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Front cover: Painted Turtle *Chrysemys picta*, see article on page 14. (© Spencer CC BY-SA 3.0)

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SHORT NOTE

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Extremely low amphibian roadkill probability on busy bicycle paths

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Road mortality can have a significant negative impact on amphibian population survival. Amphibian roadkill and how to avoid it are therefore widely studied, mostly on car roads but limitedly on bicycle paths. We investigated whether amphibian mortality on bicycle paths in Bargerveen, a Dutch Natura 2000 site, was affected by the number of passing cyclists and crossing amphibians. We investigated four transects on a daily basis during most of the amphibian spring migration in 2021. We counted and identified (to species level) all killed amphibians; further, we used cyclist counters and toad fences to assess the number of passing bicycles and crossing amphibians, respectively. We found 11 killed smooth newts *Lissotriton vulgaris*, out of 5,037 that crossed the bicycle paths. Although 11,453 anurans crossed, we found no killed anurans. The occurrence of killed smooth newts was not affected by the number of passing bicycles or crossing newts. The probability of being killed was extremely low for crossing smooth newts (0.22 %) and anurans (0 %), possibly because cyclists successfully avoid cycling over amphibians. Future monitoring should occur from early February to late November to include the complete active period of amphibians including juvenile dispersal, and across multiple successive years because amphibian numbers can vary largely between years. During our study period, however, amphibian mortality on bicycle paths in Bargerveen seems no threat to populations, despite the high numbers of cyclists.

Keywords: road ecology, animal-cyclist collisions, smooth newts, anurans, conservation

Human induced changes to the environment can have a big impact on life histories of animals. One example is road mortality, since it can be a large source of mortality which can negatively impact population survival (Sillero, 2008; Fahrig & Rytwinski, 2009). Several studies found that the mortality rate of amphibians is highest compared to other vertebrates (Glista et al., 2008; Gryz & Krauze, 2008; DeWoody et al., 2010; Attademo et al., 2011; Garriga et al., 2012; Arévalo et al., 2017). Factors determining where and when most amphibian roadkill occurs and possible mitigation measures are therefore widely studied.

Most studies on amphibian roadkill focus on car roads and found complex interactions with adjacent habitats, species composition and more (e.g. Matos et al., 2012; Meek, 2012; Heigl et al., 2017). Higher traffic intensity does not necessarily increase absolute roadkill numbers (Mazerolle, 2004; Orłowski et al., 2008), but it does increase the probability that crossing amphibians will get killed (Fahrig et al., 1995; Hels & Buchwald, 2001; Meek, 2012; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017). This is because on busy roads many amphibians are killed over time; this leads to a reduction in amphibian populations, which in turn results in lower roadkill numbers (D'Amico et al., 2015). The probability that an amphibian crossing a busy road will get killed, however, remains high (Fahrig et al., 1995; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017).

Two studies looked into amphibian mortality on bicycle paths. Allain & Smith (2016) and Heigl et al. (2017) found 17 killed smooth newts *Lissotriton vulgaris* and one green toad *Bufo viridis*, respectively, on (sub)urban bicycle paths. To our knowledge, amphibian roadkill has never been studied on bicycle paths in nature reserves, although amphibian mortality on car roads is highest on roads through protected areas, probably because of high local amphibian abundance (Garriga et al., 2012). Besides that, both aforementioned studies monitored roadkill less than once a week, which could lead to inaccurate conclusions, because amphibian roadkill disappears from the road quickly (Santos et al., 2011; 2015). Lastly, neither study corrected for local amphibian abundance, although several studies on car roads find that this affects mortality (Fahrig et al., 1995; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017).

Biking in nature is popular, resulting in the replacement of small semi-paved bicycle paths by wide concrete or asphalt paths. In 2019 there were 3,617 kilometres of concrete or asphalt bicycle path in Dutch nature areas (Weder, P., Fietsersbond, personal communication, 13 May 2022). These new paths probably attract more cyclists and allow for higher speeds because they are wide and smooth, which may increase the probability

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of amphibian roadkill. This emphasises the need for more research on amphibian roadkill on bicycle paths in nature areas.

We investigated amphibian roadkill on bicycle paths in the raised bog Natura 2000 site Bargerveen, the Netherlands (52.678267, 7.023264). In this area, all semi-paved bicycle paths have been replaced by concrete paths between 2017 and 2019. Our research aims were to investigate:

i) how many amphibians are killed on bicycle paths in this nature area,
ii) what percentage of path-crossing amphibians are killed, and
iii) whether traffic intensity and the number of crossing amphibians explain roadkill occurrence.

We defined four study transects, with a total length of 5,093 metres. On 25 February 2021 we removed all roadkill from our transects, except from the transect where we started counting later. In the period 26 February to 30 May 2021, which covers most of the amphibian spring migration, we collected the following data:

i) We counted amphibian roadkill at the end of each day (somewhere between 1600 hours and 30 minutes before sunset) because we expected most roadkill to occur during day time when most cyclists pass. We counted roadkill by walking the transects, because walking results in the most accurate monitoring (Puky, 2005). We noted the species and exact location for all amphibian roadkill and then removed them from the bicycle path. We counted daily on transects 1–3 (except, by force majeure, on transects 1 and 2 on 18 March and on transect 3 on 8 March, 10 March and 10 May). On transect 4 we counted on average twice per week during the period 24 March to 23 April and daily during 24 April to 30 May (except 13 May). On this transect we found no roadkill on the set-up day (24 March), when we would have removed all present roadkill, so this day is included in our analysis.
ii) On each transect we placed two 25-metre-long toad fences, both on opposite sides of the bicycle path, parallel to, but not opposite of each other. We left a gap of 5 metres between the fences to minimise the effect of one toad fence on the other and to prevent a small area of increased amphibian crossings, possibly leading to deviating mortality patterns. On both sides of every fence three buckets were dug in the ground, one on each end and one in the middle, to trap passing amphibians (Supplementary Fig. 2). Every morning we counted all caught amphibians and then released them on the side of the bicycle path they were headed to.
iii) We used VT300 cyclist counters to collect data on traffic intensity. Two transects were adjacently on the same path, so data from one counter was used for both transects. The counter on one transect was malfunctioning, so we have traffic data for three transects. Vehicles were counted between 4 March and 30 May 2021.

Table 1. Total number of amphibian roadkill, total number of amphibians caught in the toad fences and estimation of the total number of amphibians that crossed on all study transects, between 26 February and 30 May 2021.

	Number of roadkill	Number of caught amphibians	Estimation of number of crossing amphibians
Smooth newts	11	250	5,037
Anurans	0	562	11,453

Supplementary Figure 1 shows the location of the toad fences, the study transects on which we counted amphibian roadkill and the roadkill that we found.

In total we counted 11 killed amphibians, all smooth newts (mean per day per transect ± SD = 0.034 ± 0.20). A total of 250 smooth newts (mean per day per transect ± SD = 0.66 ± 1.85) and 562 anurans (mean per day per transect ± SD = 1.49 ± 4.46) were caught in the toad fences. We estimated total crossings on the study transects based on the counted crossings at the toad fences. We estimated that 5,037 smooth newts (mean per day per transect ± SD = 15.5 ± 42.0) and 11,453 anurans (mean per day per transect ± SD = 35.3 ± 102.0) crossed the transects. Table 1 gives an overview of the numbers of killed and crossed amphibians. The cyclist counters counted 30,843 bicycles (mean per day per transect ± SD = 123.9 ± 146.2) and 13,149 other vehicles (mean per day per transect ± SD = 52.8 ± 46.2), such as electric scooters and people on rollerblades.

We analysed, on a daily basis, the relationships between the number of passing bicycles and crossing smooth newts, and the presence of roadkill. We used roadkill presence instead of roadkill number, because roadkill number was mostly zero or one. We performed tests with the total number of passing vehicles and with cyclists only, and since the tests yield similar results, we only present results on cyclists. First, we used a generalised linear model using a Poisson distribution, as we analysed count data, to test if the numbers of passing bicycles and crossing smooth newts were correlated. These were positively correlated (Fig. 1a). Since there were many days without crossing newts, we tested the correlation excluding these days and the correlation holds (estimate = 0.0021, N = 64, p < 0.001). Second, we used a generalised linear model using a binomial distribution to test the effect of the number of passing bicycles on roadkill presence, but found no effect (Fig. 1b). Third, we tested the effect of the number of crossing smooth newts on roadkill presence with a generalised linear model using a binomial distribution. We removed an outlier with 441 crossings and one roadkill, which was just after a sudden rise in temperature leading to massive amphibian migration. We again found no effect (Fig. 1c).

Analyses and visualisation were performed in R (R Core Team, 2019), using the packages readxl (Wickham & Bryan, 2019), reshape2 (Wickham, 2007), ggplot2 (Wickham, 2016), plyr (Wickham, 2011) and ggpubr (Kassambara, 2020).

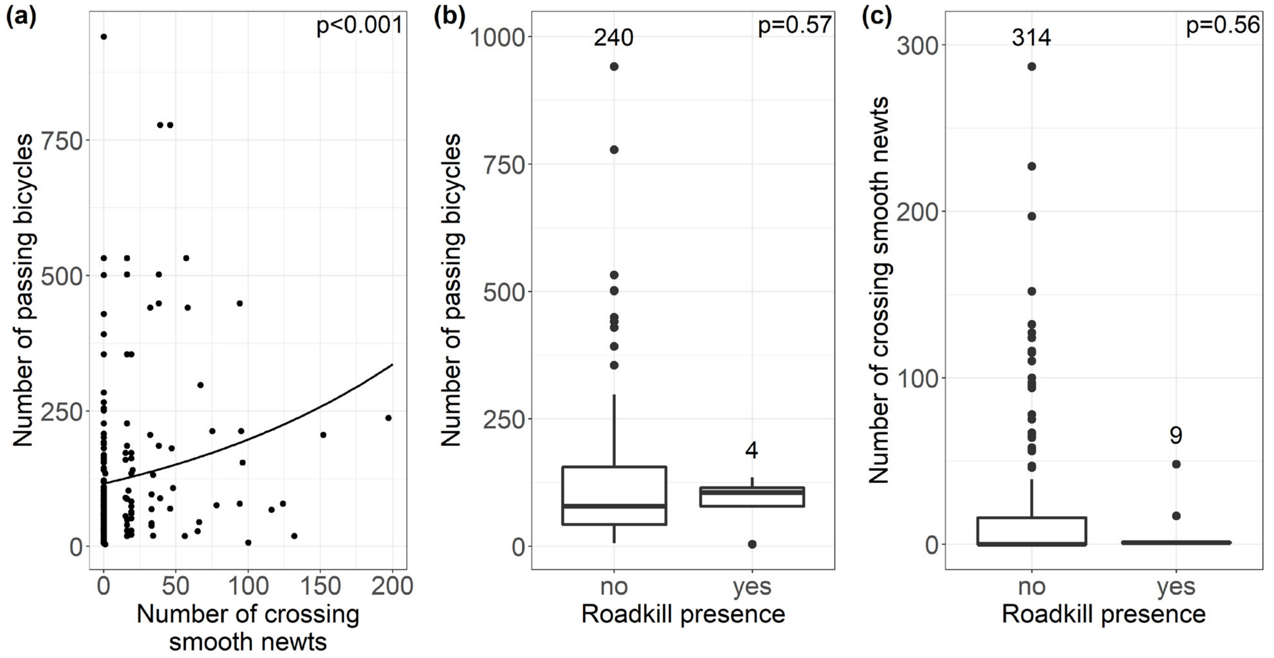

Figure 1. **(a)** The number of passing bicycles as a function of the number of crossing smooth newts (estimate = 0.0051, N = 244, p < 0.001); **(b)** The effect of the number of passing bicycles on roadkill presence (estimate =-0.0042, N = 244, p = 0.57); **(c)** The effect of the number of crossing smooth newts on roadkill presence (estimate =-0.0089, N = 323, p = 0.56). Above the boxplots sample sizes are given.

