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Multiproxy paleodietary reconstruction using stable isotopes and starch analysis: The case of the archaeological site of Playa del Mango, Granma, Cuba

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ABSTRACT

Paleoethnobotanical and stable isotope studies have demonstrated that the indigenous groups that populated the Antilles, traditionally understood as dependent exclusively on wild resources, cultivated and consumed both C_3 and C_4 plants even before the arrival of the ceramic-bearing Arawak groups. However, the relative importance of cultigens and the differential use of plants, especially maize, between populations and individuals remains unknown. In this paper we combined the analysis of stable isotopes ($\delta^{15}N$, $\delta^{13}C_{co}$, $\delta^{13}C_{en}$, $\delta^{13}C_{ap}$, $\delta^{34}S$) of 27 individuals from the archaeological site of Playa del Mango, Cuba with the identification of starch grains in dental calculus. The stable isotope results indicate that the sampled population had a 70:30 C_3/C_4 diet, where at least 65 % was based on C_3 protein. Starches from C_3 (e.g., Marantaceae, *Ipomoea batatas*) and C_4 plants (*Zea mays*) were found in similar proportions (50:50). These results support that the lack or abundance of starch grains cannot be used to infer directly the frequency at which C_3 and C_4 plants were consumed within a small population. Statistically significant differences between females and males in the carbon isotope composition of diet, and its energy portion, suggests a differential consumption of plants by sex. Playa del Mango individual diets were statistically different from those of coeval sites, supporting our previous findings that groups with different dietary traditions concurrently inhabited Cuba in precolonial times. The study demonstrates the power of combined use of stable isotope models, and starch analysis, to provide a more nuanced reconstruction of dietary practices in past human populations.

1. Introduction

The first indigenous groups that populated the Antilles, before the expansion of the later Arawak ceramic-bearing populations, were traditionally understood as highly mobile groups who depended exclusively on wild resources obtained through hunting, fishing and gathering (Rouse, 1992). During the last 15 years, the development of paleoethnobotanical and stable isotope studies in the Antilles has led to a paradigm change in understanding their subsistence strategies, food consumption patterns, and lifeways (Hofman and Antczak, 2019). While traditional narratives characterized these societies as non-agricultural food foragers (Rouse, 1972; 1986; 1992; Tabío and Rey, 1979; Wilson, 2007), new paleobotanical data have demonstrated that they engaged in the production and consumption of domestic plants and cultivars, among other local botanical resources (Newsom, 1993; Burney et al., 1994; Pagán Jiménez et al., 2005; 2015; Rodríguez Suárez, 2007; Mickleburgh and Pagán Jiménez, 2012; Chinique de Armas et al., 2015).

The earliest evidence of cultivars from the Antilles was found at the

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site of St. John, Trinidad, where plants such as maize (*Zea mays*), sweet potatoes (*Ipomoea batatas*), beans (Fabaceae, including *Canavalia* spp.), wild yams (Dioscoreaceae), chili pepper (*Capsicum* spp.), guáyiga (*Zamia* spp.), achira (*Canna* spp.) and possibly arrowroot (*Maranta arundinacea*) were found in tools from archaeological contexts dated between cal 5840 and 3350 BCE (Pagán Jiménez et al., 2015). The analysis of 43 lithic and coral ecofacts/artifacts from Plum Piece in Saba and Maruca, Puerto Ferro and Cueva Clara in Puerto Rico indicated that these ancient populations managed a suite of domestic and wild plants including maize, sweet potatoes, arrowroot, manioc (*Manihot esculenta*), common bean (*Phaseolus vulgaris*), arrowhead (*Sagittaria lancifolia*), bijao (cf. *Renealmia alpinia*) and guáyiga (*Z. portoricensis* and *Z. pumila*) (Pagán Jiménez, 2009). Maize starch grains were also documented in the dental calculus of one individual from Canashito (Aruba) whose remains were associated with a nearby burial dated to cal. BC 350 – 150 CE (Mickleburgh and Pagán Jiménez, 2012).

In Cuba, starch granules of maize, sweet potato, beans, cocoyam (*Xanthosoma* sp.), yam (*Dioscorea* sp.) and guáyiga were identified on eight artifacts from the site of Canímar Abajo on the north coast of Matanzas (Rodríguez Suárez, 2007; Morgado, 2014). These artifacts were associated with archaeological contexts dated to between cal. BCE 3330 – 1290 CE (Roksandic et al., 2015). In addition, starches consistent with guáyiga, common beans, and maize/sweet potatoes were found in the dental calculus of six individuals from this site dated to cal. BC 1380 – 800 (Chinique de Armas et al., 2015). More recently, starch grains of chili pepper, manioc, beans, maize, and possibly sweet potatoes and guáyiga, were found in two artifacts from the Caney del Castillo and Playa del Mango sites in southeast Cuba, associated with contexts of cal. BC 8 – 352 CE and cal. 318 – 429 CE, respectively (Rodríguez Suárez et al., 2020). This represents the earliest evidence of consumption of manioc and chili pepper in Cuba, and suggests that these plants were brought to the east of the archipelago by populations associated with the so called 'Banwaroid tradition' such as the ones from St. John (Trinidad) studied by Pagán Jiménez (2015).

Although there is growing evidence that both C_3 (i.e., sweet potatoes, beans) and C_4 plants (i.e., maize) are ubiquitous in most artifacts associated with these early groups (Chinique de Armas et al., 2019; Pagán Jiménez et al., 2019; Rodríguez Suárez et al., 2020), questions remain about the relative importance of cultigens, in comparison to other resources, and the differential use of plants between populations and individuals. For instance, the intake of C_4 plants, such as maize, is not apparent in the stable isotope values of some populations from western Cuba (Chinique de Armas et al., 2016; 2022; Laffoon and Chinique de Armas forthcoming). In addition, the study of plant microremains in the dental calculus from a living population of well-documented diet demonstrated that starch analysis are best suited for questions regarding the presence or absence of specific plants in the diet (Leonard et al. 2015). Combining stable isotope analysis with the identification of plant microremains has proven potential to reconstruct subsistence strategies and food consumption patterns in the past at a greater resolution than previous approaches (Chinique de Armas et al., 2015). While it is possible to identify a plant taxon by studying its starch grains (Torrence and Barton, 2006), stable isotope analyses provide the isotopic signal of the foods consumed by the individuals, and may indicate the relative importance of plant types (C_3 vs C_4) and animal protein sources (marine vs terrestrial) in their diet (DeNiro and Epstein, 1978; Schoeninger and DeNiro, 1984; Tsutaya and Yoneda, 2015). In this paper, we combine the use of different models based on stable isotope analyses with starch analyses in the dental calculus of Playa del Mango individuals, to reconstruct their dietary practices and evaluate the relative importance of C_3 and C_4 plants in their diet. In addition, the paleodietary reconstruction of Playa del Mango individuals provides us with the first opportunity to directly examine the subsistence strategies and food consumption patterns of individuals traditionally associated with the Banwaroid stone tool tradition in the Greater Antilles (Cruxent and Rouse, 1961; Boomert, 2000; Keegan and Hofman, 2017).

1.1. *The archaeological site of Playa del Mango*

The Playa del Mango site is located in the Cauto River basin within Granma province in Cuba (20°33′14.38′'N, 76°59′08.97′'W) (Fig. 1). It is situated 14 km north of the Gulf of Guacanayabo and 3.5 km east of the lagoon system of Las Playas. The site includes three mounds that cover approximately 60 000 m^2, with Mound 1 and Mound 2, 60 and 40 m in diameter and six and three meters in height, respectively. Mound 2 is adjacent to 'Laguna El Mango' that is likely a relic of an ancient channel of the deltaic system of the Cauto River. Today, although the region has been affected by several anthropogenic processes, it is possible to observe a variety of plants and animals associated with the forest and the swamp woodlands ecosystems.

