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Short communication: Estimating radiocarbon reservoir effects in Bolivian Amazon freshwater lakes

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Abstract. The Llanos de Moxos, in the Bolivian Amazon, preserves a remarkable archaeological record, featuring thousands of forest islands. These anthropogenic sites emerged as a result of activities of the earliest inhabitants of Amazonia during the Early and Middle Holocene. Excavations conducted to date on the forest islands have revealed that many assemblages contain a high number of ancient freshwater snail remains. In these shell middens, the most represented mollusc taxon, and in most cases the sole one, is *Pomacea* spp., a genus that inhabits inland shallow lakes and wetlands. Although human burials and faunal remains are typically recovered from these sites, their collagen is often not preserved or is of poor quality, and shell carbonates from *Pomacea* shells, along with carbonised plant remains, are often used for ^{14}C measurements. However, it remains undetermined if these measurements are subject to radiocarbon reservoir effect (RRE). To determine if a freshwater RRE could affect the age estimations of Amazonian archaeological and other paleoecological deposits, we collected modern coeval *Pomacea* shells and tree leaves from four locations across the Llanos de Moxos area for AMS radiocarbon dating. The radiocarbon results combined with the environmental history of Llanos de Moxos during the Holocene, do not reveal any significant RREs, and support the continued use of freshwater molluscs as viable material for radiocarbon dating in the region.

1 Introduction

Although archaeological research on the earliest human occupations in South America had traditionally prioritised coastal environments (Armesto et al., 2010; Bueno et al., 2013), recent studies have increasingly provided evidence that these populations expanded into the central regions of the continent during the Early Holocene (Lombardo et al., 2013, 2020). Archaeological research conducted in the Llanos de Moxos, a seasonally inundated tropical savannah in the Bolivian Amazon (Fig. 1A–B), has revealed that pre-Columbian communities formed artificial mounds known as forest islands since the Early Holocene (Lombardo et al., 2013). These were small forested earthen mounds for which

^{14}C radiocarbon dates constrain human occupation from approximately 11 to 2 ka cal BP (Capriles et al., 2019; Lombardo et al., 2020). Some of these forest islands are composed of shell midden stratigraphic deposits, although not exclusively, and contain a heterogeneous assemblage of archaeological remains (Capriles et al., 2019). Among them, the most common are mollusc shells of the *Pomacea* genus (Perry, 1810) (Fig. 1C). These were collected by past Amazon populations from freshwater bodies (shallow lakes and wetlands) unrelated to carbonate dissolution processes which occurred in shell middens. Human burials are also frequently found in these sites, along with animal bones and ceramic remains, which are helpful particularly for building relative chronologies. Other evidence, such as wood charcoal or carbonised seeds, may also be encountered at the forest islands, but can be rare in many depositional contexts (Capriles, 2023; Capriles et al., 2019; Lombardo et al., 2013).

The poor preservation of bone collagen (Capriles et al., 2019), along with challenges in dating bioapatite in tropical environments including the difficulties of removing contaminants and diagenesis involving isotopic exchange of dissolved carbon from shells (Cherkinsky, 2009; Fernandes et al., 2013b; Inomata et al., 2022; Zazzo and Saliège, 2011), complicates the radiocarbon dating of bones. Moreover, extensive archaeological excavations are resource-intensive, and much of the available dating evidence comes from coring and auger soil sampling. This methodological limitation significantly restricts the availability of suitable datable materials, except for *Pomacea* remains, which are abundant, particularly easy to identify and are relatively well preserved. This frequently makes the shells the most viable option for radiocarbon dating of human activities in southwestern Amazonia and other tropical settings (Lombardo et al., 2013). However, as observed in marine mollusc shells, freshwater specimens may yield radiocarbon ages older than those from coeval terrestrial organic materials (Alves et al., 2025; Culleton, 2006; Fernandes et al., 2012, 2013a; Geyh et al., 1997; Inomata et al., 2022; Philippsen, 2013). This ^{14}C offset results from the presence of ^{14}C depleted carbon in water when compared to the contemporaneous atmosphere. This carbon is assimilated by molluscs and incorporated into their shell carbonate structure (Fernandes and Dreves, 2017). Such radiocarbon reservoir effects (RRE) in lacustrine systems, also known as freshwater reservoir effects (FRE), are driven by multiple factors. Of particular relevance in our study, is the influx of water enriched with dissolved ancient carbonates (also knowns as hard-water), transported to lakes via groundwater and runoff (Yu et al., 2007) and carbon contributions from old organic matter (Fernandes et al., 2012, 2013a). Therefore, determining the magnitude of RREs across time is crucial for the accurate calibration of radiocarbon dates from subfossil shells.