During our study period, the probability that a crossing amphibian got killed was extremely low (0.22 % for smooth newts and 0 % for anurans). This was despite substantial cyclist numbers passing on the same days that amphibians were migrating in large numbers, which appeared from the positive correlation between passing bicycles and crossing newts (Fig. 1a). The roadkill probabilities may have been underestimated, because roadkill is often eaten by scavengers. These probabilities may also have been overestimated, since some amphibians may have escaped from, or may have been predated from our buckets, and higher numbers of caught amphibians result in lower roadkill probabilities. Gibbs & Shriver (2005) stated that annual roadkill probabilities of 10 % or lower result in stable populations for adult spotted salamanders *Ambystoma maculatum*. Although we studied other amphibian species and despite uncertainty around our roadkill probabilities, mortality on bicycle paths in the Bargerveen area seems no threat to amphibian populations during our study period.

However, we did not monitor during the complete active period of amphibians, so it is unknown if roadkill on bicycle paths is a threat to populations outside our study period. For example, on 25 February, the day before monitoring started, we removed 29 smooth newt mortalities. More than 50 % of amphibian roadkill disappears from the road within 24 hours (Santos et al., 2011), so the number of newts that were killed before monitoring started was probably much higher than 29.

The roadkill probability being extremely low, and roadkill presence being independent of the number of passing cyclists and crossing amphibians, could be explained by several factors. First, cyclists pass mostly during the day and amphibians migrate mostly during the night (Hels & Buchwald, 2001; Zhang et al., 2018; RAVON, n.d.c). However, a substantial part of smooth newts migrates diurnally (Jarvis et al., 2019; RAVON, n.d.c) so this is only a partial explanation. Second, amphibians migrate mostly during rainy periods (Meek, 2012; RAVON, n.d.a) which is not favourable weather for cyclists. The positive correlation between crossing smooth newts and passing cyclists (Fig. 1a), however, indicates that newts and cyclists are active on the same days, so this explanation can only be true if they are active at different hours of the day. Third, amphibians could possibly not cross bicycle paths when it is busy with cyclists. Fourth, cyclists could successfully avoid cycling over amphibians. Maybe, the chance for an individual cyclist to avoid an amphibian successfully increases with the number of cyclists present, because many cyclists could see an amphibian earlier. These group effects could possibly explain our lack of correlation between cyclists' traffic intensity and roadkill presence. Cyclists avoiding amphibians can also explain the absence of a positive correlation between crossing newts and roadkill presence (Fig. 1c) because cyclists possibly pay more attention to avoiding amphibians when many amphibians are crossing. For drivers of motorised vehicles it is harder to avoid amphibians, as shown in multiple studies (Fahrig et al., 1995; Meek, 2012; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017). Cyclists are probably able to avoid amphibians due to their lower speed. The light colour of the concrete bicycle paths in our study area might also help, since it makes amphibians on the path more visible.

As all roadkill were smooth newts, while 69 % of crossing amphibians were anurans, it could be that newts are more likely to be killed while crossing a bicycle path than anurans. Several reasons could explain this. First, newts are flatter and thinner than anurans, which makes them less conspicuous on bicycle paths; however, newts have similar body length (the length that could be struck) as anuran species common in our study area (up to 11 cm for smooth newt, common frog *Rana temporaria* and common toad *Bufo bufo* and 8 cm for moor frog *Rana arvalis* (RAVON, n.d.b)) (Allain & Smith, 2016). The least visible species having the highest mortality probability advocates that cyclists successfully avoid killing amphibians. Second, newts move slower than anurans, so they take longer to cross bicycle paths (Hels & Buchwald, 2001). Third, smooth newts migrate earliest in the year, as shown by our toad fence catches and the literature (RAVON, n.d.a), implying that newts migrate when it is dark early in the evening. Since migration generally starts from dusk (Puky, 2005), the overlap between migration and passing cyclists is probably higher for smooth newts than for anurans.

Even though our study has clear results, long term research is necessary to make hard conclusions. Future monitoring should occur from early February to late November, including the complete active period of all amphibian species (RAVON, n.d.a). It is specifically important to include the period when juvenile amphibians migrate, June–October (RAVON, n.d.a), because juvenile survival is in many cases the driving factor for population stability (Petrovan & Schmidt, 2019) and because juveniles may be more likely to become roadkill, as they are less visible and migrate more diurnally, when most cyclists pass (Vos & Chardon, 1994; Petrovan & Schmidt, 2019). Furthermore, amphibian numbers show large natural variation between years. Thus, monitoring should occur for at least 3 years in a row to draw solid conclusions on roadkill (Puky, 2005). Moreover, it may be interesting to analyse the effects of speed and group size of passing cyclists on amphibian mortality, as well as the time of the day in which cyclists pass.

We found that mortality probabilities for amphibians on bicycle paths were very low, despite substantial cyclists' traffic intensity, indicating that road mortality is, in our study period, no threat to amphibian populations.

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Data Accessibility

Data and R code are available via https://doi.org/10.34894/KYY0OX.

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Bioaccumulation of mercury in direct-developing frogs: The aftermath of illegal gold mining in a National Park

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The use of mercury in mining gold is an illegal but still common practice in developing countries and is the world's largest source of mercury pollution. The mercury released into the environment bioaccumulates in organism tissues due to its chemical properties and can adversely alter wildlife's neurological and reproductive systems. Frogs are susceptible to mercury contamination from gold mining because of their high skin permeability and association with aquatic environments. However, the effect of mercury pollution on direct-developing frogs is poorly known, particularly in tropical highlands. To understand the impact of mercury due to gold mining contamination on biodiversity of Tropical Andes, we assessed the bioaccumulation of mercury on direct-developing frogs of genus *Pristimantis* in a montane forest. We assessed bioaccumulation by comparing muscle tissue samples of frogs and sediments of streams in an area previously affected by illegal gold mining inside the Farallones de Cali National Park. Even though gold mining has not been conducted in the area for several years, we found mercury in muscle samples of direct-developing species of genus *Pristimantis* and alarming mercury concentrations in the sediment samples that exceed risk thresholds according international guidelines of the WHO (1.0749 $\mu g.g^{-1}$) and countries such as Canada, USA and Brazil (0.35 $\mu g.g^{-1}$). Our results suggest that the use of heavy metals in the gold mining can affect non-aquatic species causing bioaccumulation of heavy metals, which can be an important threat to wildlife populations, the stability of the ecosystem, and public health.

Keywords: Andean forests, mercury pollution, muscle tissue, streams pollution, sediments, total mercury

INTRODUCTION

Environmental pollution of heavy metals has become of great concern due to the adverse effects on biodiversity and public health worldwide. The mercury-based gold mining industry is the world's largest source of mercury pollution, with emissions from 410 to 1,400 tons of mercury each year, accounting for 37 % of global mercury emissions (Esdaile & Chalker, 2018). Despite technological advancements that reduce environmental impact and make mining practices more sustainable (Pantoja-Timarán et al., 2005; Rojas-Cruz & Mejía-Tobón, 2007), the use of mercury to amalgamate and concentrate precious metals is still a common practice in the mining industry of developing countries due to low costs and ease of use (Pantoja-Timarán et al., 2005; Rojas-Cruz & Mejía-Tobón, 2007; Hernandez-Cordoba et al., 2013). During artisanal and small-scale gold mining, mercury is released into rivers and streams and deposited in sediments, and methylmercury is produced, which is the most toxic and common organic mercury compound found in the environment (Pinedo-Hernandez et al., 2015; Betancur-Corredor et al., 2018). Mercury deposition in natural environments impacts the stability of ecosystems because of the reproductive and neurological consequences of exposing organisms (Fitzgerald et al., 1991; Bank et al., 2007).

Mercury bioaccumulates in organisms' tissues due to its recalcitrant properties and high affinity for organic matter and sulphur compounds (Pinedo-Hernández et al., 2015). Furthermore, methylation of inorganic mercury provides an efficient transmission pathway in food webs; thus, it is biomagnified and results in an increased risk for animals of high trophic levels (Bank, 2020). Thus, predators can be more susceptible to the adverse effects of mercury pollution. The accumulation of mercury in organisms' tissues causes behavioural, neurochemical, hormonal, and reproductive changes, directly affecting wildlife species' populations (Scheuhammer et al., 2007; Bergeron et al., 2011). Besides, methylmercury can easily pass through the blood-brain barrier and has a high affinity for brain tissue, decreasing antioxidant function and damaging the nervous system (Li et al., 2018).

The impact of bioaccumulation of mercury due to gold mining has been quantified in different organisms, including invertebrates and vertebrates (Ahumada, 1994; Bank et al., 2007; Alvarez et al., 2012; Zapata et al., 2014). Frogs, in particular, have great sensitivity to the concentration of pollutants due to their natural history traits, especially during the tadpole stage before limb formation (Rowe et al., 1996; Burger & Snodgrass, 2000). The absorption of contaminants such as mercury by frogs occurs through their permeable skin by direct contact with polluted water or sediments and by ingesting mercury-contaminated food (López-Noguera, 2015). Additionally, their trophic interactions play a crucial role in the biomagnification processes (Alvarez et al., 2012; Hernandez-Cordoba et al., 2013). Thus, frogs are excellent biological indicators for evaluating the effect of mercury in gold mining. However, the effect of mercury pollution on direct-developing frogs, which do not pass through the aquatic larval stages, is unknown.

In Colombian lowlands, mercury bioaccumulation has been reported for tadpoles in areas impacted by gold mining (Hernandez-Cordoba et al., 2013), as well as in other organisms such as fish (Alvarez et al., 2012) and turtles (Zapata et al., 2014). However, knowledge of gold mining's impacts on the highlands of the Colombian Andes, which are highly threatened by this practice, is lacking (Urbina-Cardona, 2011). Also, the mountain ecosystem of the Andes is the primary source of water supply for the most important rivers and hold a significant portion of the country's biodiversity with a high degree of endemism (Romero-Ruiz et al., 2008); thus, mercury pollution of these ecosystems can have a substantial impact on biodiversity and public health.

To understand the impact of mercury pollution due to gold mining contamination on biodiversity, we assessed the bioaccumulation of mercury on direct-developing frogs in a highly diverse forest. Hence, we took tissue samples of *Pristimantis* genus frogs and stream sediments in an Andean Forest previously impacted by illegal gold mining inside a National Natural Park (Farallones de Cali). We expected to find low mercury concentrations in tissues of the direct-developing frogs because of their independence from water sources where mercury is deposited. In addition, although large-scale mining has not been conducted in the area for several years, we expected to find high concentrations of mercury in the sediments of streams because of the excessive amounts of mercury used for gold mining throughout the area in the past.