The site was first excavated by Bernardo Utset Masía, a doctor from Manzanillo and a member of the *Junta Nacional de Arqueología*, who exhumed approximately 40 skeletons in Mound 1 (Utset, 1941). In the early 1980 s, a research team from the Academy of Sciences of Cuba excavated two new areas in Mound 1 and Mound 2, recovering faunal remains that consisted mainly of shells and terrestrial animals such as hutias (Córdoba and Arredondo, 1988) and a diverse set of flaked and ground stone tools (Febles and Godo, 1990). The recovered material culture places the Playa del Mango site within the Banwaroid tradition of the 'Archaic Age' of the Antilles (Rouse, 1992; Keegan and Hofman, 2017). According to the traditional classification systems operating in Cuba, the site would be considered either Ciboney Cayo Redondo (Rouse, 1942; Tabío and Rey, 1979), Preagroalfareros (Tabío, 1984) or 'Fisher-Gatherers': Cultural variant Guacanayabo (Guarch, 1990).

In 2014, a joint Cuban-Canadian project led by Dr. Ulises Miguel González Herrera (Cuban Institute of Anthropology) and Dr. Yadira Chinique de Armas (The University of Winnipeg) resumed excavations at the site. Some isolated human remains (teeth and bones) were found between 2014 and 2015. A funerary area uncovered at the periphery of the Mound 2 was excavated between 2016 and 2018. The 20 primary burials exhumed were all in extended dorsal decubitus position. Excavations of the top of the mound confirmed that domestic activities took place on Mound 2 (food, charcoals, and artifacts). The chronology of burials from the Mound 2 periphery suggests that this funerary area was in use from at least cal. BC 116 to 241 CE (2σ), encompassing the use of the domestic area of the mound [cal. BC 55 – 435 CE (2σ)]. A human tooth from Mound 1 [cal. 125 – 435 CE (2σ)] suggested that the associated cemetery is somewhat later and possibly coexistent with the later formation of Mound 2 (Chinique de Armas et al., 2020).

1.2. *The use of stable isotopes and starch analyses in paleodietary reconstructions*

1.2.1. *Stable isotopes*

Stable isotope analysis of archaeological skeletal remains has become a routine analytical tool for studying paleodiet, since it was first introduced over 40 years ago (Vogel and van der Merwe, 1977). The method is based on the principle that the isotopic signature of someone's diet is reflected in the isotopic signature of his/her tissues, or in other words that 'you are what you eat' (DeNiro and Epstein, 1978). Because of fractionation, however, there is a systematic offset between the isotope values of diet and body tissues, which varies between different tissue types and materials (e.g. collagen, keratin). Isotopic variation between different foods is caused by a multitude of factors, including variable photosynthetic pathways amongst plants (carbon), structural differences between aquatic and terrestrial ecosystems (carbon and nitrogen), and trophic level enrichment (carbon and nitrogen) (DeNiro and Epstein, 1978; 1981; Schoeninger et al., 1983; Schoeninger and DeNiro, 1984). There are three main photosynthetic pathways that result in pronounced differences in plant tissue carbon isotope values ($\delta^{13}C$) (Bender, 1971; Smith and Epstein, 1971; O'Leary, 1981). Most plants use the C_3 metabolic pathway (Calvin-Benson cycle) to fix atmospheric carbon and these have typical $\delta^{13}C$ of −36 to −24 ‰ (mean

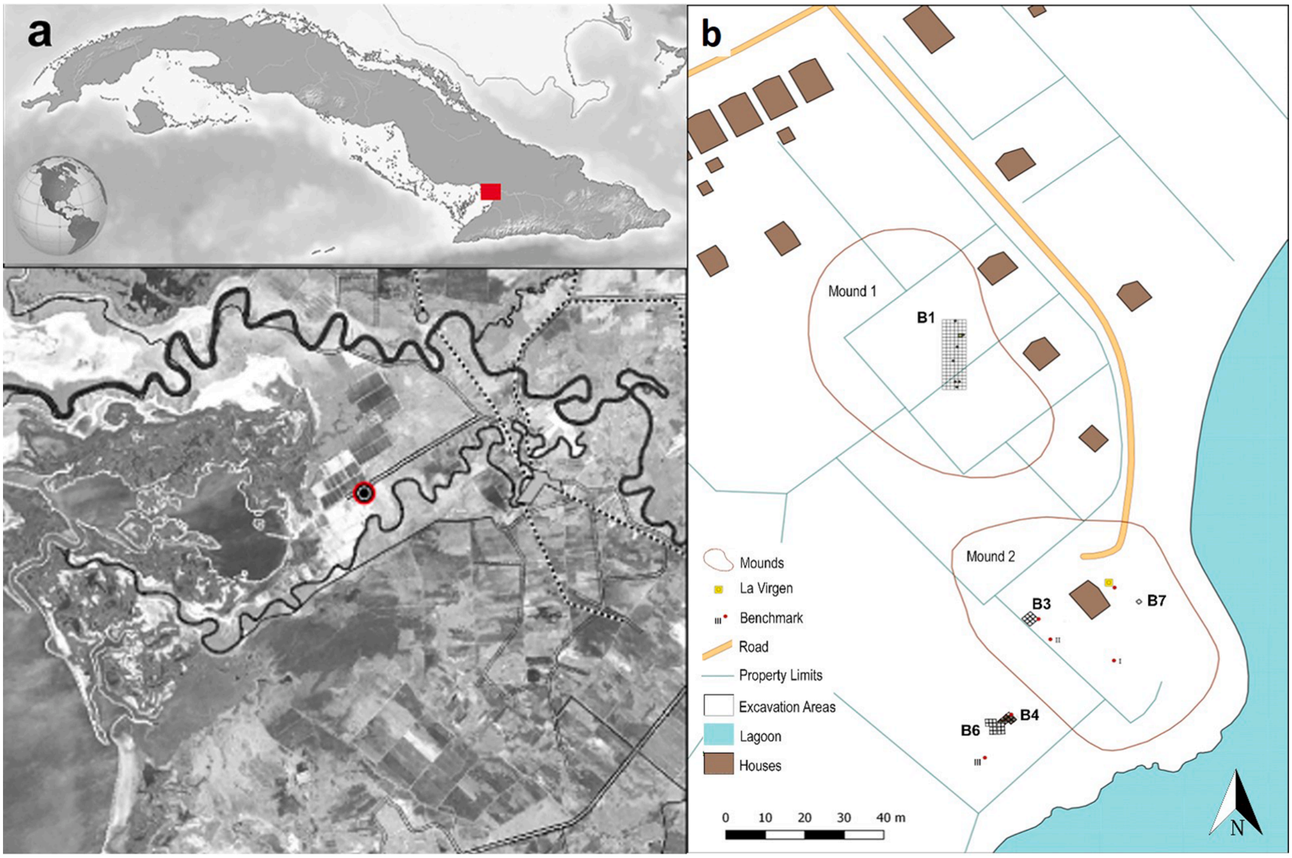

Fig. 1. (a) Geographic location of the archaeological site of Playa del Mango, The Cauto River, Granma province, Cuba. (b) Site plan with Mound 1 and Mound 2.

−26.5 ‰). A small number of plant taxa, such as sedges and some tropical grasses, use C_4 carbon fixation (Hatch-Slack) and have typical $\delta^{13}C$ of −14 to −8‰ (mean −12.5 ‰) (Bender 1971; O'Leary 1981; Smith and Epstein, 1971). Another less common group of plants, primarily succulents, uses a distinct pathway known as CAM (Crassulacean Acid Metabolism), and possesses intermediate to high $\delta^{13}C$ values that often overlap with C_4 plants (Bender et al., 1973). In the Americas, maize was (and is) the most economically important and widespread C_4 crop and probably the only C_4 plant that would have been widely consumed in the pre-colonial circum-Caribbean.

Carbon isotope values also vary between aquatic (marine and freshwater) and terrestrial ecosystems primarily resulting from differences in carbon sources at the base of the food web (DeNiro and Epstein, 1978; Chisholm et al., 1982; Schoeninger et al., 1983; Schoeninger and DeNiro, 1984) such that marine organisms generally possess higher $\delta^{13}C$ than terrestrial organisms. There are also systematic patterns in nitrogen isotope values ($\delta^{15}N$) within and between different ecosystems. In general, terrestrial plants have low $\delta^{15}N$, although some nitrogen-fixing plants, such as legumes, have even lower values. Variation within a food web is primarily the result of trophic level enrichment whereby plants possess the lowest $\delta^{15}N$ values, and these increase by ~ 3–4 ‰ at each step up the food chain between plants, herbivores, and carnivores. (DeNiro and Epstein, 1978; Minagawa and Wada, 1984). Marine ecosystems generally have higher $\delta^{15}N$ than terrestrial ones, owing to two main factors: 1) marine plants have relatively higher $\delta^{15}N$; and 2) marine ecosystems tend to have more trophic levels and thus more enrichment at the top of the food chain (DeNiro and Epstein, 1981; Schoeninger et al., 1983).

