Title: Misleading monitoring: more hatchlings do not represent turtle population recovery

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Abstract

This article critiques a recent study by Lacava et al. (2024) that suggests the population of *Podocnemis expansa* has recovered in Brazil due to an increase in hatchling releases. I argue that hatchling counts alone are insufficient to determine population trends. A new analysis of the original data, including temporal autocorrelation, only showed a sustained increase in hatchling releases at five of the eleven areas studied, which goes against the original claim that most areas showed population recovery. I propose three main challenges to the original study. Firstly, hatchling counts should not be used in isolation to assess population recovery. The focus should instead be on hatchling relative abundance. Secondly, the study failed to consider alternative hypotheses that could explain the observed increases, including changes in methods and effort over time. Lastly, there was an incorrect use of statistical significance and a failure to address biological relevance of the results. The original study did not account for temporal autocorrelation in the data. Reanalysis shows that changes in at least one area were due to changes in methods/management. I argue that the conservation program needs to broaden its scope to include adult populations and other factors, as the current number of hatchlings released is likely insufficient to replace the number of adults consumed annually. The study by Lacava et al. (2024) requires significant correction and clarification to avoid misleading conclusions about the effectiveness of conservation efforts.

Keywords: correction, freshwater turtle, Brazil, Podocnemis expansa, Programa Quelônios da Amazônia, reptile

Recently, Lacava et al. (2024) showed that the number of *Podocnemis expansa* hatchlings had increased across nesting beaches over four decades. The authors show that currently the Brazilian Amazon Chelonian Program (Programa Quelônios da Amazônia - PQA) typically releases more than two million hatchlings per year, which is truly impressive. While the study presents a positive trend in hatchling releases in some nesting areas, the data collected and the results presented do not support the authors' conclusions regarding population recovery. New analysis including temporal autocorrelation shows evidence of a sustained increase in hatchlings released at only five of the 11 areas, which challenges the authors' conclusion of population recovery in "most" areas. Here I identify where corrections are required grouped into three general lines: i) hatchling counts in isolation do not represent turtle population trends, ii) authors fail to consider equally plausible alternative hypotheses, and iii) incorrect use of statistical significance.

Firstly, the authors use annual hatchling counts in isolation to evaluate population trends. This is not appropriate for turtle species. While authors identify hatchling counts as the most consistently collected data available, this does not mean it should be used to assess population trends. Studies with *P. expansa* and marine turtle species with similar social nesting patterns demonstrate that while hatchling counts can be part of turtle population monitoring, they're inadequate in isolation (Eisemberg et al. 2019; Forero-Medina et al. 2021; Hays et al. 2025; Hendrix and Pérez-Espona 2024). A more appropriate approach would be to simply reframe the text to refer to hatchlings released instead of population trends/recovery. The potential of hatchlings released to reflect population trends could then perhaps be discussed. For example, reinforcing limitations and the need to include other factors like adult survival for assessing turtle population trends (Gibbs and Amato 2000; Páez et al. 2015).

Turtle population dynamics are strongly dependent on adult survival, as such indicators are required that are in some way calibrated with adult populations e.g. hatchlings per nest/hatchlings per female (Eisemberg et al. 2019; Moll and Moll 2004). The failure to include such indicators invalidates the authors' conclusions regarding population trends. As reinforced by Eisemberg et al. (2019), the number of hatchlings released is a poor indicator of *P. expansa* population trends due to a dependence on environmental and climate factors (e.g. temperature and river levels). In the case of protected areas such as in Lacava et al. (2024), the number of hatchlings released will also depend on the conservation management actions and efforts (techniques, number of staff, etc.).

Before drawing any conclusions about trends based on hatchling counts, key assumptions need to be more rigorously tested. By emphasizing population recovery, the study assumes that an increased number of hatchlings released translates to increases in the adult breeding population. This assumption needs to be tested, for example by conducting post-release monitoring to assess actual hatchling survival rates in the wild (Mogollones et al. 2010). To address the limitations of using hatchling counts in isolation, the study could be refocused to assess changes in hatchling relative abundance over time, without drawing direct conclusions about overall population trends. This would reduce reliance on untested assumptions. By refocusing the study in this way, the authors could still provide valuable information about the impact of the PQA on hatchling production, while avoiding overinterpretation of the results in terms of population recovery. This would provide a more cautious and scientifically sound assessment of the program's effectiveness.