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MATERIALS & METHODS

Study area

This study was conducted in Alto del Buey - Minas del Socorro area at Farallones de Cali National Park, a protected area on the Colombian Western Andes in Valle del Cauca, Colombia (Fig. 1). Study locality has 196,364.9 ha that includes Tropical Rainforest, Sub-Andean Forest, High-Andean Forest, and Paramo ecosystems, and the site of born of the Cali River, one of the main water supplies for the municipality of Cali. Since the last century, Alto del Buey has been an area of

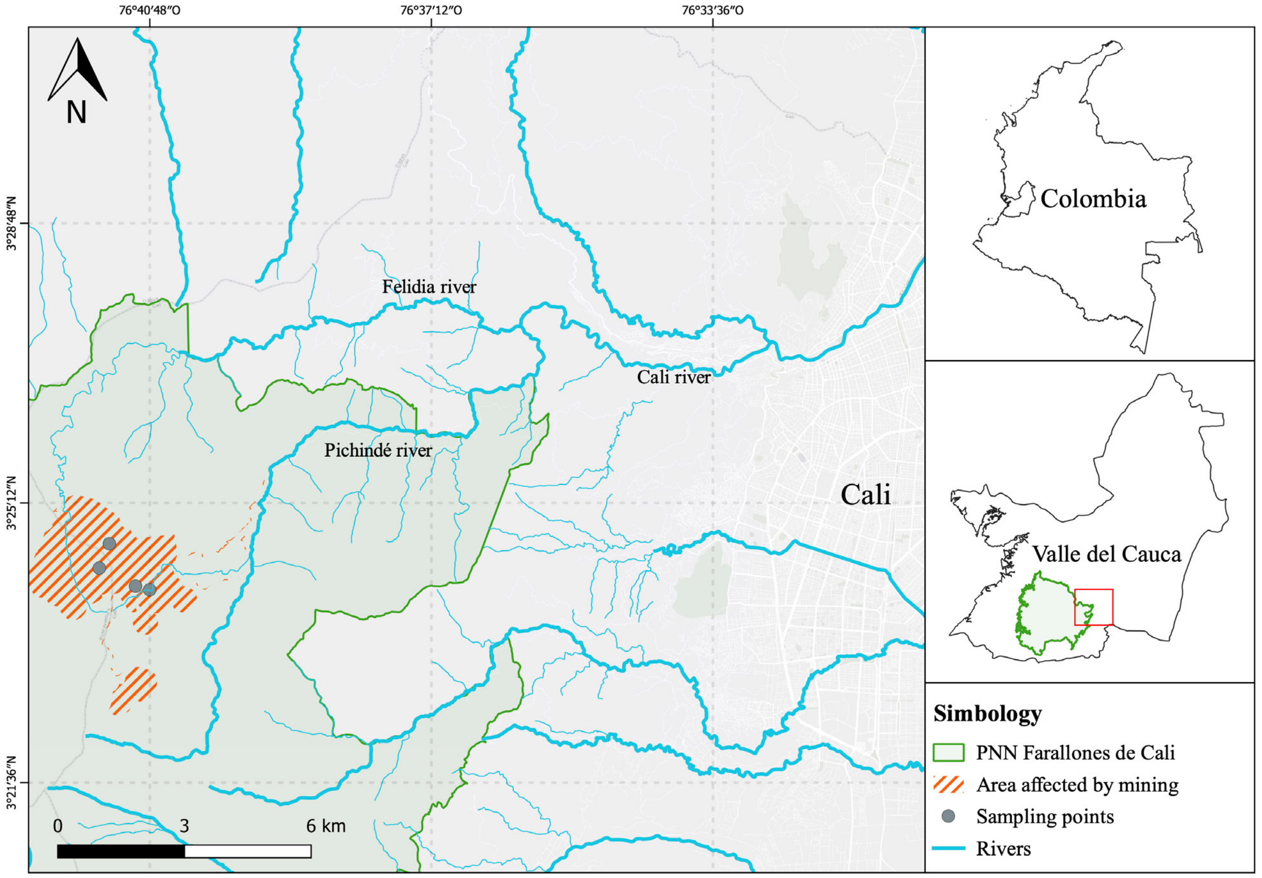

Figure 1. Alto del Buey study area (PNN Farallones de Cali, Valle del Cauca, Colombia), showing the location of the sampling points and the associated rivers.

illegal gold mining in the headwaters of the Felidia and Pichindé rivers, the main tributaries of the Cali River. Besides, no biological information from this area existed until a few years ago (e.g. Cuellar-Valencia et al., 2020; 2021).

Since 2010, park staff visits have evidenced the environmental deterioration caused by mining, which included 676.7 ha affected by more than 400 mining sinkholes, several camps, access roads that lead to deforestation, heavy machinery, and evidence of chemical contaminants used, such as cyanide and mercury that end up in water sources. Following the dismantling of the mining camps, the whole area is in natural regeneration.

Fieldwork

We sampled sites with noticeable effects of illegal gold mining inside the study area between September and December 2017, covering an altitudinal range between 3,000 and 3,500 m. The streams of four sites (El Feo, Campamento base, La Cruz, Sendero) were sampled in the study, exhibiting notable environmental deterioration in deforestation, chemical contamination, and solid waste despite two years without large-scale mining (Fig. 2); Thus, we looked for frogs along the streams using the visual encounter survey method (VES). We captured adult frogs and identified them to the lowest possible taxonomic level. Several individuals of the largest and most abundant species were transported alive to the facilities of the Universidad del Valle for further analysis. Additionally, we took a sediment sample from the streams where each individual was collected. Finally, we took an additional sediment sample from a site where a mill for gold mining had been located in the past. All sediment samples were handled with latex gloves and stored in hermetically sealed bags without being sieved and with some stream water.

Laboratory analysis

We assessed mercury accumulation for eight samples of four species of *Pristimantis*: *P. brevifrons* (1), *P. buckleyi* (3), *P.* aff *calcaratus* (2), and *P.* sp (2). To obtain these samples were needed 13 individuals, one for each sample for *P. buckleyi* (Campamento base) and *P.* aff *calcaratus* (Sendero), two individuals for the sample of *P. brevifrons* (El Feo) and three individuals for each sample of *P.* sp (La Cruz). All individuals that were used for one sample were collected in the same place. As the sediment samples were taken by each individual, the tissues samples where more than one individual was used, the sediment samples were combined to form a unique sample. To avoid overestimating the mercury measurement, we kept individuals alive 48 hours before analyses to eliminate their intestinal contents (Hernandez-Cordoba et al., 2013). Then each individual was euthanised following the protocol proposed by Cortez-F et al. (2006). We used only muscle tissue to avoid overestimation of residues in the digestive tract. We removed all the muscle tissue and dried it in an oven at 60 °C for 24 hours. The dried tissues were macerated until obtaining 1.0 g for each sample and were subjected to acid digestion to convert all forms of mercury into inorganic mercury (Hg^{+2}). For this, 10 mL of 55 % nitric acid (HNO_3) and 2 mL of 30 % hydrogen peroxide (H_2O_2) were added to each tissue sample in a reflux system for 1 hour at 60 °C and 5 hours at 100 °C (Campbell et al., 1986; Escobar-Sánchez, 2010).

Figure 2. Environmental impact caused by illegal gold mining at Alto del Buey, PNN Farallones de Cali: **a)** deforestation and mining camp remains; **b)** remains of heavy machinery used for mining; **c)** abandoned mining sinkhole; **d)** stream affected by chemical contamination (Arrows point to drops of liquid mercury in water).

Table 1. Total mercury concentration [Hg^{+2}] found in frog tissues and sediments, and bioaccumulation index (BI) of mercury in frogs collected at PNN Farallones de Cali, Colombia.

Species	Site	[Hg^{+2}] tissue ($\mu g.g^{-1}$)	[Hg^{+2}] sediment ($\mu g.g^{-1}$)	BI
Pristimantis sp	La cruz	0.0016	0.0053	-0.0037
Pristimantis sp	La cruz	0.0052	0.0105	-0.0053
Pristimantis brevifrons	El Feo	0.0027	0.0053	-0.0026
Pristimantis buckleyi	Campamento base	0.0177	0.4415	-0.4238
Pristimantis buckleyi	Campamento base	0.0046	0.2835	-0.2789
Pristimantis buckleyi	Campamento base	0.0426	2.200	-2.1574
Pristimantis aff *calcaratus*	Sendero	0.0004	0.0206	-0.0202
Pristimantis aff *calcaratus*	Sendero	0.0004	0.0588	-0.0584
-	El Feo	-	1.400[a]	-

[a]Sediment sample taken from a mill for gold mining

The sediment samples were treated according to the protocol developed by IDEAM (2009) to analyse heavy metals in sediments. The samples were opened and dried at room temperature for 24 hours, and then in an oven at 60 °C for 12 hours. They were macerated until obtaining 4.0 g for each sample. They were subjected to acid digestion as above for 2 hours at 100 °C. Finally, both sediment and tissue samples were diluted with 25 ml of deionised water, filtered, and stored in plastic bottles at 0 °C. To determine the concentration of mercury (Hg^{+2}) in tissue and sediment samples, we follow Shaw et al. (1988), so we used cold vapour atomic absorption spectrophotometry (CVAAS) with sodium borohydride ($NaBH_4$) in the Laboratorio de Análisis Industriales of the Universidad del Valle (LAI), which is certified by national and international agencies regulating laboratory certification of high quality (Icontec, Icontec International, IQNet). Quality control samples included calibration curve and lab duplicates for each sample, without anomalies reported by the LAI.

Data analysis

The mercury bioaccumulation index, the ratio between tissue sample's metal concentration and the concentration in the corresponding sediment sample (Ahumada, 1994), was calculated for each frog sample. We assessed differences in mercury concentration between the tissue samples and sediment samples by applying a U-Mann Whitney test since parametric assumptions were not met for data (Shapiro-Wilk normality test: tissues W = 0.68085, p-value = 1.365×10^{-3}, and sediments W = 0.57804, p-value = 8.784×10^{-5}). We performed a Spearman correlation to determine the correspondence between mercury concentration in sediment and the tissues. Analyses were performed with the CAR package v.3.0-3 (Fox et al., 2021) in the R programming version 4.0.5 (R Core Team, 2021).

RESULTS

The streams sampled correspond to small water bodies that are tributaries of main rivers such as the Felidia and Pichindé rivers, which in turn are tributaries of the Cali River. We found an assemblage of amphibians composed of arboreal species and notably dominated by directly-developing frogs of the genus *Pristimantis* (family Strabomantidae).

Total mercury concentrations found in muscle tissues were lower (mean ± standard deviation, 0.0094 ± 0.0145) and less variable (range: 0.0004 - 0.0426 $\mu g.g^{-1}$) than those found in sediments (mean: 0.4917 ± 0.7829, range: 0.00532 and 2.2 $\mu g.g^{-1}$). The bioaccumulation indexes exhibited negative values (Table 1) since mercury concentrations in the muscle tissues were lower than in the sediment samples (Mann Whitney U = 57, p = 9.972×10^{-3}). However, alarming mercury concentrations were found in two sediment samples, one taken from a place where there was a mill for gold mining two years ago. The mercury concentration in sediments was not significantly associated with tissues, but the correlation coefficient was moderated and positive (Spearman's correlation $\rho = 0.55$, p = 0.154), probably due to the small sample size (n = 8).

DISCUSSION

The mercury concentration in muscle tissues of direct-developing frogs in the high elevation of Colombian Andes was lower than in strictly aquatic animals (Alvarez et al., 2012; Palacios-Torres et al., 2018), other anurans with larval stage in lower elevation sites affected by gold mining in Colombia (Hernandez-Cordoba et al., 2013), and the control treatment of controlled experiments with dietary mercury in wood frog tadpoles (Wada et al., 2011). Nevertheless, they were similar to the methylmercury concentration in the tissues of adult wood frogs in forested landscapes in the north-eastern US (Faccio et al., 2019). Besides, we found high mercury concentrations in sediment samples from streams in the study area, which are comparable to the concentrations of mercury found in sediments of areas with high impact of mining activity (Olivero et al., 1998; Hernandez-Cordoba et al., 2013). Differences in mercury concentrations between sediment samples and muscle tissues resulted in negative bioaccumulation indexes. Therefore, results suggest that direct-developing frogs are not exempt from contamination by heavy metals released into the environment; however, the bioaccumulation levels differ from frogs with aquatic larval stages due to the different life histories and behaviours that determine the exposure to contaminants.

