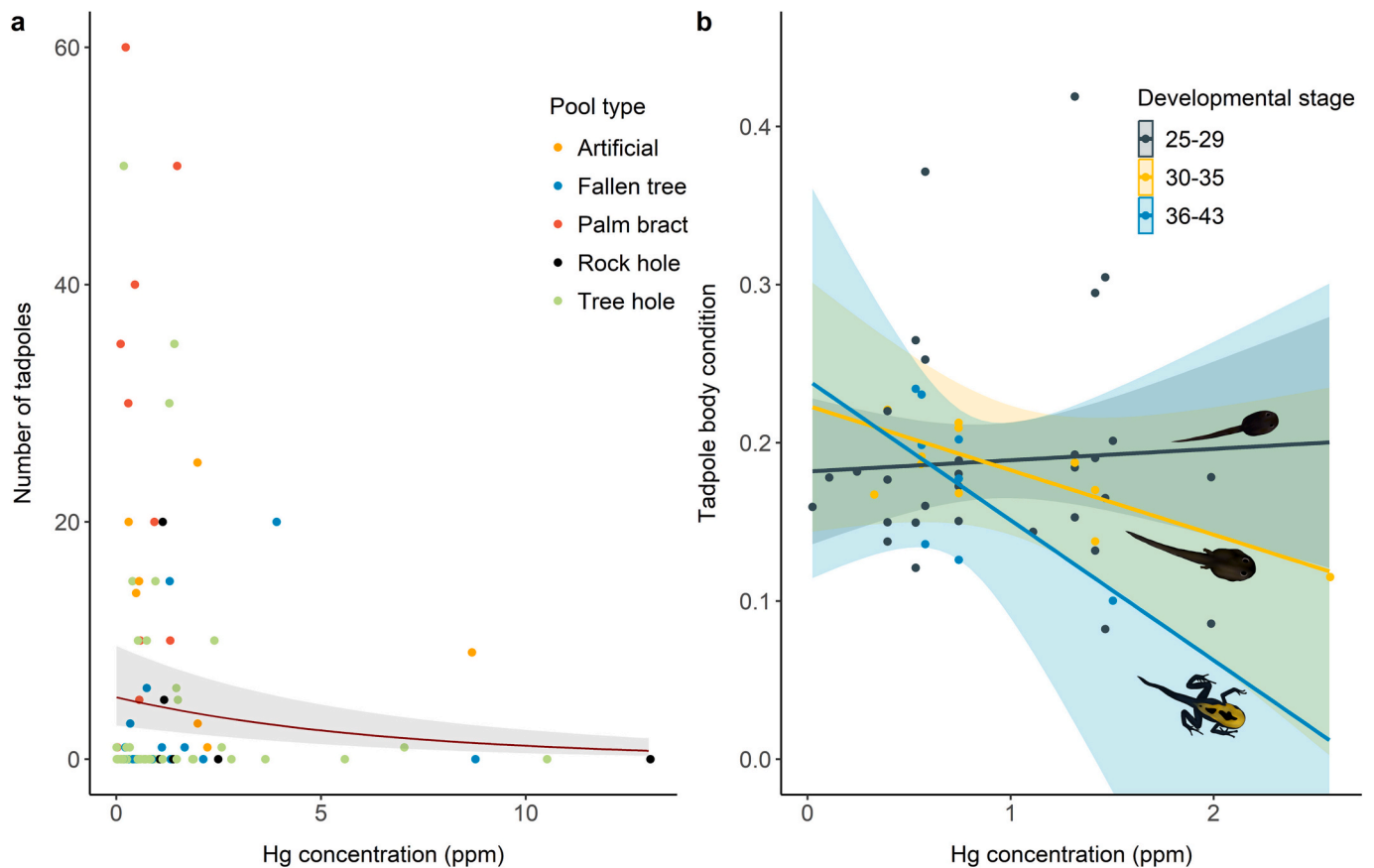
**Fig. 2.** The predicted (large symbols and lines) Hg concentration (ppm) for (a) the distance to the closest known ASGM, (b) the surface area per water volume ratio (SA/V), (c) height above ground, and (d) the different types of phytotelmata (fallen trees, palm bracts, and tree holes) and other pool types (artificial pools and rock holes). In plot d, each colour represents one study site and filled/empty dots represent presence/absence of *D. tinctorius* tadpoles in the pool. The dashed line represents the severe effect level (2 ppm); Hg concentrations above this are considered to be heavily polluted. The 95 % confidence intervals are shown as shading (a-c) or lines (d). Note that for plots a-c, the y-axis is on the log-scale for easier visualization.