Secondly, several equally plausible alternative hypotheses could also explain the observed trends in the data. These hypotheses need to be tested to avoid known pitfalls in simplistic

monitoring (Christie et al. 2021; Christie et al. 2019; Wauchope et al. 2021), and include i) improved methods/techniques in nest and hatchling management over time leading to increases compared with the early years/decades, ii) increased number of staff/workers, iii) improved technical experience/capacity of staff/workers, iv) improved data collection and reporting, and v) expansion in the size/number of nesting areas included in conservation actions over the years etc. As authors fail to provide key methodological details it is impossible to assess the validity of the methods. For example, there is no detail on when the fencing of the hatchlings started in the different areas, the annual coverage of the fenced areas at the nesting beaches, or if the coverage changed over time. Indeed it is unclear what was actually measured as in the methods authors state "the number of hatchlings released annually at each reproductive site", whereas the legend for Figure 2 states: "Fig. 2 The number of Giant South American River Turtle (*Podocnemis expansa*) hatchlings born". In addition, authors should also consider the total nesting area available each year that was fenced/monitored. This variable strongly influences the number of nests and therefore hatchlings per nesting area. There is also no indication if management efforts changed over time. Without testing these alternative hypotheses, it is impossible to separate real trends from artifacts and systematic biases caused by variations in monitoring/management intensity and techniques.

Authors suggest in the discussion that the more positive trends they found in comparison to earlier studies were explained by the inclusion of more years: "Based on a more extended data set than previous studies on the Brazilian PQA public dataset (Eisemberg et al. 2019; Forero-Medina et al. 2019), our results show a far more positive trend of increase over 43 years in most areas and for all the nesting sites pooled together.". This is not the case. Running the same analysis using subsets corresponding to the years in earlier studies the trends can worsen or

improve over time at different beaches (Supplemental Material S1). The contrasting results

presented most likely come from the far more robust analysis presented in the earlier studies. The

earlier studies used a combination of indicators not only hatchling counts and attempted to

correct methodological differences over time (Eisemberg et al. 2019; Forero-Medina et al. 2021).

As such the more cautious conclusions presented in the earlier studies appear to have much

stronger support based on the available data. It is unclear why Lacava et al. (2024) did not repeat

the same analysis as presented in the earlier studies. One possible explanation is that a lack of

robust monitoring data comes from severe budgetary cuts and limited operational cover, which

limits the scope and activities of the PQA (Eisemberg et al. 2019).

Finally, there is an equivocal interpretation of statistical significance, with the authors also

failing to appropriately address the biological significance of the results. As the authors'

objective is to assess conservation impact it is worth remembering that they identify increases in

hatchling counts in 6 of the 11 areas. This proportion means that overall, there was only a 50:50

chance for an area to experience increases in the number of hatchlings released (Chi-squared = 0,

df = 1, p-value = 1). Statements such as "efforts for turtle conservation over four decades to

protect the *P. expansa* were efficient in most PQA-protected nesting areas", are therefore

potentially misleading. As the study had no control, i.e. areas without protection, it is impossible

to know if this overall proportion of areas with increases provides evidence of conservation

success. In other words, the simple study design cannot be used to assess conservation

effectiveness (Christie et al. 2021; Christie et al. 2019; Wauchope et al. 2021).

The authors identify six areas where "populations increased over time with statistical

significance". The six areas were Abufari and Embaubal ("more subtle increase"), and Monte

Cristo, Guaporé, Camaleões, and Walter Bury with a "very evident increase of hatchlings

released over the 40 years.". Both areas with "more subtle increase" had very low deviance explained (12.9 and 18.2 % model deviance explained for Abufari and Embaubal respectively), with the lower confidence limits at the end of the monitoring period appearing to overlap the upper confidence limits at the start in both cases (Figure 2 in the article). The overlap in confidence intervals over time raises questions regarding statistical significance (Greenland et al. 2016; Halsey 2019; Muff et al. 2022).