Two sediment samples had high mercury concentrations (1.4 and 2.2 $\mu g.g^{-1}$) that exceed the risk threshold of 1.0749 $\mu g.g^{-1}$ proposed by the World Health Organization (Betancur-Corredor et al., 2018) and are similar to samples from sites with a high impact of gold mining (Olivero et al., 1998; Hernandez-Cordoba et al., 2013). These samples came from places very close to mills that used mercury during the process of gold amalgamation (Palacios-Torres et al., 2018), and their concentrations are alarming since they were found in sediments of streams that are tributaries of Felidia, Pichindé, and Cali rivers, which provide untreated water to more than 7,000 people in the rural areas of Cali. It should be noted that for Colombia there is no guideline that estimates the maximum threshold allowed for mercury in sediments, however, other countries such as Canada, USA, Brazil estimate thresholds that do not exceed 0.35 $\mu g.g^{-1}$ (Burton, 2002; CONAMA, 2004)

We found mercury in adult frogs of the genus *Pristimantis*, which have a reproductive mode by direct development (Crump, 1974; Duellman, 1992), although they are not in direct contact with the habitats where the mercury is discharged at any stage of their life; therefore, the bioaccumulation of mercury in tissues occurs indirectly. The low organism-environment interaction explains the lower concentration of mercury we found in muscle tissues than in sediments, resulting in negative bioaccumulation indexes for the different species. However, there is a marginally significant association between tissue and sediment samples, indicating that as mercury concentration increases in the environment, it is higher in animal tissues. Thus, although direct-developing species do not directly contact stream sediments, the mercury concentration in these samples represents the mercury used or remaining in the study sites environment. Species of *Pristimantis* may have obtained mercury from the prey they consume, and the biomagnification processes may occur in the study area since amphibians are at the base of the terrestrial food chain (Rimmer et al., 2009; Liu et al., 2020). Mercury can also be released into the environment as vapour at a lower percentage during the amalgamation process (Betancur-Corredor et al., 2018). Thus, the mercury bioaccumulated by frogs can be in part derived from vapours released from this process.

Another possible explanation for the low concentration of mercury we found in muscle tissues than in sediments is the nature of the mercury. The ratio of methylmercury to total mercury in tissues of frogs can be more than 80 %, depending on the species and season (Wang et al., 2005), while this ratio in sediments can be even lower than 1 % (Shi et al., 2005), thus having a huge amount of non-bioaccumulative inorganic mercury. Then, comparing mercury concentrations in tissues of frogs and sediments using total mercury measurements may be underestimating the actual values of forms of mercury that bioaccumulate as methylmercury. Thus, sediments do not correspond to an accurate point of comparison for measurements of mercury bioaccumulation in the tissues of frogs as has already been mentioned in previous works (Smalling et al., 2019; 2021), especially when using measurements of total mercury and frogs of direct development that present a low interaction with this source.

In conclusion, gold mining negatively affects ecosystems by indiscriminate deforestation and contamination of water sources with heavy metals (Campbell et al., 1986; Swenson et al., 2011; Esdaile and Chalker, 2018; Palacios-Torres et al., 2018). In the high elevations of the Colombian Andes, we found mercury accumulation in an assemblage dominated by direct-developing frogs of genus *Pristimantis*. The total mercury concentrations we recorded in frog tissues were lower than those reported from other localities with gold mining and larval-stage frogs (Hernandez-Cordoba et al., 2013) and controlled experiments with mercury diet in larval-stage frogs (Wada et al., 2011), but similar to the methylmercury levels found in the tissues of adult wood frogs (Faccio et al., 2019). The bioaccumulation index was negative, which can be related to the fact that we compared tissue samples obtained from direct-developing frogs with stream sediment samples, an aquatic environment with which these frogs do not have a close relationship. Also, another possible explanation is because the methylmercury is the form of mercury founded mainly in the tissues of frogs (Wang et al., 2005), which can present low levels in sediment samples (Shi et al., 2005), then the use of total mercury measurement comparing frog tissues and sediments may not correspond to an accurate way of comparison (Smalling et al., 2019; 2021).

Results suggest that the assemblage is indirectly accumulating heavy metals, probably by feeding insects with aquatic life-stages. The accumulation of mercury in animals that do not have direct contact with aquatic environments, where heavy metals are dumped due to gold mining, is a threat to the ecosystems' stability. In particular, the accumulation of heavy metals in frogs can produce a process of biomagnification since amphibians are at the base of the terrestrial food chain, which can threaten biodiversity due to the adverse effects of heavy-metals contamination on the survival and reproduction of wildlife (Rimmer et al., 2009; Hernandez-Cordoba et al., 2013; Liu et al., 2020).

Although our small sample size does not allow us to know the variability of mercury concentrations in the study site and makes it difficult to carry out statistical analyses, these results represent valuable information on little-known dynamics in high mountain ecosystems that are strongly intervened by actions of illegal mining in Colombia. This demonstrates the importance of control actions by environmental authorities in national parks to prevent this type of activity and, in this way, mitigate the harmful effects on the environment. In the same way, this work represents a starting point for future studies in this field in high mountain ecosystems. For future studies we recommend expanding in a gradient system that allows the comparison of species of direct-developing frogs with species with aquatic larval stages; consider different sources of mercury in the environment in addition to sediments such as waters and potential diet of the frog species examined; and not be limited to total mercury measurements but also measure methylmercury of the different samples.

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Authors' Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Oscar Mauricio Cuellar-Valencia and Gustavo Adolfo Rodriguez-Salazar. The first draft of the manuscript was written by Oscar Mauricio Cuellar-Valencia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Statement

This research was conducted under the authorisation of the National Authority of Environmental Licenses and the Ministry of Environment and Sustainable Development of Colombia (Resolución 1070 del 28 de Agosto de 2015), and Parques Nacionales Naturales de Colombia (aval No. 20172200004353 del 26 septiembre del 2017).

Data accessibility

The data that support the findings of this study are openly available in Figshare at https://figshare.com/s/8fa3463ef88a7ad20b1c.

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Demography of a painted turtle intergrade (*Chrysemys picta picta* X *C. p. marginata*) population from an altered wetland

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The demography of a painted turtle *Chrysemys picta picta* X *C. p. marginata* population from a eutrophic habitat was examined at a wetland site in south-central Pennsylvania (USA) during 2011–2019. Males reached sexual maturity at 90 mm carapace length (CL) in half the time taken, but at the same size, as painted turtles studied elsewhere in the north-eastern portion of the United States. Females matured at 130 mm CL at our site, which was larger and began at an earlier age than conspecifics. Our data corroborate findings of faster growth in *C. picta* juveniles resulting in earlier maturity at body sizes equal to or larger than slower growing juveniles. Our results also conform to previous findings linking wetlands altered by added nutrient input to increased growth patterns of their resident painted turtle population. Rapid growth rates for aquatic turtles are likely to become more common globally as urbanisation continues to expand and alter wetland habitats.

Keywords: Growth, population size, population structure, survivorship, urban

INTRODUCTION

The painted turtle *Chrysemys picta* Schneider 1783, is a polytypic aquatic species found across central and eastern North America (Powell et al., 2016) with three recognised subspecies (Uetz et al., 2021). In Pennsylvania, the range of the midland painted turtle, *C. p. marginata* Agassiz 1857, extends through much of the western and extreme northern portions of the state. Intergradation by *C. p. marginata* with the eastern painted turtle *C. p. picta* occurs throughout the eastern and south-eastern portion of the state (Ernst & Ernst, 1971). The lower sub basin of the Susquehanna River is the centre of the intergradation zone in the state (Hulse et al., 2001). Within this watershed is Wildwood Park in Harrisburg, Dauphin County, where intergrades of *C. p. picta* X *C. p. marginata* are abundant in a eutrophic canal and an artificial lake (Wingert & Meshaka, 2021).

Demographic studies of *C. picta* are common (e.g. Gibbons, 1968; Mitchell, 1988; Zweifel, 1989; Frazer et al., 1991; Congdon & Gibbons, 1996), and two studies have examined demographic patterns in Pennsylvania populations (Ernst, 1971a,b; Hughes & Meshaka, 2020). Studies by Ernst (1971a,b) were conducted on an intergrade population in south-eastern Pennsylvania, and a study by Hughes & Meshaka (2020) was conducted on *C. p. marginata* in an artificial wetland complex in south-western Pennsylvania. Nutrient levels within the aquatic habitats occupied by this species can affect demographic traits within populations (Ernst & Lovich, 2009). Although neither of the wetland habitats in the two Pennsylvania studies were described as altered, three studies, one on *C. p. bellii* in Iowa (Quinn & Christiansen, 1972) and in Michigan (Gibbons, 1968), and one on *C. p. picta* in Maryland (Ernst & McDonald, 1989), explicitly examined growth in habitats altered by eutrophic conditions. Rapid growth was common to turtles of all three studies, larger adult body sizes was found in two studies (Quinn & Christiansen, 1972; Gibbons, 1968), and early maturity with an effect on minimum body size was detected by one study (Ernst & McDonald, 1989). More broadly, Congdon et al. (2018) identified a connection between fast growth of juveniles and early maturity at larger or similar sizes compared to slower-growing juveniles of three turtle species, including *C. picta*.

Altered aquatic habitats are highly influential in shaping variation in several important life-history traits and such demographic responses are likely common among urban populations of aquatic turtles worldwide. Within this context, we recognised the opportunity to comprehensively evaluate responses in multiple demographic traits by a single population of *C. p. picta* X *C. p. marginata* over a 9-year period to an artificially eutrophic and human-made wetland imbedded within a city in south-central Pennsylvania. Our research approach, in turn, provides findings that are not only globally relevant but also becoming increasingly common.

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MATERIALS & METHODS

Study area

Our study was conducted at Wildwood Park, a 93.5 ha county park located in Harrisburg, Dauphin County, Pennsylvania (40.310, -76.883). Approximately 60 % of the park is comprised of a shallow artificial lake that is fed by Paxton Creek (Fig. 1). An accumulation of detritus has resulted in a gradual filling-in of the lake with much of it converting to a marsh dominated by cattail *Typha* sp. During the time of this study, only a section of Paxton Creek (0.34 ha) at the south end, the spillway area (0.29 ha), a channel (0.99 ha) running more or less parallel to the tow path on the west end, and another channel (1.91 ha) running along the eastern edge of the lake, were deep enough to be habitable by *C. picta*. A section of the Pennsylvania Canal ran along the western boundary of the park adjacent to the lake and was separated by a tow path (Fig. 1). The canal measured 1,934.65 m in length, had an average width of 23.8 m, and an area of 26,467.6 m^2 (2.65 ha). The canal depth changed significantly from one to two metres from the west side of the towpath into the canal (Russell et al., 2014). A cleared utility right-of-way averaging 16.3 m borders the west side of the canal and separates it from a two-lane paved road. The main water lily found in the canal was spadderdock *Nuphar advena* L., and the dominant submergent aquatic macrophyte was coontail *Ceratophyllum demerseum* L. Captured turtles frequently passed spadderdock seeds. Duckweed *Lemna* sp. was the common floating plant. Small painted turtle juveniles were seen feeding on duckweed at the surface. There are also algae species in the water which have not yet been identified (Russell et al., 2014).