Sulphur isotope analysis ($\delta^{34}S$) is a novel method that is used to estimate the aquatic and marine contribution to the weaning process, a distinction that is not apparent in carbon or nitrogen isotope ratios (Nehlich et al., 2011). The $\delta^{34}S$ values of tissue protein reflect those of dietary protein, as sulphur in animal tissue protein exists in the form of methionine and cysteine amino acid residues whose proportions differ in mammals (Tanz and Schmidt, 2010; Tcherkez and Tea, 2013). The $\delta^{34}S$ of animals from marine ecosystems cluster between + 17 ‰ and + 21 ‰ (VCDT) while in terrestrial ecosystems they can vary from + 22 to −22 ‰ because of the wide range of $\delta^{34}S$ values in terrestrial organic matter (Peterson and Fry, 1987; Krouse et al., 1991).

1.2.2. *Starch analysis*

Starch analysis provides insights into the use of plants by populations in the past (Lieverse, 1999; Hardy et al., 2009). These microscopic grains are energy molecules stored within the cells of some plant types (Torrence and Barton, 2006), and an important source of carbohydrates for humans (Laden and Wrangham, 2005; Hardy et al., 2015). The grains vary widely in both size and shape among plant taxa, and are often identifiable at several taxonomic levels, including sometimes genus and species (Torrence and Barton, 2006). Grains can be found in multiple contexts, including the cracks and fissures of stone tools, food-holding vessels (Ugent et al., 1982; Piperno and Holst, 1998; Piperno et al., 2000; Berman and Pearsall, 2020) and human dental calculus (Hardy et al., 2009; Piperno and Dillehay, 2008; Mickleburgh and Pagán Jiménez, 2012; Chinique de Armas et al., 2015; Barton and Torrence, 2015). While samples from tools may represent non-dietary plants, or exclude unprocessed vegetable resources, starch from within dental calculus is unique since it directly places the plant within an individual's mouth (Hardy et al., 2009; Barton and Torrence, 2015).

Dental calculus is formed when calcium and phosphorus, found within oral fluid, precipitates out of solution and binds with the biofilms sustained on teeth during life (Leiverse, 1999). As this process occurs slowly, particles of food, and other detritus within the mouth can become trapped in the growing matrix (Henry and Piperno, 2008). Once these deposits harden, these micro-botanicals are better protected from

Table 1
General information of the Playa del Mango (Granma Cuba) individuals included in this study.

ID	Block/Grid	Level (m)	Age	Sex	Chronology
PM2_E-1	B4/C-5; C-14; C-8	0.10 – 0.25	Adult	M	AD 48–235
PM2_E-2	B4/C-5; C-8	0.10 – 0.25	Adult	F (XX)	AD 15–225
PM2_E-3	B4/C-11; C-10	0.10 – 0.25	Adult	M (XY)	BC 116 - AD 75
PM2_E-4	B4/C-11; C-4	0.10 – 0.25	Adult	F	BC 52 - AD 58
PM2_E-5	B4	0.20	Adult	M	n/a
PM2_E-6	B4	0.15	Non-adult	F (XX)	n/a
PM2_E-7	B6/C-I; C-VI	0.10 – 0.25	Adult	M	AD 57–177
PM2_E-8	B6/C—H; C-K	0.10 – 0.20	Non-adult	I	n/a
PM2_E-9	B6/C—H; C-K	0.10 – 0.20	Adult	F (XX)	BC 114 - AD 25
PM2_E-10	B6/C-J; C-III	0.10 – 0.25	Adult	M (XY)	AD 79–216
PM2_E-11	B6	0.10 – 0.25	Adult	M	n/a
PM2_E-12	B6/C-III; C-G	0.10 – 0.25	Adult	M	AD 15–91
PM2_E-13	B6/C-F; C-VI	0.10 – 0.25	Adult	M	AD 127–241
PM2_E-14	B6/C-III	0.10 – 0.20	Adult	M (XY)	AD 47–140
PM2_E-15	B6/C-J	0.10 – 0.20	Adult	F (XX)	BC 3 - AD 81
PM2_E-18	B6	0.10 – 0.20	Non-adult	I	n/a
PM2_E-19	B6/C-III	0.10 – 0.20	Adult	M	AD 23–135
PM1_M1	B1	Level 1	Adult	I	n/a
PM1_M2	B1	Level 1	Adult	I	n/a
PM1_M43	B1	Level 1	Adult	I	n/a
PM1_M46	B1	Level 1	Adult	I	n/a
PM1_R4	B1/R4	Level 1	Adult	I	n/a
PM1_C-E5	B1/E5	Level 1	Adult	I	n/a
PM1_C-T4	B1/T4	Level 2	Adult	I	n/a

both the salivary amylase during life and taphonomic processes after death (Henry and Piperno, 2008; Mickleburgh and Pagán Jiménez, 2012). However, factors controlling the rate and intensity of calculus growth are poorly understood (Lieverse, 1999). Consequently, the ubiquity or amount of starches identified in a sample cannot directly indicate the frequency of starches consumed by individuals or populations (Weslowski et al., 2010; Leonard et al. 2015; Radini et al., 2016; Mariotti Lippi et al., 2017). These limitations can be overcome by combining the identification of starches with stable isotope analysis of the individual's tissues. Applied together, these two methods provide much more detail and a higher resolution in the reconstruction of dietary practices of ancient populations.

2. Materials and methods

2.1. The sample

Human samples from 27 individuals excavated by the team at Playa del Mango were processed for stable isotope analysis (Table 1). Bone collagen from four adult individuals from Mound 1, and 17 individuals from Mound 2 (14 adults and 3 non-adults), were processed for carbon ($\delta^{13}C_{co}$) and nitrogen ($\delta^{15}N$). Within this sample, $\delta^{13}C_{ap}$ (carbon from apatite) was quantified in four individuals from Mound 1 and 14 from Mound 2. Additionally, eight individuals were analyzed for sulfur ($\delta^{34}S$) (four for each mound). A total of 25 tooth enamel samples from 15 individuals were studied for $\delta^{13}C_{en}$ (Mound 1: 5 individuals; Mound 2: 10 individuals, 20 teeth). In order to exclude isotope values possibly reflecting bone collagen diagenetic alterations, only samples with minimum carbon and nitrogen concentrations of 13 % and 4.8 %, respectively (Ambrose, 1990) and whose C/N ratio was within the range of 2.9 to 3.6 (DeNiro, 1985) were considered in this study. A strong positive linear correlation was observed between wt. % C and wt. % N ($R^2 = 0.9551$) as would be expected for well-preserved collagen. Additionally, dental calculus from six individuals (22 % of the total sample) was available to conduct starch grain analysis (Table 1).

Sex was assessed for Mound 2 individuals by using established morphometric methods for pelvic, cranial, and postcranial remains (Buikstra and Ubelaker, 1994; Bruzek, 2002). A genetic study of some of those individuals (Nägele et al., 2020) confirmed the sex assessment in 100 % of cases (Table 1), which indicates a high level of confidence in the sex estimations when using morphometric methods. Five females and 10 males were included in this study (Table 1). Only isolated teeth and bones were recovered from Mound 1, making it impossible to assess the sex of the individuals.

Samples were divided in two general categories: adults and non-adults, based on dental eruption and developmental/senescence stage of the postcranial bones (Roksandic and Armstrong, 2011). In order to examine differential food consumption based on age we analyzed longitudinal as well as cross-sectional data of carbon from enamel. Enamel is formed during childhood and it does not undergo further turnover once it is fully formed (Nanci, 2013), and thus it retains the isotopic and elemental dietary signals acquired during growth and development. The ages at the start and end of enamel formation differ among tooth types, and in deciduous teeth (Hillson, 1996; Reid and Dean, 2006; Reid et al., 2008). In this study, $\delta^{13}C_{en}$ from incisors (I) and first permanent molars (M1) were considered to better represent the isotopic signals of infancy since their formation starts roughly at birth and ends between 3 and 4 years of age (Hillson, 1996; Reid and Dean, 2006). On the other hand, second molars (M2) and premolars (PM) were considered to better represent stable isotope values during childhood since their crowns form roughly between 2 and 8 years (PM1: 2–3 yrs. – 6 yrs., M2: 2–3 yrs.–7yrs) and third permanent molars (M3) are expected to represent adolescence since their formation completes between seven and 16 years (Hillson, 1996).