which had the lowest Hg concentrations, were mostly represented by palm bracts (Table S2; Fig. S1). Palm bracts are typically ephemeral and may dry out and decompose rapidly (Fouilloux et al., 2021), having relatively little time to accumulate Hg through rain and leaf litter. In contrast, water pools formed in rock holes may have naturally higher levels of Hg due to both, their permanent nature when compared to degrading plant structures, and their direct contact with Hg-rich soils (Roulet et al., 1998; Fadini and Jardim, 2001).

Artificial pools were common in the disturbed sites of Cacao and Petit Matoury, and often used by *Dendrobates tinctorius* as tadpole-rearing sites. No other species was recorded using this pool type. Although anthropogenic litter accumulated in disturbed areas increases the availability of pools for tadpole deposition, they could potentially act as evolutionary traps if the higher Hg levels recorded, compared to most phytotelmata, negatively affect tadpole development and/or survival. For instance, the highest Hg concentration in the presence of a *D. tinctorius* tadpole was measured in an artificial pool (8.68 ppm). Furthermore, in contrast to other studies where frogs have shown to modify their oviposition behaviour to avoid pesticides, presumably by detecting chemical pesticide cues (Takahashi, 2007; Vonesh and Buck, 2007), we did not observe any differences in the presence/absence or number of *D. tinctorius* tadpoles in relation to Hg concentrations

(Table S3). This is in line with previous findings indicating the surprisingly wide range of pool chemical properties that *D. tinctorius* tadpoles can withstand (Fouilloux et al., 2021), which may even extend to potentially harmful substances, and suggests that phytotelm-breeding frogs might be unable to identify and/or avoid highly Hg-polluted pools for oviposition or tadpole deposition. Some Australian species, for instance, have been shown to struggle to develop in polluted wetlands, yet adults do not seem to discriminate between polluted and unpolluted pools (Sievers et al., 2019). Alternatively, it could indicate that these frogs are selecting pools on the basis of other factors such as inter-/intraspecific competition and pool stability, as is the case in several other species (Lehtinen, 2004; Summers and McKeon, 2004; Brown et al., 2008; Ryan and Barry, 2011; Schulte et al., 2011; Fouilloux et al., 2021). However, understanding the influence of Hg contamination on pool selection requires further research on parental decision-making under controlled conditions.

When accounting for all species recorded within the pools, which included *Allobates femoralis*, *Rhinella* spp., and *Osteocephalus oophagus*, we found a decrease in tadpole number (but not in presence probability) with increasing Hg concentrations (Table S4; Fig. 3a). However, the number of tadpoles was significantly higher in palm bracts (Table S4), consistent with the restrictions of these species to lower pools for egg/



**Fig. 3.** (a) The predicted number (line) of tadpoles from all species in relation to Hg concentration (ppm) and (b) predicted body condition of *Dendrobates tinctorius* tadpoles (lines) in relation to the Hg concentration (ppm), shown for the different developmental stage categories. Note that in this analysis, one tadpole outlier (stage 25–29) found in a pool with Hg concentration of 7.03 ppm was removed from our model. Raw data are represented as dots and 95 % confidence intervals as shading.

tadpole deposition compared to *D. tinctorius* (Fouilloux et al., 2021). Thus, the selection of low polluted pools may be due to a biological restriction to palm bracts, which showed lower Hg levels compared to other pool types, rather than an active avoidance of pools with higher Hg levels. In addition, the number of tadpoles also increased in pools closer to the ground, with smaller SA/V and higher pH (Table S4).