Figure 1. A section of the Pennsylvania Canal and Wildwood Lake at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania. Photograph taken 3 October 2018 by W.E. Meshaka, Jr.

The canal received extensive runoff from the adjoining road and industrial warehouses that run parallel and west of it, to the extent that much of the lake has converted to cattail marsh. The eutrophic condition of our site is quantified by water quality data recorded by the Susquehanna River Basin Commission's Paxton Creek monitoring station (ID 01571005, coordinates 40.306, -76.856) located upstream from Wildwood Park, its period of record having encompassed the duration of our study. The extent of eutrophication in Paxton Creek expressed in normalised concentration (mg/L) was available for total Nitrogen (0.676), dissolved Nitrogen (0.833), total Phosphorus (0.598), dissolved Phosphorus (0.618), total Ammonia (0.833), dissolved Ammonia (0.539), and total Suspended Solids (0.578). The topic of the watershed's impairment was addressed at both the state level by the Pennsylvania Department of Environmental Protection (DEP) and at the federal level by the Environmental Protection Agency. The DEP listed Paxton Creek in Harrisburg, Dauphin County, as impaired after studies in 2004, 2005, and 2006 based on siltation and the source as urban runoff/storm sewers, primarily phosphorus. In 2010, the DEP delisted the watershed that includes Paxton Creek (Shearer, 2012), but the EPA later determined that delisting of Paxton Creek for nutrient impairment was not appropriate (Sauro, 2019; DeJesus, 2021). As of 2013, DEP listed Paxton Creek as impaired for sediment but not for nutrients. During the latter 20th century, the lake depth significantly decreased with sediment deposition. It originally averaged four to 152.4 cm in depth and as of 2015 Wildwood Lake averaged approximately 15.2 cm (Herbert et al., 2015). From 2003 through 2018, the lake depth diminished from shallow open water to mudflat and cattail marsh (Fig. 2).

Trapping and Processing Method

Six baited hoop-nets were set for five consecutive days in spring, summer, and autumn during 2011–2014 and opportunistically in 2015 (54 trap days), 2016 (30 trap days), and 2019 (30 trap days). The traps (Memphis Net and Twine Co., Memphis, TN) were 2.0 m x 1.0 m with 2.54 cm mesh. The traps were set at fixed locations near the shoreline of the canal, and the sites remained constant for the duration of the study. Traps were baited with a partially opened sardine can or with chicken gizzards which were changed daily after traps were checked.

We used a 61 cm aluminum sliding caliper, accurate to 0.5 mm, to measure straight-line carapace length (CL) and plastron length (PL). The sex for adult turtles was determined by foreclaw length (longer in males relative to CL) and by the location of the cloaca relative to the carapace (i.e. the cloaca extends beyond the carapace in males; Ernst & Lovich, 2009). New turtles were individually marked using two methods. Each new

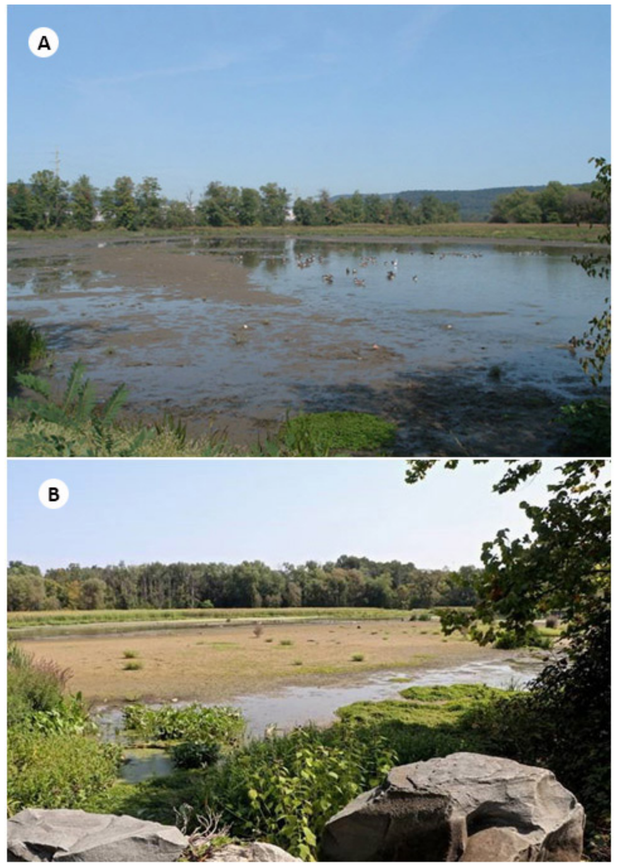

Figure 2. Wildwood Lake in 2003 **(A)** with open water and visited by wading birds and dabbling ducks, and in 2018 **(B)** having succeeded to mudflats and cattail marsh. Property of Wildwood Lake Nature Center Archives.

turtle was permanently marked using the Proximate Binary Coded Decimal (PBCD) scute-notching system of Buhlmann et al. (2008). The notches which displayed a unique number were made with a Dremel tool. Each new turtle was also given a Passive Integrated Transponder (PIT) tag inserted through the left thigh along the bridge of the carapace. The tags were HPT12 preloaded sterile tags from BioMark (Boise, Idaho), inserted using a BioMark MK-25 Rapid Implant Gun. Recaptured turtles were measured, scanned for the PIT tag, and carapacial notches were renewed as needed before releasing turtles on the same day of their capture.

Our study site supported three other turtle species, which were captured in the traps during this study. eastern musk turtles *Sternotherus odoratus* (n = 2) and snapping turtles *Chelydra serpentina serpentina* (n = 62), were individually marked using the same methods applied to painted turtles and released. Exotic red-eared sliders *Trachemys scripta elegans* also established at Wildwood, were captured (n = 22) and euthanised, and a sample of them was deposited in the State Museum of Pennsylvania, Harrisburg, Pennsylvania (Russell et al., 2014).

Determination of Sexual Maturity

Long foreclaws are associated with mature males in this species (Ernst & Lovich, 2009). Foreclaws of at least 7 mm were common in males ranging 93.0–121.3 mm CL. Foreclaws of at least 8 mm were common in males ranging 93.5–141.8 mm CL. Foreclaws of at least 9 mm were common in males ranging 90–140.5 mm CL. Among four males larger than 90 mm CL, one had foreclaws of 6 mm, and three had foreclaws of 5 mm. No foreclaws were larger than 6 mm among the five males smaller than 90 mm CL. Thus, we concluded that males of at least 90 mm CL evidenced clear sign of sexual maturity in this secondary sexual characteristic (Fig. 3).

We used body size of dissected and nesting females during 2011–2021, as the criterion of female shell length at sexual maturity. During 2016–2017, seven females (132.9–154.2 mm CL) were removed from the population to ascertain minimum shell length at sexual maturity. Specimens were deposited in the section of Zoology and Botany of the State Museum of Pennsylvania. Opportunistic observations of 15 nesting females during 2011–2014 (143–155 mm CL) and 2018, 2019, and 2021 (130–165.1 mm CL) provided additional data to determine shell length of the smallest mature female. The smallest sexually mature female measured 130 mm CL (124 mm PL) and was seen nesting on 2 July 2018. A female (SMP-H-9230) measuring 132.9 mm CL (124.6 mm PL) captured on the tow path on 8 July 2016 was found to contain luteal scars and yolking ovarian follicles. Based upon this sample, 130 mm CL was accepted as the cut-off for smallest sexually mature female in this study.

Growth and Age Estimation

In most species of turtles, age can be reasonably estimated in young individuals using growth annuli on epidermal scutes (Spencer, 2002). However, counting growth rings has been found to be unreliable in providing accurate age estimates in adult turtles (Wilson & Tracy, 2003), especially for estimating ages in adult *C. picta* (Brooks et al., 1997). Lindeman (1996), in particular, showed that counting growth rings for individuals of *C. picta* becomes inaccurate around age 7 (i.e. counts of scute annuli are useful in age determination only among juveniles, and unreliable, if even readable, thereafter). Alternatively, we set out to establish sex-specific, length-at-age relationships for our samples based on repeated records of age and length from known-age individuals that had a more conservative value of 5 or fewer estimated growth rings when first captured (37 females captured 58 times; 60 males captured 94 times). We used a multi-model approach to compare three well established growth models (von Bertalanffy, Gompertz, and Logistic) using the 'AquaticLifeHistory' package (Smart, 2019a) in R (version 4.0.5; R Core Team, 2021). We rooted the models using the carapace length of a hatchling found at the site (24.8 mm). We assessed the best fitting models using an information theoretic approach (Akaike Information Criterion AIC; Burnham & Anderson, 2002). The best fitting model for each sex was then used as input to build length-at-age curves using a Bayesian Markov chain Monte Carlo process with the 'BayesGrowth' package in R running for 5,000 iterations (Smart, 2019b). Growth was also calculated as the difference in carapace length between captures divided by the interval in years between captures, which we plotted against carapace length at first capture. Carapace size intervals in the bar histogram of body size distribution were determined using the equation of Sturges (1926). Summary statistics of body size were performed using Microsoft Excel 365 (Microsoft Inc., Redmond, Washington, USA).

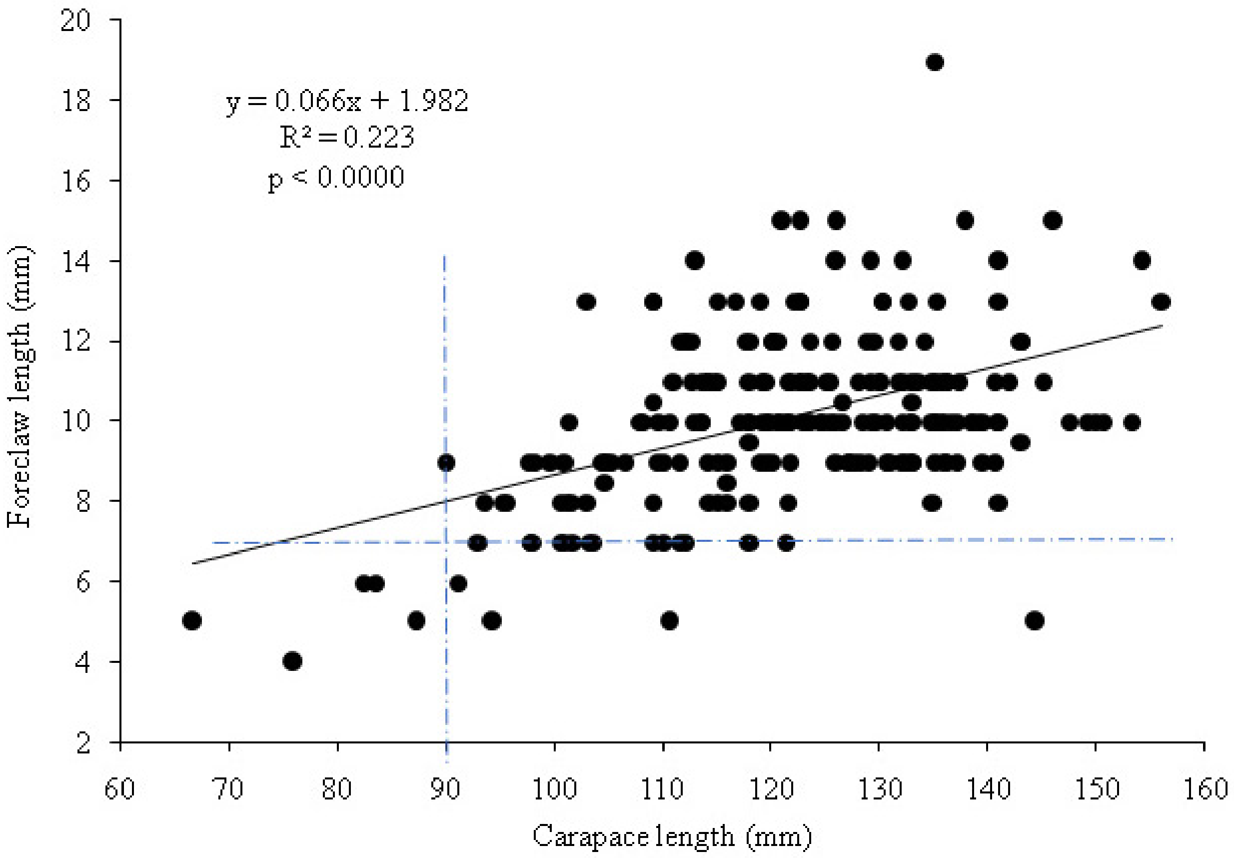

Figure 3. Relationship between foreclaw length and carapace length (CL) in 215 male painted turtles *C. picta picta* X *C. p. marginata* at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019. Blue crosshairs indicate thresholds associated with minimum foreclaw length associated with sexual maturity.