2.2. Stable isotopes analysis

After collagen extraction for radiocarbon dating of Playa del Mango individuals (Chinique de Armas et al., 2020), samples from nine individuals were sent to the Ján Veizer Stable Isotope Laboratory (formerly the G.G. Hatch Stable Isotope Laboratory) at the University of Ottawa in Canada, to be analyzed for $\delta^{13}C_{co}$, $\delta^{15}N$ and $\delta^{34}S$ stable isotopes. The other collagen, apatite and enamel samples were processed, by the first and last author of the paper, at the Faculty of Archaeology, Leiden University and analyzed at the Stable Isotope Lab of the Free University Amsterdam. These included two of the individuals previously sent to the Ján Veizer Lab to control for possible inter-laboratory variations. Previous research has demonstrated only minimal inter-laboratory differences in collagen stable isotope values (Pestle et al. 2014). In this study, absolute differences were also negligible (0.70 and 0.59 ‰ for $\delta^{13}C_{co}$; −0.49 and −0.05 ‰ for $\delta^{15}N$).

Samples sent to the Ján Veizer Stable Isotope Laboratory followed the Longin (1971) collagen extraction method and the protocol outlined in Crann et al. (2017). The samples were decalcified with 0.5 N hydrochloric acid (HCl) until translucent. Then, they were treated with 0.1 N sodium hydroxide (NaOH) to remove humic acids during 30 min at room temperature, and 0.5 N HCl again (30 min, room temperature) to remove any CO_2 absorbed during the base wash. Every step was followed by three rinses with Milli-Q water. The samples were gelatinized in a pH 3 solution at 60 °C overnight, filtered using a cleaned glass Whatman autovial syringeless filter and freeze-dried.

Collagen samples were weighed into tin capsules. Calibrated internal standards were prepared with every batch of samples for normalization of the data. The isotopic composition of collagen carbon and nitrogen was determined by the analysis of CO_2 (and N_2) produced by combustion on an Elementar VarioEL Cube Elemental Analyser followed by "trap and purge" separation and on-line analysis by continuous-flow with a DeltaPlus Advantage isotope ratio mass spectrometer coupled with a ConFlo III interface. The routine precision of the analyses is 0.2 ‰ for both carbon and nitrogen, VPDB and AIR, respectively. The isotopic composition of collagen sulfur was determined by an Isotope Cube autosampler coupled with a Thermo Finnigan DeltaPlus XP IRMS via a Conflo IV for analysis. The routine precision of the sulfur analyses is 0.4 ‰, VCDT.

Samples measured at the Stable Isotope Lab, Faculty of Science, Free University Amsterdam followed standard analytical protocols for stable isotope analysis of archaeological collagen and enamel, and these are described in greater detail elsewhere (Laffoon et al., 2013; 2016). Samples of cortical bone were mechanically cleaned and manually crushed with a pre-cleaned mortar and pestle. For collagen samples, processing and preparation for stable isotope analysis followed a modified version of the Longin (1971) method (Brown et al., 1988). Crushed bone was demineralized in 0.6 M HCl at 4° C with the acid being refreshed every 2–3 days. After thorough rinsing in DDI-H_2O (distilled, deionized water), samples were treated in 0.125 M NaOH for 20 h to remove potential organic contaminants and then again rinsed to neutral with DDI-H2O. The extracted collagen was then gelatinized in 0.001 M HCl at 80 °C for 24–48 h, filtered with Ezee filters (Elkay©), frozen, and then freeze-dried.

For apatite and enamel isotope analysis, sample processing was done according to the protocol reported in Bocherens et al. (2011). Bone/enamel fragments were mechanically cleaned with a hand-held drill mounted with a diamond-tipped drill bit and then rinsed with DDI-H2O. Rinsed samples were then chemically oxidized in pre-cleaned vials for 4 h with 2.5 % sodium hypochlorite (NaOCl), rinsed to neutral with DDI-H2O, placed in 0.1 M calcium-acetate buffered acetic acid (CH_3COOH) solution for 4 h, rinsed to neutral again, and dried down.

Collagen isotope measurements were conducted on a Thermo Quest Delta XP IRMS plus connected to a Flash Elemental Analyzer. International standards USGS40, USGS41, and USGS42 were used for sample calibration. Enamel and bone apatite isotope measurements were conducted on a Finnigan Delta Plus IRMS, connected to a GasBench II universal automated interface. The international reference material (NBS19) was used to monitor long-term reproducibility for $\delta^{13}C$ (<0.1 ‰). Carbon and nitrogen isotope results are reported in parts per thousand or permil (‰), in the δ notation, relative to the international VPDB and AIR standards, respectively. Typical analytical uncertainty for collagen carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is 0.2 ‰, and for apatite carbon ($\delta^{13}C$) is < 0.15 ‰.

2.3. Starch analyses

Dental calculus samples were extracted from six individuals (three adult males, one adult female, one non-adult female and an isolated tooth) from Playa del Mango and analyzed for starch microremains. The protocol for starch granule recovery was adapted from Mickleburgh and Pagán Jiménez (2012). Dental calculus was removed with a sterile razor blade. Samples were demineralized in 1 M HCl over a 24-hour period, or until calculus had dissolved. The resultant solution was agitated, diluted with distilled water, and centrifuged at 4500 rpm for 3 min. The solution was then reduced and a drop of glycerol was mixed into the samples to increase the viscosity of the media. This facilitates the rotation of recovered starches, aiding in their identification. Blanks were processed along with the samples to control for possible contamination during the laboratory procedure. Additionally, samples from all materials and instruments used during the sample processing, and water tramps placed in different places of the lab, were examined for starch grains. No modern starches were present.

Samples were observed at 400X magnification under transmission light microscopy, both with and without a polarising filter. Recovered granules were photographed and described with respect to the following features: size, shape, extinction cross location and shape, hilum location, presence of lamellae, presence and shape of fissures, granule border characteristics, and presence of pressure facets (Reichert, 1913; Pagán Jiménez, 2007). All tentative and secure granule identifications were based upon comparative material of the reference collection at the University of Winnipeg and the Laboratory of Archaeometry of the Cuban Institute of Anthropology (including references of different members of the order of all taxonomic plants identified). Other published reference collections (Pagán Jiménez, 2007; 2015; Sívoli et al., 2009) and archaeological starch grains were taken into account.

2.4. Mixing models and statistical analysis

We applied tissue-specific discrimination factors to the collagen and apatite isotope values in order to estimate the isotope values of food sources. Here we combined the formula for estimating $\delta^{13}C$ of dietary protein from Pestle et al. (2014): $\delta^{13}C_{protein}$ (‰) = ($0.78 \times \delta^{13}C_{co}$) – ($0.58 \times \Delta^{13}C_{ap\text{-}co}$) – 4.7 ($r^2 = 0.86$) and the formulas for estimating $\delta^{13}C$ from energy sources and whole diet published by Kellner and Schoeninger (2007): $\delta^{13}C_{energy}$ (‰) = ($1.1 \times \delta^{13}C_{ap}$) – 8.4 ($r^2 = 0.59$); $\delta^{13}C_{diet}$ (‰) = ($1.04 \times \delta^{13}C_{ap}$) – 9.2 ($r^2 = 0.97$).

The $\delta^{13}C_{co}$ and $\delta^{13}C_{ap}$ values were plotted against the model regression lines proposed by Froehle et al. (2010) to differentiate C_3 and C_4 protein sources when controlling by protein diet type. In addition, we combined $\delta^{13}C_{co}$, $\delta^{13}C_{ap}$ and $\delta^{15}N$ values using the multivariate model proposed by Froehle et al. (2012). For that, we calculated the discriminant function scores [Carbon: Carbon F1 = ($0.322 \times \delta^{13}C_{ap}$) + ($0.727 \times \delta^{13}C_{co}$) + ($0.219 \times \delta^{15}N$) + 9.354; Nitrogen: F2 = ($-0.393 \times \delta^{13}C_{ap}$) + ($0.133 \times \delta^{13}C_{co}$) + ($0.622 \times \delta^{15}N$) – 8.703] for all individuals where the three variables ($\delta^{13}C_{co}$, $\delta^{13}C_{ap}$ and $\delta^{15}N$) were available and plotted them against the clusters obtained by Froehle et al. (2012).