From all the tadpoles found, we sampled a total of 34 *D. tinctorius* tadpoles from developmental stages 25–29, 12 individuals from stages 30–35 and 8 from stages 36–43. This reduction in the number of tadpoles encountered with increasing developmental stages might be due to chance (as we sampled relatively few tadpoles per pool), detection bias due to increased flight behaviour (Warkentin, 1999), or mortality over time due to predation or mercury bioaccumulation. Further research should explore whether this pattern could also be explained by a lower survival of tadpoles to later stages as a consequence of longer exposure times to high mercury levels, with detrimental effects on their survival, or antipredatory behaviour. For example, Lefcort et al. (1998) reported reduced predator avoidance behaviour of tadpoles exposed to different heavy metals.

Tadpole body condition was best explained by the intercept only model (Table S5). However, there was a visual tendency for a negative correlation between Hg concentration and body condition of tadpoles at later developmental stages (Fig. 3b). In addition, when excluding from our model early stage tadpoles, which have been exposed to mercury for a few weeks only (Mean  $\pm$  SD =  $4.62 \pm 0.99$ ; C. Fouilloux and B. Rojas, unpublished), mercury was included in the second-highest ranking model within  $\Delta$  AIC < 4 (Table S5). After model averaging, there was a negative correlation of Hg and tadpole body condition (estimate  $\pm$  SE =  $-0.03 \pm 0.01$ ,  $Z = 3.47$ ,  $p \leq 0.001$ ; Table S6). More measurements

from a wider range of Hg concentrations, especially of older tadpoles, would be needed to further investigate this pattern.

One question that arises from this study is how much of the Hg detected in phytotelmata is ingested and accumulated in the tissues of developing tadpoles, as well as which are the potential repercussions on their development, ecology and behaviour. Bank et al. (2007) found a significant correlation between Hg levels in ponds and Hg levels in tadpole tissue, with much lower concentrations than those found in our study ( $1.3e^{-6}$ – $8.4e^{-6}$  ppm). In addition, they found that Hg concentrations increased with tadpole body length, pointing towards bioaccumulation of Hg as a function of age (Bank et al., 2007). These results are in line with our findings, which point towards a detrimental effect of Hg on body condition in late developmental stages. Further, Urine et al. (2004) found adverse effects on growth and development of Southern leopard frog larvae exposed to dietary Hg in concentrations similar to those reported here. The data from these studies suggest that the Hg concentrations detected in phytotelmata could also bioaccumulate in phytotelm-breeding frogs, negatively affecting the development at particularly sensitive developmental stages. Finally, given the neurotoxic and endocrine-disrupting nature of Hg (Tan et al., 2009; Crump and Trudeau, 2009), we urge to investigate the possible repercussions not only on the development of tadpoles, but also on their behaviour. To that end, experimental studies will be crucial to quantify the negative effects of various Hg concentrations on tadpole development, physiology, behaviour, and survival under standardised conditions, and to understand the mechanisms involved without the need to account for the myriad of confounding effects (such as predation, competition, pool type, water chemistry, etc.) that impede our ability to draw stronger conclusions from wild populations. Nevertheless, our results provide



valuable baseline data of mercury concentrations in the wild and lay the foundation for future work on this important environmental issue.

### CRedit authorship contribution statement

**Lia Schlippe-Justicia:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Jérémy Lemaire:** Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Carolin Dittrich:** Formal analysis, Investigation, Writing – review & editing. **Martin Mayer:** Formal analysis, Investigation, Writing – review & editing. **Paco Bustamante:** Resources, Writing – review & editing. **Bibiana Rojas:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

### Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

### Data availability

Data will be deposited in the Figshare repository upon acceptance.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.169450>.

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