Llanos de Moxos is a noncalcareous region and the river catchment basin in the Andes drains almost exclusively through siliciclastic rock (Gómez Tapias et al., 2019). This has led to a widespread practice of radiocarbon dating lake

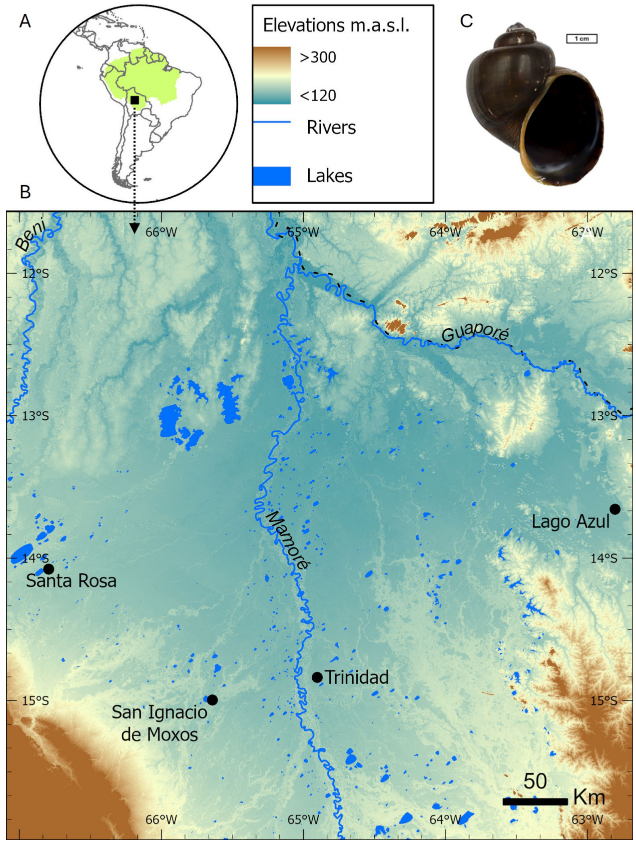

Figure 1. **(A)** Location of the study area in South America. **(B)** Llanos de Moxos in the Bolivian Amazon and the four lakes from which samples were collected. **(C)** A modern specimen of the freshwater *Pomacea* genus from Lago Azul. Maps were created by UL using ArcGIS software, and the photograph of the *Pomacea* shell was taken by AGE.

sediments palaeosols and shells from the Bolivian Amazon without RRE corrections (Carson et al., 2014; Lombardo et al., 2013, 2018; Whitney et al., 2014). However, RREs may originate also from oxidation of old organic matter (Fernandes et al., 2012, 2013a, 2016). Thus, testing for an absence of freshwater RREs in the Bolivian Amazon remains necessary. Here, we report nine accelerator mass spectrometry (AMS) radiocarbon dates from *in vivo* collections of *Pomacea* shells and coeval terrestrial plant samples from four different locations across the Llanos de Moxos area.