Population Size and Survivorship

We calculated apparent annual survival (Φ) and recapture rates (p) using open population Cormack-Jolly-Seber models (CJS; Lebreton et al., 1992) in the program MARK (White & Burnham, 1999). To test for differences in Φ and p between sexes, we generated CJS models to examine whether Φ or p differed based on sex, time, or a sex-time interaction. We based model selection for all analyses on AICc (corrected AIC for small sample sizes) values, with lower values denoting greater parsimony (Burnham & Anderson, 2002). We calculated population abundance for adults using POPAN parameterisation of Jolly-Seber models (Jolly, 1965; Seber, 1965) in MARK (White & Burnham, 1999).

Encounter histories to calculate demographic traits estimate the probability that an individual will leave a population. With encounter rates reversed, the probability of an individual entering the population was estimated (Pradel, 1996), whereby λ = rate of individuals entering a population. Pradel's λ differs from traditional estimates of λ as no fecundity values are included in its calculation, so is not necessarily equivalent to a true population growth rate. Pradel's λ was estimated in Program MARK in conjunction with the CJS models described above. Measure of central tendency was expressed as mean and standard deviation unless otherwise noted. Statistical significance was recognised at a p value of < 0.05.

RESULTS

Population Structure

Juveniles comprised 14.1 % of 375 new captures, outnumbered by adults at 6.08:1.00. The adult male:female sex ratio of this sample was 2.93:1.00. Adult male body size averaged 121.4 mm CL (std. dev. = ± 14.1; min-max = 90.0–156.0; n = 240) and 111.6 mm PL (std. dev. ± 12.8; min-max = 84.0–142.2; n = 240). Adult female body size averaged 147.8 mm CL (std. dev. ± 8.7; min-max = 129.7–171.0; n = 82) and 138.3 mm PL (std. dev. ± 8.4; min-max = 120.1–165.0; n = 82). Many of the 240 males (40 %) fell in the 111.0–126.0 mm CL range, and most of the 82 females (59.8 %) fell into the 143.0–158.0 mm CL range (Fig. 4). Plastron length was strongly related to CL in adults of both males ($r^2 = 0.95$, F = 4357, $p < 0.001$; mm PL = 0.8857 (mm CL) + 4.1118) and females ($r^2 = 0.89$, F = 627.69, $p < 0.001$; mm PL = 0.906 (mm CL) + 4.4546).

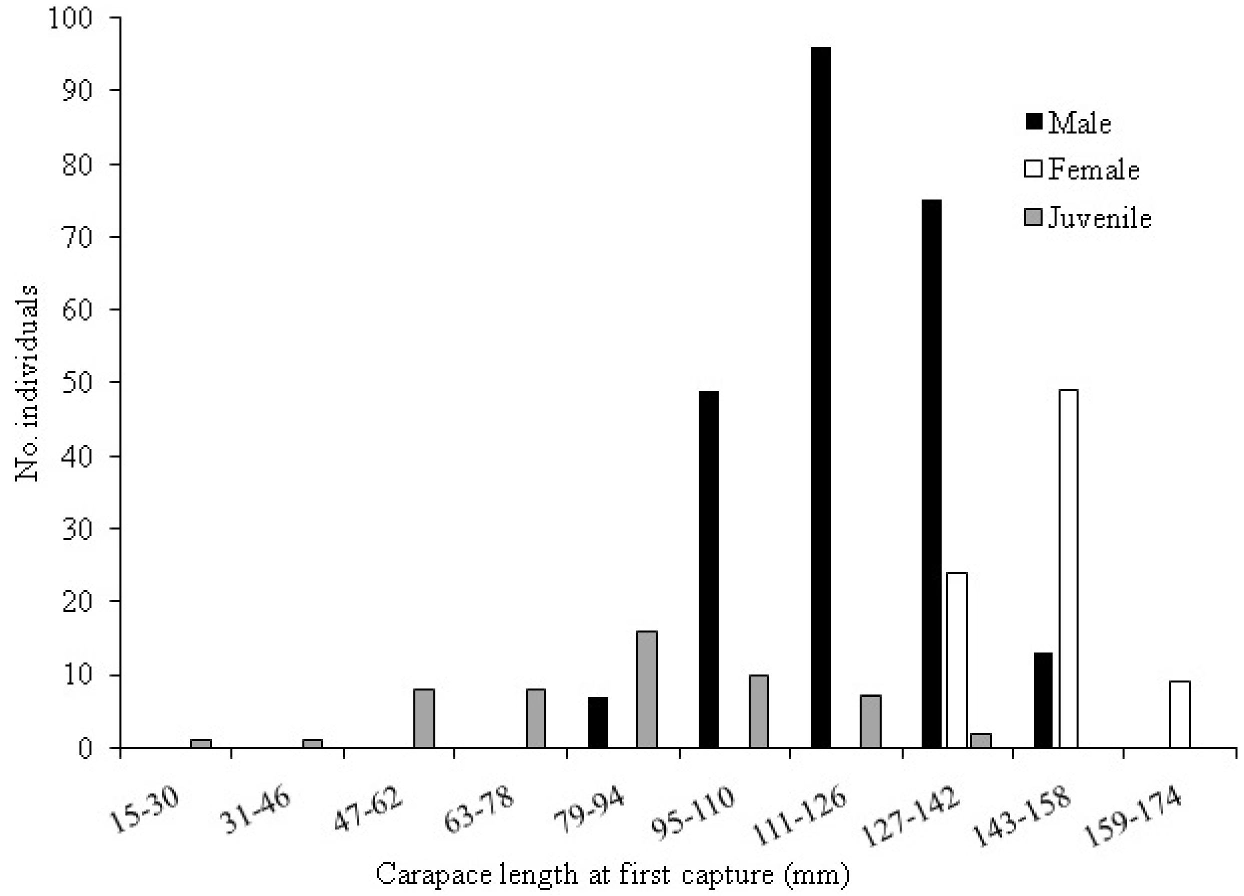

Figure 4. Body size distributions of 240 adult male, 82 adult female, and 53 juvenile painted turtles *C. picta picta* X *C. p. marginata* at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019.

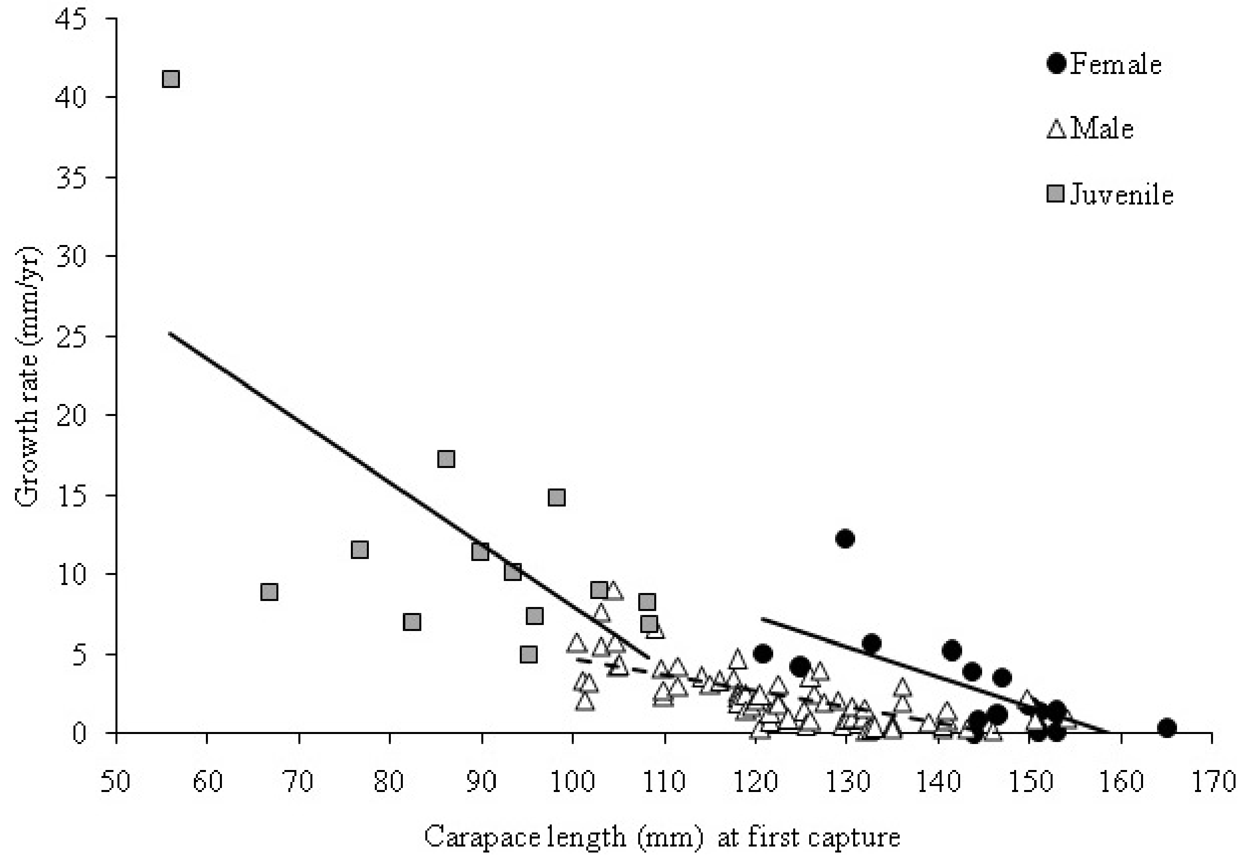

Figure 5. Annual growth rate plotted against carapace length at first capture for males, females, and juveniles of the painted purtle *C. picta picta* X *C. p. marginata* at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019.

Table 1. Multi-model comparisons for determining sex-specific, best-fitting growth curves for *Chrysemys picta* at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019. Asymptotic body size (*A*) and characteristic growth constant (*k*) ± 1 standard error.