In order to estimate the relative importance of food sources in individuals diets, and the probabilistic variations in dietary practices between infants, children, adolescents and adults, the Bayesian mixing model MixSIAR (3.1.12) (Stock and Semmens, 2013; 2017) was used to account for both process and residual sampling errors that can arise due to consumers sampling food sources from different locations, and any inherent isotopic variability among consumers, respectively (Stock and Semmens, 2016). We included into the model the sources that had been previously identified as potential food for adults and non-adults in the region (Pestle, 2010; Mickleburgh and Pagán Jiménez et al., 2012; Pagán Jiménez et al., 2015; Chinique de Armas et al., 2015; 2017; Chinique de Armas and Pestle, 2018). The isotopic composition of Caribbean food sources (flora and fauna), and their concentration dependencies, were taken from Pestle (2010) and cited literature (Table 2). Differences between protein sources of diet and collagen ($\Delta^{13}C_{col\text{-}prot}$), were estimated from the difference between collagen values of samples and their calculated $\delta^{13}C$ of dietary protein ($\Delta^{13}C_{col\text{-}prot} = 4.49 \pm 0.47$ ‰). Differences between sources rich in carbohydrates and collagen were calculated from the difference between collagen values of the samples and their calculated $\delta^{13}C_{diet}$ ($\Delta^{13}C_{col\text{-}diet} = 3.30 \pm 0.91$ ‰). $\delta^{13}C_{ap}$ and $\delta^{13}C_{en}$ values were adjusted according to the most recent estimate of the offset between whole diet and bioapatite for humans:

Table 2

Isotopic values and standard deviations (‰ Air for nitrogen; VPDB for carbon) for probable source diet components used in the MixSIAR calculations for Playa del Mango individuals. Source isotopic compositions and concentration dependencies were taken from Pestle (2010) and cited literature. Estimated diet-col fractionation offsets for carbon (VPDB for carbon) are based on the difference between collagen and the calculated $\delta^{13}C_{protein}$ and $\delta^{13}C_{diet.}$ The $\Delta\delta^{15}N_{diet\text{-}col}$ and $\Delta\delta^{13}C_{diet\text{-}ap}$ were taken from Ambrose (2000) and Fernandes et al. (2012), respectively.

Source	Source Isotopic Compositions (‰)				Concentration Dependences (%)				Diet-Collagen Fractionations (‰)					
	$\delta^{15}N$	**Sd**	**$\delta^{13}C$**	**Sd**	**N**	**Sd**	**C**	**Sd**	**$\Delta\delta^{15}N_{diet\text{-}col}$**	**Sd**	**$\Delta\delta^{13}C_{diet\text{-}col}$**	**Sd**	**$\Delta\delta^{13}C_{diet\text{-}ap}$**	**Sd**
Mol	4.2	3.7	−13.0	4.6	10.9	2.9	39.7	5.3	3.6	1.2	4.5	0.5	–	–
MFish[a]	8.4	2.94	−11.3	2.9	15.01	1.0	45.0	1.0	3.6	1.2	4.5	0.5	–	–
ERFish	9.4	1.70	−16.3	2.6	13.28	1.2	47.8	2.5	3.6	1.2	4.5	0.5	–	–
TAnim	5.9	2.44	−21.6	2.6	13.16	0.8	44.9	0.6	3.6	1.2	4.5	0.5	–	–
Maize	3.3	1.63	−9.8	0.7	7.12	0.2	42.0	0.4	3.6	1.2	3.3	0.9	10.1	0.4
RC[b]	3.8	1.51	−22.4	1.2	1.09	0.1	41.4	1.3	3.6	1.2	3.3	0.9	10.1	0.4
Leg	2.2	1.16	−22.9	0.8	3.82	0.3	41.8	0.4	3.6	1.2	3.3	0.9	10.1	0.4
Zamia	−0.6	0.24	−25.6	0.9	1.5	0.7	42.6	3.4	3.6	1.2	3.3	0.9	10.1	0.4
TFruits			−23.1	3.5			43.46	4.50	3.6	1.2	3.3	0.9	10.1	0.4
BMilk	5.1	0.2	−22.5	1.1	49.13	3.63	1.61	0.27	5.5	0.7	4.4	0.1	10.1	0.4

[a] Includes Fish (2, 3 carnivores and reef fish).

[b] Root Cultigens and legumes were also analyzed in combination by averaging original values.

+10.1 ± 0.4 ‰ (Fernandes et al., 2012). To account for the difference in $\delta^{15}N$ between diet and collagen $\Delta^{15}N_{col\text{-}diet}$, we applied the value of 3.6 ± 1.2 ‰ as reported by Ambrose (2000).

Since samples were normally distributed, we used a T-test (t) to compare carbon and nitrogen isotopic means between samples from Mound 1 and Mound 2, and between adult female and male individuals from Mound 2. To compare more than two samples, one-way ANOVA (F) with a Tukey-Kramer post-hoc test was used for normally distributed samples. When the sample did not fit the normal distribution, we used a Kruskal Wallis (H) with a Dunn test. Statistical significance was set at $\alpha = 0.05$ for all tests performed.

3. Results

3.1. Stable isotopes

Stable isotope results are summarized in Table 3. Collagen carbon isotope values of Mound 1 and Mound 2 adults range between −18.6 ‰ and −17.3 ‰ ($\delta^{13}C_{co}$ mean: −18.2 ± 0.7 ‰) and −18.9 ‰ and −15.9 ‰ ($\delta^{13}C_{co}$ mean: −17.8 ± 1.0 ‰), respectively. Stable isotope nitrogen values of adult individuals from Mound 1 were between 9.7 ‰ and 10.7 ‰ ($\delta^{15}N$ mean: 10.1 ± 0.5 ‰), while in Mound 2 they had values between 8.4 ‰ and 9.6 ‰ ($\delta^{15}N$ mean: 9.1 ± 0.4 ‰). Carbon apatite values of Mound 1 adults were between −12.0 ‰ and −11.3 ‰ ($\delta^{13}C_{ap}$ mean: −11.7 ± 0.3 ‰) and between −13.2 ‰ and −9.6 ‰ ($\delta^{13}C_{ap}$ mean: −11.4 ± 1.0 ‰) for adults from Mound 2. No statistically significant variations were found between adult individuals from the two funerary areas for $\delta^{13}C_{co}$ ($t = -0.79$, $df = 3.67$, $p = 0.48$), $\delta^{13}C_{ap}$ ($t = 3.09$, $df = 2.46$, $p = 0.07$) or $\delta^{15}N$ ($t = -1.00$, $df = 15.52$, $p = 0.33$). Three individuals from Mound 1 had a $\delta^{34}S$ average of 5.5 ± 1.0 ‰ (ranging from 4.4 to 6.1 ‰).

Non-adults from Mound 2 had $\delta^{13}C_{co}$ and $\delta^{13}C_{ap}$ isotope values ranging between −18.1 ‰ and −17.0 ‰ ($\delta^{13}C_{co}$ mean: −17.7 ± 0.6 ‰) and −12.5 ‰ and −9.3 ‰ ($\delta^{13}C_{ap}$ mean: −10.9 ± 2.2 ‰), respectively. Non-adult nitrogen values were found to be between 8.1 ‰ and 13.0 ‰ ($\delta^{15}N$ mean: 10.2 ± 2.5 ‰). Carbon enamel values ($\delta^{13}C_{en}$) of adult teeth formed during infancy were between −13.1 ‰ and −10.0 ‰ for Mound 1 individuals ($\delta^{13}C_{en}$ mean: −11.9 ± 0.4 ‰) and between −13.6 ‰ and −10.9 ‰ ($\delta^{13}C_{en}$ mean: −12.3 ± 1.0 ‰) in those from Mound 2. Tooth enamel of Mound 1 samples formed during childhood and adolescence averaged −10.5 ± 0.1 ‰ and −12.1 ± 2.3 ‰ ($\delta^{13}C_{en}$: max: −10.5 ‰, min:-13.7 ‰), respectively, while Mound 2 children and adolescents had values of −12.8 ± 0.7 ‰ ($\delta^{13}C_{en}$range: from −13.6 ‰ to −12.3 ‰) and −12.5 ± 0.6 ‰ ($\delta^{13}C_{en}$ range from −13.3 ‰ to −11.6 ‰), in that order. No statistically significant variations were found between age ranges (Infancy vs Childhood vs Adolescence) for $\delta^{13}C_{en}$ isotope values ($F = 0.14$, $df = 2$, $p = 0.87$).