2 Materials and methods

The freshwater golden apple snail genus *Pomacea* (Fig. 1C) is native to South America and has rapidly spread worldwide (Céspedes et al., 2024; Hayes et al., 2008; Seuffert and Martín, 2024). Apple snails of the *Pomacea* genus are part of the *Ampullariidae* family, which includes the largest freshwater snails, reaching up to 17 cm in length (Azmi et al., 2022). Previous research on this genus showed that it has high growth rates, which are primarily dependent on temperature and mollusc ontogeny (Seuffert and Martín, 2013; Sutton et al., 2017). *Pomacea* specimens are primarily macrophytophagous, thus preferring floating or submersed plants over emergent ones, although some species, such as *P. canaliculata* also feed on animal matter (Estebenet and Martín, 2002).

In this study, modern *Pomacea* spp. specimens were collected alive from four freshwater continental systems located across the Llanos de Moxos region (Fig. 1B) in 2023 and 2024. In both years, collections took place in October, coinciding with the lowest water level during the dry season. The molluscs were immediately sacrificed to prevent additional calcium carbonate deposition. This was done by immersion in boiling water for up to three minutes, which facilitated the removal of the edible portion of the mollusc. We obtained a sample from the shell edge (or lip), which represents the most recent shell growth, in order to analyse the carbonate deposited during the last few weeks or months prior to collection. The shell periostracum was manually removed using a dental microdrill equipped with a 1 mm tungsten bit. Additionally, tree leaves of terrestrial trees, which are replaced in less than one year, were collected near where molluscs were collected and at the same time. This allows for a comparison of coeval ^{14}C values for the atmosphere and precipitated shell carbonate.

A total of five mollusc shells and four tree leaves were subject to sample pre-treatment, combustion, and graphitization at the CIRAM laboratory (Martillac, France) while AMS measurements were carried out at BARNAS mass spectrometry (Vilnius, Lithuania). To remove surface contamination, mollusc shells were treated with hydrochloric acid (HCl, 1 M) at room temperature. Plant leaves were placed in HCl (1 M) at 80 °C for one hour and then treated with sodium hydroxide (NaOH, 0.1 M) at room temperature for 10 min. Subsequently, they were placed again in HCl (1 M) at 80 °C to remove absorbed atmospheric CO_2. Following chemical pre-treatments, both shell and leaf samples (weights ranging from 3.5 to 15.4 mg) were combusted at 920 °C using an elemental analyser (EA) (Elementar, Vario ISOTOPE Select) at the CIRAM laboratory. Samples were weighed into tin capsules and combusted in the EA using oxygen mixed with helium carrier gas (O_2 dosing time: 100 s). The carbonates were combusted following a similar procedure, but these were ground into powder before being weighed into tin capsules and subject to a longer combustion time (O_2 dosing time: 120 s). The CO_2 emerging from the EA was split with ca. 90 % of this captured by a zeolite trap in an Automated Graphitization Equipment (AGE) (IonPlus AG, AGE 3) and underwent catalytic conversion into graphite, using the hydrogen reduction method based on Vogel et al. (1984).

Radiocarbon content of both shell and leaf samples was measured using a 50 kV accelerator mass spectrometer Low-Energy Accelerator (LEA, IonPlus AG) at the BARNAS laboratory. Corrections for isotopic fractionation were applied following Stuiver and Polach (1977), based on the comparison of $^{13}C/^{12}C$ and $^{14}C/^{12}C$ AMS measurements. The analytical precision of the Fraction Modern (F^{14}C), which expresses ^{14}C concentration normalised to the standard ^{14}C atmospheric level in 1950 (Reimer et al., 2004) is reported here at 1σ. Measurements of stable carbon isotope ratios for leaf and shell samples were carried out at the CIRAM laboratory using ca. 10 % of the EA produced CO_2 (see previous paragraph) which flowed into a coupled isotope ratio mass spectrometry (IRMS) (Elementar, isoprime precisION). Raw isotope data were normalised against international standards (caffein USGS61 [$\delta^{13}C = -35.05 \pm 0.04$ ‰ VPDB, Schimmelmann et al., 2016] and glucose BCR-657 [$\delta^{13}C = -10.76 \pm 0.04$ ‰ VPDB, European Commission certificate EUR 20064 EN]) and δ^{13}C results expressed in per mil (‰) relative to the VPDB (Vienna Pee Dee Belemnite) standard.