	Model	AICc	ΔAIC	AICc Weights	*A*	*k*
Males	von Bertalanffy	645.77	0	0.77	116.4 ± 1.75	0.6 ± 0.055
	Gompertz	648.48	2.71	0.20	114.8 ± 1.53	0.97 ± 0.105
	Logistic	652.25	6.47	0.03	113.9 ± 1.44	0.79 ± 0.079
Females	Gompertz	482.46	0	0.44	142.7 ± 6.04	0.59 ± 0.107
	Logistic	482.84	0.38	0.36	138.8 ± 5.07	0.81 ± 0.143
	von Bertalanffy	484.11	1.65	0.19	152.9 ± 8.86	0.34 ± 0.065

Growth and Age Estimation

Growth rates decreased with increasing carapace length (Fig. 5). The average growth rate was highest for juveniles (12.2 mm/yr, min-max = 4.9–41.2 mm/yr, n = 13) and much lower for females (2.8 mm/yr, min-max = 0–12.2 mm/yr, n = 17) and males (2.3 mm/yr, min-max = 0.2–9.1 mm/yr, n = 68). Based on AICc values, the top models for growth differed between the sexes: von Bertalanffy for males and Gompertz for females (Table 1). Estimates of asymptotic body size (*A*) and characteristic growth constant (*k*) returned the following values based on the top models for each sex: males (*A* = 116.4; *k* = 0.6) and females (*A* = 142.7; *k* = 0.59). The growth trajectory of males rose more rapidly than females, such that males reached maturity faster, at two years of age, but males exhibited almost no noticeable growth beyond an estimated age of four years (Fig. 6). Males were also smaller at their asymptotic body size compared to females. Females, in contrast, appeared to grow at a steadier pace until reaching maturity. Based on visual inspection of the growth curve and female body sizes (Fig. 6), a few females reached maturity at three years of age, many at four years, and by five years of age all females were mature. Thereafter, growth continued, albeit very slowly.

Survivorship, Detection, and Population Size

Female survivorship (0.80 ± 0.04) was lower than that of males (0.89 ± 0.02), although recapture probabilities were similar (Table 2). For the most parsimonious model, Φ differed between sexes and p was time dependent (Table 3). As expected, recapture probabilities increased with sampling intervals and numbers of individuals marked. Pradel's λ was stable to slightly increasing for females and stable to slightly decreasing for males. Since Pradel's λ values are based on probability of new animals entering the population, these values are indicative of the probability of capturing an unmarked individual entering the population. The population was male biased at roughly 2.1:1 among initial captures, which is reflected in the population estimates (Table 2). Population density in the canal (2.65 ha) and the lake (3.53 ha) combined was 52.3 turtles/ha. Trends in recapture rates between sampling intervals increased over time (Fig. 7).

Table 2. Population dynamics of the painted turtle *Chrysemys picta* at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019. Parameters include apparent survivorship (Φ), recapture probability (p), Pradel's Lambda (λ), and population size (n) ± one standard error and 95 % confidence intervals.

	Φ	p	*λ*	n
Female	0.80 ± 0.04 (0.74, 0.93)	0.13 ± 0.03 (0.08, 0.19)	1.02 ± 0.02 (0.97, 1.06)	96 ± 13 (73, 128)
Male	0.89 ± 0.02 (0.84, 0.93)	0.17 ± 0.02 (0.14, 0.21)	0.98 ± 0.01 (0.95, 1.01)	227 ± 24 (185, 283)

DISCUSSION

We found that several demographic traits of the turtle population at our site were accelerated, and when examined in the context of other demographic studies in the mid-Atlantic region (Ernst & Lovich, 2009), it appears that differences in nutrient levels in the wetlands may be a likely factor. In Pennsylvania, for example, Ernst (1971a,b) provides a reasonable comparison to our results because he also studied a population of *C. p. picta* X *C. p. marginata* intergrades from a more natural wetland on Big Chickees Creek, which is only about 45 km south-east of our site. Our study site, on the other hand, was quantifiably eutrophic and subjected to continuous nutrient enrichment (see Methods). This apparent difference in resource availability, in turn, provided us with a variable to consider in the following discussion as an effect on comparative growth rates, body sizes, and ages at maturity between the populations. However, we note that meaningful interpopulation differences, possibly associated with hybrid vigour, cannot be ruled out, nor canotherabiotic differences between the two

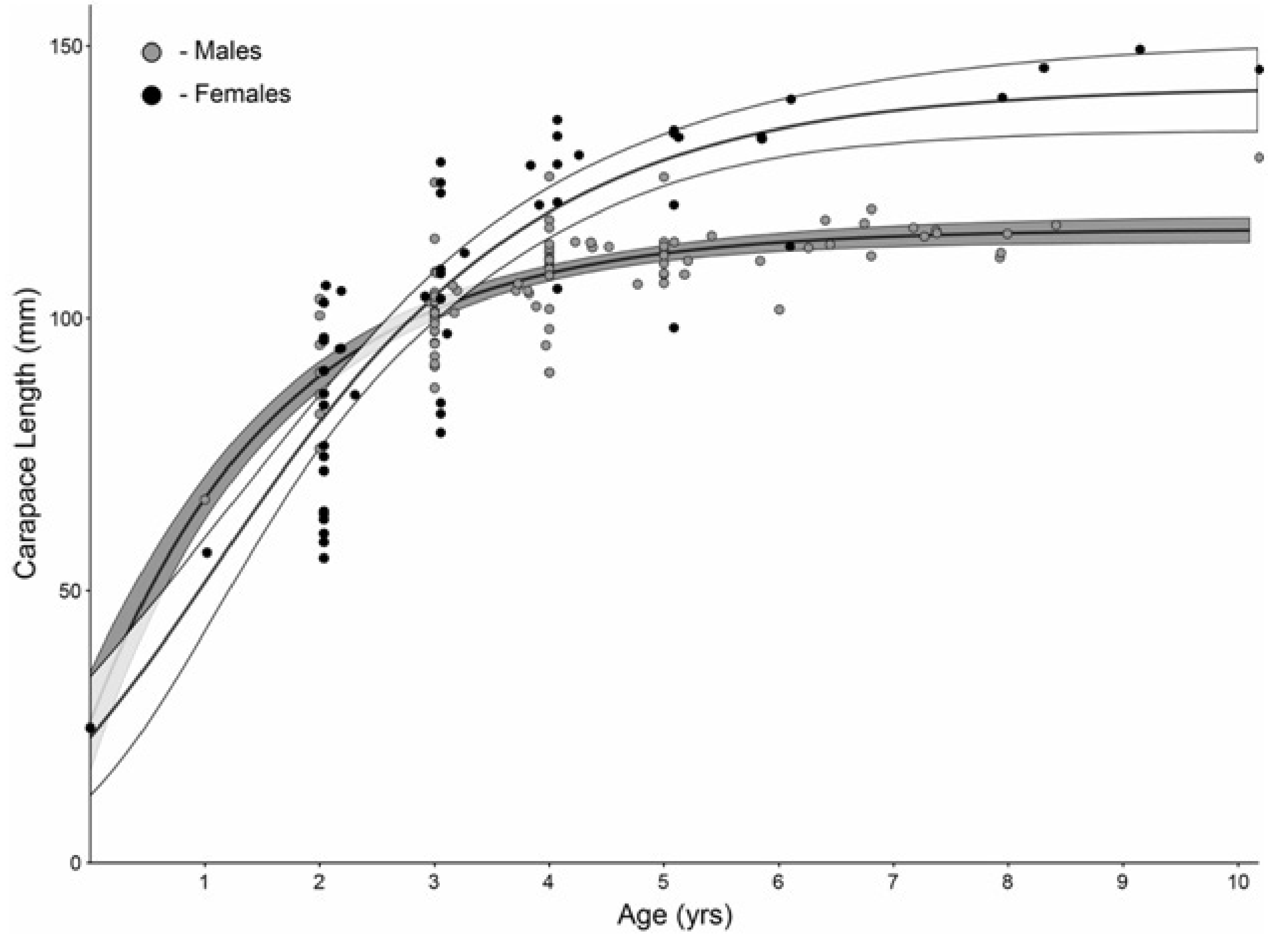

Figure 6. Best-fitting, sex-specific growth curves for the painted turtle *C. picta picta* X *C. p. marginata* using known length-at-age data collected from wild individuals at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019. Dark lines indicate age-length relationships and shaded areas confidence bands, generated by a Bayesian Markov chain MonteCarlo process.

habitats unrelated to nutrient levels, which may have accounted for the demographic differences we detail below.

Male painted turtles at Wildwood Park reached a larger minimum PL of 83.8 mm than the 70 mm PL reported by Ernst (1971a). Likewise, respective mean PL (111.6 mm, 96 mm) and maximum PL (142.2 mm, 121.0 mm) of adult males were larger at our eutrophic site than those reported by Ernst (1971a). Elsewhere in the mid-Atlantic region, sexual maturity in males of *C. p. picta* was reached at > 71 mm PL in Myrtle Grove Wildlife Management Area, a natural habitat in which turtles exhibited normal growth, Charles County, Maryland (Ernst & McDonald, 1989), and 71mm PL (77.7 mm CL) in a lake, creek and beaver ponds in Henrico County, Virginia (Mitchell, 1988). However, at a sewage treatment plant in Charles County, Maryland, the smallest mature male measured 87.1 mm PL (Ernst & McDonald, 1989). These comparisons between our study and more natural sites corroborate the conclusions by Ernst & McDonald (1989) that males exhibit plasticity in body length at sexual maturity. Our data and those of Ernst & McDonald (1989) on minimum PL at sexual maturity provide a PL range (83.8–87.1 mm) as a general response to eutrophic conditions in males of mid-Atlantic populations, such that recently matured males from eutrophic systems are approximately 1.2 times the size of their counterparts from less altered systems.

The minimum PL associated with sexually mature females from our site (122.2 mm) was larger than the minimum (100.8 mm) reported by Ernst (1971a) from a natural setting. The same was true of respective mean PL (138.3 mm, 116.9 mm) and maximum PL (165.0 mm, 145.4 mm) of adult females (Ernst, 1971a). In Henrico County, Virginia, minimum size at sexual maturity in females of *C. p. picta* was reached at 97.2 mm PL (Mitchell, 1988). However, among four females collected at a sewage lagoon in Charles County, Maryland, two females measuring 127 mm and 129 mm PL were not yet mature, and 132 mm and 139 mm PL may or may not have been mature, but none of these females contained shelled eggs or corpora lutea, nor were collecting dates provided (Ernst & McDonald, 1989). An examination of the ovarian follicle size-classes and widest diameters indicated that the two largest females collected by Ernst & McDonald (1989) may have been developing their first clutch, or, if mature, their first clutch of the season. Our data and those of Ernst & McDonald (1989) suggest that females subjected to eutrophic conditions exhibit a larger body size at sexual maturity resulting in a primiparous shell length at 1.3 times the size of their counterparts from more natural systems.

Table 3. Comparison of Cormack-Jolly-Seber models for apparent annual survival (Φ) and recapture probability (p) between male and female painted turtles *Chrysemys p. picta* X *C. p. marginata* at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019. Models differ in whether Φ and p are assumed to be constant (.), fully time dependent (t), or differ between sexes (g), and whether there are interactions (*) among these factors.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par.	Deviance
Φ(g) p(t)	1098.16	0	0.997	1	12	350.6368
Φ(g) p(g*t)	1110.135	11.975	0.0025	0.0025	22	340.9789
Φ(t) p(t)	1113.476	15.3153	0.00047	0.0005	19	350.9133
Φ(t) p(g*t)	1119.644	21.4839	0.00002	0	29	334.7405
Φ(g*t) p(t)	1125.847	27.6863	0	0	29	340.9429
Φ(g*t)p(g*t)	1136.363	38.2029	0	0	38	330.4361
Φ(t) p(g)	1154.61	56.4493	0	0	12	407.0861
Φ(g*t) p(g)	1173.009	74.8488	0	0	22	403.8528
Φ(g) p(g)	1177.345	79.1844	0	0	4	446.4368
Φ(.) p(.)	1178.767	80.607	0	0	2	451.9216

Mean values of shell dimensions can reflect differences in the environment, especially nutrient inputs. For example, in Virginia mean adult PL of males and females, respectively, were larger (103.1 mm and 124.1 mm) from a more eutrophic site (Mitchell, 1985a,b) than those in a less nutrient-rich site nearby (96.2 mm and 120.5 mm; Mitchell, 1988). To that end, we note that the mean PL of adult males (96.0 mm) and females (116.9 mm) from Ernst's (1971a) natural site were much smaller than those from our site (111.6 mm and 138.3 mm, respectively). Within a single study, Gibbons (1967) found decreasing size in longest shell lengths in both sexes from three sites in Michigan that varied in nutrient load: polluted river, eutrophic lake, and clean marsh. Carnivory in turtles from Gibbons (1967) also increased with increasing eutrophication, suggesting dietary differences may contribute to growth responses of individual males and females. Thus, rapid growth and overall larger body size is associated with more nutrient-rich sites, and larger body size at sexual maturity is more pronounced in females than in males.