Within Mound 2, no statistically significant differences were found between adult females ($\delta^{13}C_{co}$ mean: −17.4 ± 1.1 ‰) and males ($\delta^{13}C_{co}$ mean: −17.7 ± 1.1 ‰) for $\delta^{13}C_{co}$ ($t = 0.86$, $df = 4.84$, $p = 0.43$). In contrast, statistically significant differences were found in $\delta^{13}C_{ap}$ (F mean: −10.4 ± 0.7 ‰; M mean: −11.9 ± 0.7 ‰) and $\delta^{15}N$ (F mean: 8.6 ± 0.2 ‰; M mean: 9.3 ± 0.2 ‰) isotope values between females and males ($\delta^{13}C_{ap}$: $t = 3.63$, $df = 5.85$, $p = 0.01$; $\delta^{15}N$: $t = -5.19$, $df = 5.76$, $p = 0.00$) (Fig. 2: Row A). No differences by sex were found in tissues formed during infancy ($t = 1.02$, $df = 4.85$, $p = 0.35$) and adolescence ($t = 0.41$, $df = 2.93$, $p = 0.71$) for $\delta^{13}C_{en}$. No more than three samples per category (female and male) were available for comparisons between children.

The estimated value of $\delta^{13}C_{diet}$ was −21.4 ± 0.3 ‰ for Mound 1 individuals (ranging between −21.7 ‰ and −21.0 ‰) (Table 3). In the case of Mound 2 adults, the $\delta^{13}C_{diet}$ had a mean value of −21.1 ‰ ± 1.0 (min: –22.9 ‰, max: −19.2 ‰). According to the models used, Mound 1 individuals had diets with a mean $\delta^{13}C_{energy}$ of −21.3 ± 0.3 ‰ (min: −21.6 ‰, max: −20.8 ‰) while Mound 2 adults exhibited a mean value of −21.0 ± 1.1 ‰ (ranging from –22.9 ‰ to −19.3 ‰). In terms of the carbon composition of the protein portion of diet, Mound 1 individuals have a mean value of −22.6 ± 1.0 ‰ ($\delta^{13}C_{protein}$ min: –23.4 ‰, max: −21.5 ‰) while Mound 2 adults had a mean value of –22.2 ± 1.1 ‰ (ranging from −23.8 ‰ to −20.1 ‰). Carbon isotopic values of diet, dietary energy and protein sources portion of diet in Mound 2 non-adults had average values of −20.5 ± 2.3 ‰ ($\delta^{13}C_{diet}$ min: −22.7 ‰, max: −18.9 ‰), −20.4 ± 2.4 ‰ ($\delta^{13}C_{energy}$ min: –22.1 ‰, max: –18.6 ‰) and –22.3 ± 0.2 ‰ ($\delta^{13}C_{protein}$ min: –22.4 ‰, max: –22.1 ‰), respectively (Table 3). No statistically significant differences were found for the carbon composition of the whole diet ($t = -1.00$, $df = 15.47$, $p = 0.33$), energy ($t = -1.00$, $df = 15.51$, $p = 0.33$) and protein portions ($t = -0.65$, $df = 3.21$, $p = 0.56$) between Mound 1 and Mound 2 individuals (Fig. 2: Row B). However, statistically significant differences were found between males and females for the carbon composition of diet ($t = 3.65$, $df = 5.88$, $p = 0.01$) and its energy portion ($t = 3.65$, $df = 5.90$, $p = 0.01$). The protein portion of diet ($t = -0.22$, $df = 5.36$, $p = 0.83$) had similar mean carbon isotopic values for both females and males (Fig. 2: Row C).

Playa del Mango data plots are closer to the C_3 protein line (100 % C_3 diet) in the model of Froehle et al. (2010) although some individuals are out of the 95 % credibility intervals line (Fig. 3A). In addition, Playa del Mango individuals fall within Cluster 4 of Froehle et al. (2012) model. This cluster is consistent with populations with a 70:30 C_3:C_4 diet with at least 65 % C_3 protein (Fig. 3B) where C_3-based sources were likely consumed (Fig. 3C). One of the non-adults showed higher $\delta^{15}N$ values than adults (Fig. 3C).

The MixSIAR mixing model, combining carbon and nitrogen stable isotopes, suggested that root cultigens, terrestrial animals, and legumes had a major contribution to most individuals' diets (median between 14 %, and 35 %) (Table 4). The mode for root cultigens is notably high in

Table 3
Stable isotope values of the Playa del Mango (Granma, Cuba) individuals (‰ Air for nitrogen; VPDB for carbon).