3 Results and discussion

The radiocarbon activity of modern *Pomacea* shells, collected *in vivo* from four lakes, ranged from 1.0001 ± 0.0037 to 1.0303 ± 0.0036 F^{14}C, while the terrestrial leaves from neighbouring locations ranged from 1.0018 ± 0.0037 to 1.0155 ± 0.0036 F^{14}C (Table 1). Significant differences in F^{14}C values for terrestrial plant samples collected in 2023 and 2024 partly reflect the decline in atmospheric ^{14}C levels following nuclear weapons testing during the 1950s–1960s (Hua et al., 2022). No statistically significant differences were observed between the F^{14}C values obtained from coeval lacustrine and terrestrial samples, except for mollusc LA.1 (Table 1). This specimen exhibited a radiocarbon activity higher than that the reference terrestrial leaf LA.2 and shell LA.3 retrieved from the same lake. The F^{14}C values for LA.2 and LA.3 were not statistically different (Table 1). The reason for the discrepancy observed for LA.1 is presently indetermined. Although the results could arise statistically (F^{14}C values for LA.1 and LA.2 overlap at a 3σ range) we cannot fully exclude local variations in ^{14}C carbon sources available to the two molluscs or ^{14}C differences arising from differences in mollusc carbon metabolism. The higher δ^{13}C value in LA.3, when compared to LA.1, may be related to a higher incorporation of carbon sourced from organic matter by LA.1 (mollusc shell LA.1 also showed the lowest δ^{13}C value of all shell samples) (Fernandes and Dreves, 2017). In this case, carbon from older organic matter present at the lake would have to have a higher F^{14}C value than inorganic carbon resulting from the incorporation of a bomb signal.

Our results are overall consistent with the hypothesis that no significant RRE is expected for modern freshwater *Pomacea* shells within the Bolivian Amazon. However, we must also consider potential temporal variations in RRE values, as a result of climate change, hydrological dynamics, and human impacts (Geyh et al., 1997; Philippsen, 2013). Varia-

Table 1. Radiocarbon and stable isotope results for modern mollusc and plant samples measured using AMS and IRMS, respectively. Chi-square tests compare mollusc radiocarbon results with paired plant samples.

Collection site	Collection date	ID Code	Lab Code	Material	$F^{14}C$	$\chi 2$ test results $df = 1$ (5 %, 3.8)	$\delta^{13}C$ (‰) (by IRMS)
Trinidad	October 2023	CHU.100	CIRAM-8348	Shell	1.0154 ± 0.0036	$T = 0.8$	−7.8
		CHU.101	CIRAM-8349	Tree leaf	1.0108 ± 0.0035		−32.4
Lago Azul	October 2023	LA.1	CIRAM-10931	Shell	1.0303 ± 0.0036	$T = 8.5$	−17.5
		LA.3	CIRAM-13341	Shell	1.0137 ± 0.0035	$T = 0.1$	−14.6
		LA.2	CIRAM-10932	Tree leaf	1.0155 ± 0.0036		−30.2
San Ignacio	October 2024	SI.1	CIRAM-12310	Shell	1.0001 ± 0.0037	$T = 0.0$	−14.9
		SI.2	CIRAM-12311	Tree leaf	1.0004 ± 0.0034		−29.6
Santa Rosa de Yacuma	October 2024	SRO.100	CIRAM-12312	Shell	1.0024 ± 0.0034	$T = 0.0$	−14.1
		SRO.101	CIRAM-12313	Tree leaf	1.0018 ± 0.0035		−32.4