As the analysis was based on counts over time, temporal autocorrelation needs to be explicitly included in the models. There are several reasons why this is important but generally, the most important reason is to avoid type I errors (Carroll and Pearson 2000; Keith et al. 2015; Zuur et al. 2010). Reanalysis of the data including temporal autocorrelation with Generalized Linear Mixed Models provided evidence of increases in hatchling counts from 5 of the 11 nesting areas (Supplemental Material S1). Abufari beach was no longer "statistically significant". The "increase" in numbers at this beach was due to a jump in the number of hatchlings released around 1990. Until 1990 there was an average of approximately 50 thousand hatchlings released, then after 1990 the number suddenly quadrupled to approximately 200,000 hatchlings. Such a sudden and large jump is not consistent with the population dynamics of turtle species and would not be reflected by the simplistic GLM approach adopted by the authors. The most likely explanation for such a jump is a change in management such as increasing the area protected, change in counting technique, etc. Unfortunately, this example highlights that a lack of methodological details makes it impossible to know how much of the observed increases are due to methodological differences or biologically meaningful changes in the numbers of hatchlings released.

How the numbers of hatchlings released translate into meaningful results depends among other things on the biological relevance of the statistical significance. For example, while Walter Bury was the nesting area with the strongest increase in hatchlings released, local communities that live around this nesting area identified the sale of adults as the main reason for declines in turtle populations in the region (de Paula Nascimento et al. 2024). The same study identified a lack of ethics among beach guards and a lack of governance as problems around this nesting area. Indeed, locals report beach guards selling adult turtles to middle-class buyers (de Paula Nascimento et al. 2024). This contrast between the increasing number of hatchlings and reports of reduced adult populations supports previous studies that highlighted a lack of correlation between hatchling counts and adult populations and the urgent need to redirect conservation efforts (Eisemberg et al. 2019; Páez et al. 2015). Recent declines over the past decade in another area (Guapore) were not represented by the GLM model chosen by the authors (Figure 2 in the published article). These declines are thought to be associated at least in part with extreme climatic events (Energisa 2024), which highlights how sensitive hatchling counts are to external factors and reinforces the need to not only appropriately quantify environmental and methodological differences among beaches and over time, but to also expand monitoring to include samples of different stages including nests and adult females (Eisemberg et al. 2019; Forero-Medina et al. 2021; Páez et al. 2015). Although I illustrate my points using recently published examples, the authors have decades of experience so were aware of these issues.

I grouped corrections into three different sections, yet there are important considerations that can be found across all. The activities of PQA remain strongly focused on protecting beaches without considering the adult population. Despite calls for changes, the PQA does not appear to have evolved to meet 21st-century challenges of rapid expansion of human-induced impacts

across Amazonia, including increased extreme climatic events, habitat loss, etc (Eisemberg et al. 2019; Páez et al. 2015). Without adapting the monitoring protocols to include samples of nests and adult females it remains impossible to assess population trends. Authors show that there are typically more than two million hatchlings released per year. Unfortunately, considering what is known of the species population demographics, this is still well below the number required to replace individuals consumed annually in urban and rural areas in the Brazilian Amazon (Chaves et al. 2021; Peres 2000). Using what is known about turtle population dynamics, it is likely that 200 – 1000 hatchlings are needed to replace 1 adult for this long-lived and late-reproducing species. Although there is no published estimate covering the whole Brazilian Amazon, based on published data (Chaves et al. 2021; Peres 2000) it is plausible that somewhere around 300,000 adult *Podocnemis expansa* are consumed annually in Brazil – therefore at least 60 million hatchlings need to be released per year to replace the adults consumed.

In conclusion, while hatchling counts can be part of turtle population monitoring, they're inadequate on their own to assess the effectiveness of conservation actions. Turtle species require not only long-term but also multi-faceted integrated approaches to both assess population trends and to guide and evaluate conservation efforts. The study published by Lacava et al. (2024) requires correction/clarification to avoid ingenuous and potentially misleading conclusions and to more closely reflect the reality of 21st-century freshwater turtle conservation efforts.

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