ID	$\delta^{13}C_{co}$ (‰)	$\delta^{15}N$ (‰)	C (%)	N (%)	C/N	$\delta^{13}C_{ap}$ (‰)	$\delta^{34}S$ (‰)	$\delta^{13}C_{diet}$ (‰)	$\delta^{13}C_{energy}$ (‰)	$\delta^{13}C_{protein}$ (‰)	Teeth	$\delta^{13}C_{en}$ (‰)
PM2_E-1	−18.4 −18.6[a]	9.2 9.8[a]	40.7 45.3	15.0 45.3	3.2 3.4	–	–	–	–	–	M1 M3	−12.2 −12.7
PM2_E-2	−18.6	8.4	24.8	9.2	3.2	−10.8	–	−20.4	−20.2	−23.8	M1 M3	−12.3 −13.3
PM2_E-3	−16.1	9.5	43.4	15.2	3.3	−11.1	–	−20.7	−20.6	−20.1	M1 M2 M3	−11.7 −12.6 −11.9
PM2_E-4	−17.6	8.9	21.9	8.1	3.2	−11.1	–	−20.8	−20.6	−22.2	–	–
PM2_E-5	−18.2	9.2	26.2	9.5	3.2	–	–	−21.4	−21.3	−22.6	–	–
PM2_E-6	−18.1	13.0	39.5	14.1	3.3	−12.5	–	−22.1	−22.1	−22.1	m2 M1	−13.6 −13.8
PM2_E-7	−16.7 −17.3[a]	9.6 9.6[a]	36.6 27.0	13.3 9.9	3.2 3.2	−11.6	–	−20.8	−20.6	−21.0	–	–
PM2_E-8	−17.0	8.1	32.2	11.5	3.3	−9.3	–	−18.9	−18.6	−22.4	–	–
PM2_E-9	−15.9	8.6	25.3	9.2	3.2	−9.6	–	−19.2	−19.0	−20.8	M1 M3	−10.9 −11.6
PM2_E-10	−18.9	9.0	32.1	11.7	3.2	−13.2	–	−22.9	−22.9	−22.7	M1	−13.6
PM2_E-11	−18.6	9.2	40.3	15.0	3.1	−11.8	–	−21.4	−21.3	−23.2	M1 M3	−11.9 −12.7
PM2_E-12	−17.4	9.3	32.5	11.9	3.2	−12.4	–	−22.0	−22.0	−21.2	–	–
PM2_E-13	−18.9	9.4	36.6	13.4	3.2	−12.2	–	−21.9	−21.9	−23.3	M2	−13.6
PM2_E-14	−18.2	9.0	29.5	10.8	3.2	−11.2	–	−20.9	−20.8	−22.9	M2 M3	−12.3 −12.97
PM2_E-15	−17.3	8.4	31.4	11.5	3.2	−9.9	–	−19.5	−19.3	−22.5	M1 I M3	−12.0 −12.5 −12.3
PM2_E-18	−18.0	9.6	41.0	16.0	3.0	–	–	–	–	–	–	–
PM2_E-19	−18.4	9.0	33.5	12.3	3.2	−12.4	–	−22.1	−22.0	−22.5	–	–
Total Adult Mean (SD)	**−17.8** **(1.0)**	**9.1** **(0.4)**	32.5 (6.5)	11.9 (2.3)	3.2 (0.1)	**−11.4** **(1.0)**		**−21.1** **(1.0)**	**−21.0** **(1.1)**	**−22.2** **(1.1)**	**Inf.** **Chil.** **Juv.**	**−12.3** **(1.0)** **−12.8** **(0.7)** **−12.5** **(0.6)**
Max/Min	**−15.9/ −18.9**	**9.6/ 8.4**	43.4/ 21.9	15.2/ 8.1	3.3/ 3.1	**−9.6/ −13.2**		**−19.2/ −22.9**	**−19.0/ −22.9**	**−20.1/ −22.4**		
FemaleMean (SD)	**−17.4** **(1.1)**	**8.6** **(0.2)**	–	–	–	**−10.4** **(0.7)**		**−20.0** **(0.7)**	**−19.8 (0.7)**			
MaleMean (SD)	**−17.7** **(1.1)**	**9.3** **(0.2)**	–	–	–	**−11.9** **(0.7)**		**−21.6** **(0.7)**	**−21.5 (0.7)**			
Non-adultMean (SD)	**−17.7** **(0.6)**	**10.2** **(2.5)**	37.5 (4.7)	13.9 (2.2)	3.2 (0.2)	**−10.9** **(2.2)**		**−20.5** **(2.3)**	**−20.4** **(2.4)**	**−22.3** **(0.2)**		
Max/Min	**−17.0/ −18.1**	**13.0/ 8.1**	41.0/ 32.2	16.0/ 11.5	3.3/ 3.0	**−9.3/ −12.5**		**−18.9/ −22.1**	**−18.6/ −22.1**	**−21.5/ −23.4**		
PM1_M1	−17.3	9.8	41.4	14.7	3.3	–	5.9	−21.3	−21.2	−21.5	–	–
PM1_M2	−19.0	10.6	41.2	15.0	3.2	−12.0	4.4	−21.7	−21.6	−23.0	–	–
PM1_M43	–	–	–	–	–	–	–	–	–	–	M1	−13.1
PM1_M44	–	–	–	–	–	−12.0	–	−21.6	−21.6	–	–	–
PM1_M46	−18.6	9.7	23.9	7.9	3.6	−11.3	–	−21.0	−20.8	−23.4	–	–
PM1_R4	–	–	–	–	–	–	–	–	–	–	M3	−13.7
PM1_CE5	–	–	–	–	–	−11.7	–	−21.4	−21.3	–	M1	−12.5
PM1_CT4	–	–	–	–	–	–	6.2	–	–	–	M3	−10.7
PM1_eT4	–	–	–	–	–	–	–	–	–	–	PM1	−10.5
Total Mean (SD)	**−18.2** **(0.7)**	**10.1** **(0.5)**	35.5 (10.0)	12.5 (4.0)	3.4 (0.2)		**5.5** **(1.0)**	**−21.4** **(0.3)**	**−21.3** **(0.3)**	**−22.6** **(1.0)**	**Inf.** **Chil.** **Juv.**	**−11.9** **(0.4)** **−10.5** **(0.1)** **−12.1** **(2.3)**
Max/Min	**−17.3/ −18.6**	**10.7/ 9.7**	41.4/ 23.9	15.0/ 7.9	3.6/ 3.2		**6.2/ 4.4**	**−21.0/ −21.7**	**−20.8/ −21.6**	**−21.5/ −23.4**		

[a] Samples processed for $\delta^{13}C_{co}$ and $\delta^{15}N$ at the Hatch Lab, University of Ottawa. All the other samples were processed at the Free University Amsterdam.

Mound 2 males with a median value representing 34.9 % (Table 4, Fig. 4: Row A). In contrast, the protein contribution of legumes for Mound 2 females has a median value representing 35 % (Table 4, Fig. 4: Row A). Mound 1 individuals' diets showed a higher contribution of mollusks and marine fish than Mound 2 individuals, especially males. The other resources showed lower median percentage values (Table 4; Fig. 4: Row A).

The MixSIAR model, using $\delta^{13}C_{ap,}$ showed less variations between the probabilistic proportions of the different dietary carbohydrate sources (Fig. 4: Row B). C_3 plants, especially root cultigens and *Zamia*, showed higher probabilistic values than C_4 plants (Table 5). In the case of the females from Mound 2, maize seems to have had a contribution similar to root cultigens and *Zamia* (Table 5). However, the median probability for the general consumption of C_3 plants among females is still higher than for C_4 plants (85 % vs 15 %). This difference is even higher for Mound 2 males (95.9 % vs 4.1 %) (Table 5, Fig. 4: Row B).

MixSIAR model values using $\delta^{13}C_{en}$ also showed less differences between different C_3 resources (median values higher than 14 %) (Fig. 4:

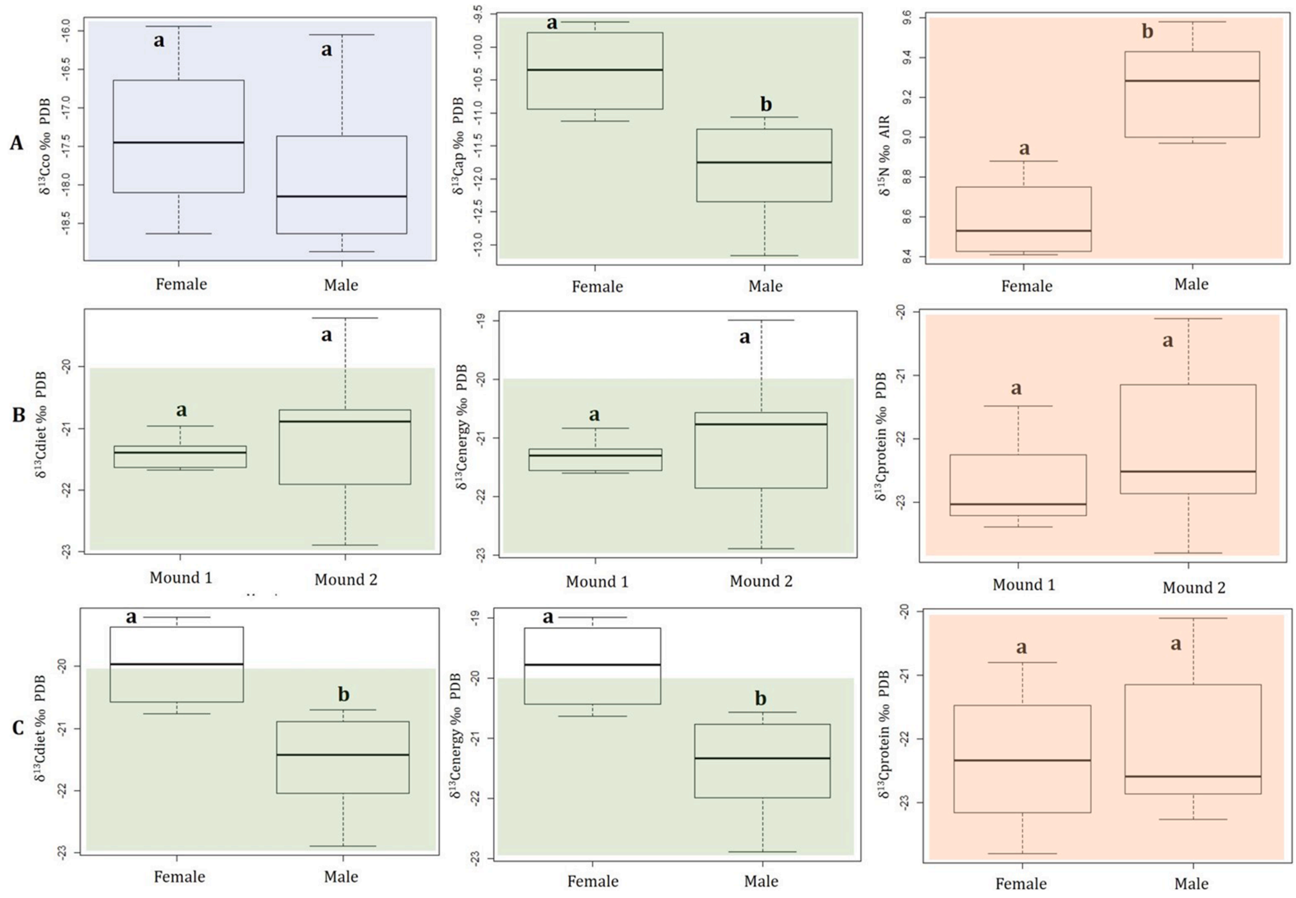

Fig. 2. $\delta^{13}C_{co}$, $\delta^{13}C_{ap}$ and $\delta^{15}N$ isotopic variations between females and males of Mound 2 (Row A). Isotopic variations in the carbon composition of diet ($\delta^{13}C_{diet}$), energy ($\delta^{13}C_{energy}$) and protein portions ($\delta^{13}C_{protein}$) between Mound 1 and Mound 2 individuals (Row B) and females and males from Mound 2 (Row C). Different letters mean statistically significant differences.