We also wanted to know if growth rates and the minimum age at sexual maturity differed between eutrophic and natural sites. Quinn & Christiansen (1972) documented faster growth by western painted turtles *C. p. bellii* from Iowa in eutrophic systems than in those with demonstratively less organic matter in the substrate. Likewise, the nutrient level, adult body size, and carnivory of the turtles at Gibbons' (1967) sites were associated with differential growth rates in those in increasingly eutrophic waterbodies. Ernst & McDonald (1989) corroborated faster growth in both sexes from a eutrophic site and determined that sexual maturity of males at a sewage treatment site was reached in two years, as was ours, instead of four years from natural sites in south-eastern Pennsylvania (Ernst, 1971a) and central Virginia (Mitchell, 1988), where all males had foreclaws of at least 8 mm. Sexual maturity of females was reached at 3–5 years of age at our site, but females from a sewage treatment plant in Maryland may have been mature at three years (Ernst & McDonald, 1989). Ernst & McDonald's (1989) uncertainty regarding a connection between enhanced growth and early maturity in females from the sewage lagoon is understandable considering their unavoidably small sample size of dissected turtles. However, because Ernst & McDonald's (1989) site was presumed to have been much more nutrient-rich than ours, we consider it probable that the large 3-yr old females at their site were mature. Comparatively, females from more natural sites in the mid-Atlantic region matured at an age of five (Ernst, 1971a) or eight years (Mitchell, 1988). Some females from our site reached the minimum body size at sexual maturity in three years, followed by many at four years, and all by five years. Whereas males and females consistently matured at larger sizes at our site, earlier age at sexual maturity varied more in females than in males. Fast growth in juvenile turtles in our study, linked to elevated nutrient input, results in earlier maturation at similar or larger body sizes compared to slower growing counterparts of *C. picta*, as well as blanding's turtle *Emydoidea blandingii* and *C. serpentina* (Congdon et al., 2018).

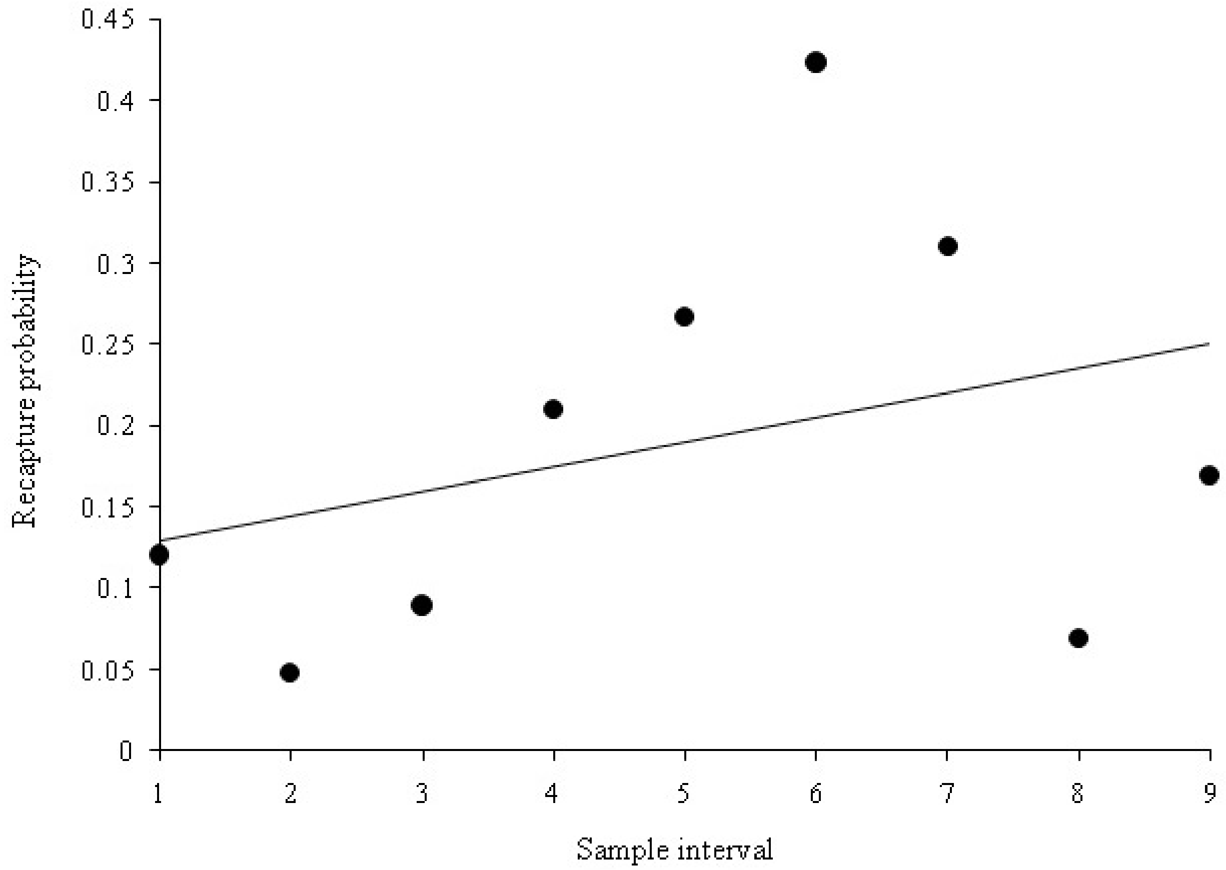

Figure 7. Trends in recapture rates between samples of the painted turtle, *C. picta picta* X *C. p. marginata*, at Wildwood Park, Harrisburg, Dauphin County, Pennsylvania, during 2011–2019.

Other demographic measures of our study population were in general agreement with those of other populations of *C. p. picta* and *C. p. picta* X *C. p. marginata* intergrades. Survivorship of both sexes was high at our site, a finding typical of *C. p. picta* (Mitchell, 1988; Zweifel, 1989) and *C. p. marginata* (Hughes & Meshaka, 2020) in the north-east and mid-Atlantic. Population density, however, can range widely across and within regions. Our estimate of 52.3/ha exceeded 25/ha of *C. p. picta* in a New York pond (Bayless, 1975) and 13.9/ha of *C. p. marginata* in a Pennsylvania pond (Hughes et al., 2016). In three Michigan ponds, population densities of *C. p. bellii* ranged from 39.9 to 89.5/ha (Congdon et al., 1986). Seasonal population density estimates range even more widely, including a published range of 137–248/ha for *C. p. picta* in New York ponds (Zweifel, 1989), and estimates as high as 590.4/ha for *C. p. picta* X *C. p. marginata* in a marsh and pond in south-eastern Pennsylvania (Ernst, 1971b) and 590/ha for *C. picta* in a Michigan pond (Gibbons, 1968).

The adult:juvenile ratio of a population also varies widely among populations, across habitats, and over time within a population. Our adult:juvenile ratio of first captures (6.08:1.00) was comparable to values of 5.0:1.0 from a pond (Bayless, 1975), and 4.20:1.00 from a marsh and pond (Ernst, 1971b). The ratio was much lower at a lake (1.1–1.3:1.00) studied by Mitchell (1988). On the other hand, an 18-year study in New York ponds yielded an average adult:juvenile ratio of 2.16:1.00 and range 0.45–6.30:1.00 (Zweifel, 1989). In general, hoop-net based population inferences tend to be skewed towards trapping larger species (Ennen et al., 2021) and mostly adults of smaller species (Tesche & Hodges, 2015). For example, it was recently shown that the average CL of 95 *C. picta* caught in hoop-nets with a mesh width size of 5.08 cm was significantly larger than the mean CL of 231 individuals caught in hoop-nets with a mesh size of 2.54 cm (Gulette et al., 2019). We suggest that future efforts employ a variety of turtle sampling methods to determine if the patterns we found reflect the size-class distribution of this turtle population (e.g. Ream and Ream, 1966).

Adult sex ratios of *C. picta* are often even (Bayless, 1975; Ernst, 1971b, Mitchell, 1988), however, they can vary over time (0.62–1.80:1.00) (Zweifel, 1989) and subjected to differences based on sampling technique used (Ream & Ream, 1966). To that end, our hoop-nets could have drawn males to a single female already in the traps resulting in a male bias. We also consider a potential combined effect of early sexual maturity in males and differential mortality in nesting females that may have influenced the sex ratio we found. Many of the well-known mesopredators of nesting females and their eggs (Ernst & Lovich, 2009) were regularly encountered at Wildwood Park: raccoons *Procyon lotor*, red foxes *Vulpes vulpes*, mink *Neogale vison*, striped skunks *Mephitis mephitis*, and long-tailed weasels *N. frenata* (W.E.M. and E.W., pers. obs.).

Fully comparable, systematic comparisons of physical and chemical parameters among sites, or data on temporal transitions within sites, as in comparisons with Ernst's (1971a,b) sites are lacking; therefore, we cannot rule out alternative explanations for the observed pattern. However, the population of *C. picta* at our site displayed demographic patterns consistent with those of populations in other eutrophic systems, suggesting a general effect of water quality that we suggest should be investigated experimentally. Both sexes grew faster and matured at longer shell lengths than conspecifics from less nutrient-rich habitats. Comparatively, males showed less plasticity in body size at sexual maturity and a narrower range in reduced age at maturity than females at our site. From a 33-yr study in Michigan, Congdon et al. (2018) demonstrated that the juvenile growth rate in *C. picta* females has a profound effect on age at sexual maturity, such that faster growing juveniles matured up to 6-yrs sooner than slower growing females and that post-maturity growth had very little effect on size-dependent reproductive traits, such as clutch size. Consequently, the earliest-maturing females, such as those that matured in 3-yrs at our site, can potentially have more lifetime egg output than the oldest-maturing individuals at Wildwood (5-yrs). Based on average clutch sizes of primiparous and older female *C. picta* in Congdon et al. (2018), the earliest-maturing females at Wildwood could produce 12 eggs over two years before the oldest-maturing females first laid eggs, and in turn, it would take these females decades until they caught up in lifetime egg output to the earliest-maturing females. One question that remains to be answered in life history theory for *C. picta* is whether there is a strong relationship between age at maturity and longevity, such that the turtles at Wildwood may mature earlier compared to elsewhere but die sooner, a phenomenon that could impact population dynamics in a long-lived, often abundant species that is ever more subjected to altered environments in expanding urban ecosystems across its geographic range. This last point, and the potential effects of human-altered habitats on chelonian demography in general, can by extension apply as testable hypotheses to aquatic turtle species worldwide, which are facing increasing contact with human impacts to their remaining aquatic habitats.

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