tions in lake sediment sodium bicarbonate have been linked to evaporation rates (Geyh et al., 1997). However, the absence of carbonate sources in investigated lakes and climatic stability observed for Llanos de Moxos via palaeoecological records likely exclude climate as a potential source for RRE temporal variations (Brugger et al., 2016; Mayle et al., 2000). RREs may also reflect geothermal activity (Ascough et al., 2010), which has not been reported for Llanos de Moxos. Fluvial dynamics in Llanos de Moxos did change significantly during the Holocene, particularly between 4 ka and 2 ka cal BP, when heightened river activity is recorded (Lombardo et al., 2018). Nevertheless, the Bolivian lowlands and their river catchment areas, with the exception of a carbonate outcrop in the region of Torotoro (Apaéstegui et al., 2018) within the catchment of the Rio Grande river, are mostly devoid of limestone rocks. This allows us to suggest that changes in hydrological dynamics are unlikely to have impacted temporal variations in RRE values for most of the Llanos de Moxos. As for the Rio Grande river, it deposits its sediments in the Santa Cruz region, where its discharge is significantly reduced as water flows underground (Lombardo, 2016). Rio Grande feeds into the Mamoré river, south of the Llanos de Moxos. However, until approximately 4000 years ago, the Rio Grande flowed northward, depositing a large sedimentary lobe in southern Llanos de Moxos and likely contributed significantly to sediments that now cover the northeastern part of the region (Lombardo, 2014; Lombardo et al., 2012). Although the carbonate section of the Rio Grande catchment is extremely small compared with non-carbonate rocks, we cannot fully exclude a negligible RRE value in shells dating older than 4000 years in the eastern part of the Llanos de Moxos.

Humans have impacted the landscapes within our study region to some extent and could have, in theory, locally influenced RRE values. Different studies show that pre-Columbian populations actively managed their surroundings; modifying hydrological conditions to retain water longer into the dry season or drain water more effectively during the wet season (Lombardo et al., 2025), increasing fire activity (Brugger et al., 2016; Duncan et al., 2021), and constructing geometric earthworks (Carson et al., 2014), among others landscape modifications. However, these human activities primarily took place during the Late Holocene (Erickson, 2000; de Souza et al., 2018; Whitney et al., 2013), following the formation of forest island shell middens, suggesting that their impact during the Early and Middle Holocene, if any, was minimal. Regarding the Late Holocene, these pre-Columbian earthworks influenced the runoff of rain waters, either by speeding up the drainage or via water retention, on clayish and impermeable soils, with little to no exchange with underground water, as attested by the oxidative patterns of the subsoil (Lombardo et al., 2015). We therefore exclude a notable impact of pre-Columbian human activities on RREs.

In conclusion, while our sample size is still relatively modest, our radiocarbon results, together with an assessment of the stability of the conditions impacting RRE values, are consistent with the hypothesis that there is an absence of significant freshwater RREs in the Llanos de Moxos area. This adds support for the reliability of existing radiocarbon chronologies based on ^{14}C measurements from *Pomacea* shells and incentivises wider use of freshwater molluscs in future radiocarbon dating projects. Nevertheless, evaluating potential reservoir effects using archaeological indicators should still be considered in further studies, for example by analysing paired shell carbonates and carbonised plant remains, or other materials of well-constrained chronological age (e.g., Alves et al., 2025). We also note that our results are based on modern mollusc shells. Ancient shells deposited in soils could be subject to diagenetic processes that may produce erroneous radiocarbon results. Thus, radiocarbon dating of ancient mollusc shells requires the application of appropriate pre-screening protocols to assess shell preservation and of pre-treatment protocols to remove contaminant carbon (Douka et al., 2010).

Data availability. All data can be found in the Table 1.

Author contributions. AGE and UL designed the experiment. AGE, UL, KD, and CDS collected the samples radiocarbon dated in this experiment. PMBA and AC provided scientific support. RF verified the results, experiments, and other research outputs. AGE prepared the manuscript with contributions from UL, JMC, and RF. All co-authors have reviewed and edited the final version of the manuscript.

Competing interests. The contact author has declared that none of the authors has any competing interests.

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