Table 4
MixSIAR model percent estimates for potential diet protein sources for Playa del Mango individuals combining $\delta^{13}C_{co}$ and $\delta^{15}N_{co}$. The shaded columns indicate the median estimates (highest potential diet protein sources in bold).

Food	Mound 1					Mound 2 Females					Mound 2 Males				
Sources[a]	5 %	25 %	50 %	75 %	95 %	5 %	25 %	50 %	75 %	95 %	5 %	25 %	50 %	75 %	95 %
Mol	0.3	2.6	6.3	11.0	18.7	0.5	2.5	5.0	8.8	21.5	0.5	1.1	1.8	3.6	7.3
MFish	0.6	3.0	7.1	13.6	24.4	0.0	0.8	2.0	4.2	9.4	0.1	0.4	0.9	3.4	7.2
ERFish	0.8	3.9	8.9	14.3	24.5	0.6	2.7	5.4	8.0	12.9	0.7	4.4	8.1	11.6	17.4
TAnim	2.9	7.9	**14.0**	24.2	50.9	2.2	7.5	**15.8**	22.9	34.0	4.7	12.3	**16.9**	26.7	49.1
Maize	0.4	2.5	5.2	9.4	17.0	0.0	2.1	5.1	9.0	15.8	0.3	0.8	1.9	3.5	5.7
RC	5.4	15.9	**25.0**	38.6	53.3	5.4	16.3	**26.8**	37.7	57.5	11.3	21.9	**34.9**	51.3	71.8
Leg	0.2	6.9	**18.6**	32.9	47.9	11.2	22.4	**35.0**	45.5	56.5	3.9	13.0	**24.3**	40.6	51.8

[a] Mol: molluscs, MFish: marine fish, ERFish: estuarine/riverine fish, TAnim: terrestrial animals, RC: root cultigens and Leg: legumes.

Row C). The consumption of breastmilk decreased during childhood, whereas the percentage of terrestrial fruits increased, reaching higher values in adolescence. Maize median values were consistently lower than other resources for infants, children and juveniles (Table 6, Fig. 4: Row C).

The $\delta^{15}N$ and $\delta^{13}C_{co}$ isotopic composition of Playa del Mango is statistically significantly different from other populations from the Antilles, including other ‘Archaic Age’ related sites from western Cuba such as Cueva del Perico I (CP), Cueva Calero (CC) and Canímar Abajo (CA) (Fig. 5). The $\delta^{13}C_{en}$ composition of the third molar is statistically significantly different from that of Canímar Abajo (Fig. 5C).

3.2. Starch analysis

A total of 33 starch grains, from both C_3 and C_4 plants, were recovered from dental calculus from Playa del Mango individuals in a proportion of 50:50 C_3/C_4 (Table 7). From them, 13 grains were not identified due to damage in their structure and/or lack of diagnostic traits. The characteristics of the grains with visible traits were summarized in Table 8.

Several starch grains, recovered in the dental calculus of E-1, showed characteristics consistent with starches from the Marantaceae family (Table 8, Fig. 6A-D). Grain 6A was identified as *Maranta* sp. (Table 8). It has a width of 20 μm and a length of 25 μm, oval/transovate shape, eccentric circular hilum, eccentric linear fissure and border with a simple dark line. Other grains were consistent with *Calathea* sp. (Fig. 6: C and D). They showed oval/transovate shapes, with a maximum length between 29.5 and 30 μm, eccentric circular hilum, absence of fissure and border with simple dark line. Grain 6D has concentric B rings and other traits consistent with *Calathea allouia* (Table 8).

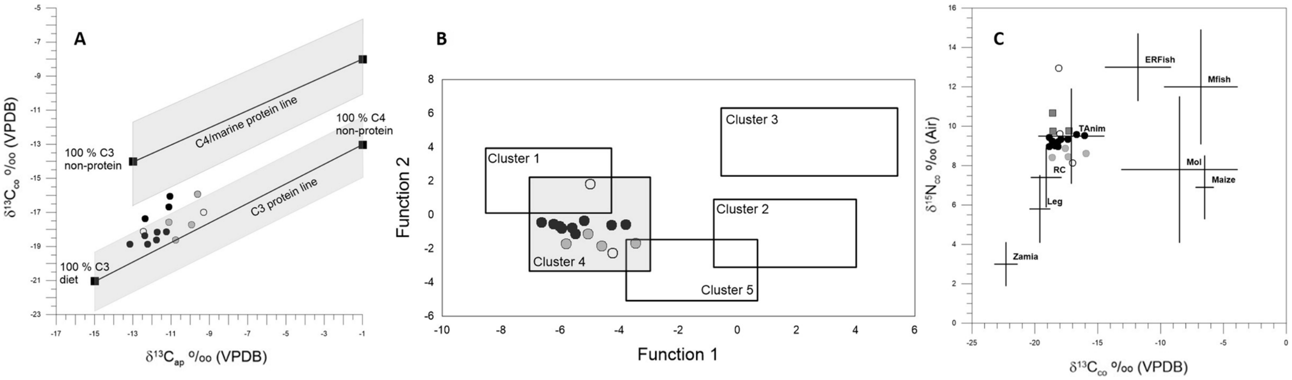

Fig. 3. Mean $\delta^{13}C_{co}$ and $\delta^{13}C_{ca}$ values of Playa del Mango individuals plotted against Froehle et al. (2010) (A), Froehle et al. (2012) (B) and MixSIAR (C) models. C: $\delta^{13}C_{co}$ and $\delta^{15}N$ values of Playa del Mango individuals against the stable carbon and nitrogen values of food sources. Mol: molluscs, MFish: marine fish, ERFish: estuarine/riverine fish, TAnim: terrestrial animals, RC: root cultigens, and Leg: legumes. Grey circles: Mound 2 females, black circles: Mound 2 males, empty circles: Mound 2 non-adults, Quadrant: Mound 1 adults.

Three starch grains from the dental calculus of E-11 were potentially identified as *Ipomoea batatas* (sweet potatoes) (Fig. 6: F, G and H). Maximum length observed was between 11.2 and 14.1 μm. Starch grains 6F and 6H showed polygonal shape, central and open circular hilum, double-border and three to four pressure facets. Grain 6G presented a truncated shape, centric circular hilum and a central linear fissure (Table 8). A partially depressed extinction cross was observed in three of them. One grain with features of *Xanthosoma* sp. was identified in the dental calculus of E-3 (Fig. 6: E). This starch exhibited irregular rectangular shape, linear A fissure and a double-border.

Starch grains consistent with *Zea mays* were found in the dental calculus of at least 80 % of the individuals studied (Table 7). Typical size ranged from 9 to 27 μm, with one grain measuring 41 μm (Table 8, Fig. 7B). Shapes were variable, being oval irregular (Fig. 7 B, C, D), circular (Fig. 7 C, G, H, I), and polygonal (e.g., Fig. 7A, D, E, J). Circular hilum, radial and stellar fissures were observed (Fig. 7 A, B, G). A double border was detected in all cases (Fig. 7). No blacks presented starches.

4. Discussion

Stable isotope values for Playa del Mango individuals are indicative of a diet rich in C_3 terrestrial-based resources and C_3 plants. According to Froehle et al. (2010) model, most values are within the 95 % credibility interval for a C_3-based protein diet (Fig. 3A). This is consistent with the estimated $\delta^{13}C$ protein values of diet for all individuals (<-20 ‰) and with the fact that Playa del Mango stable isotope values cluster together with populations that had a 70 % C_3 and a 30 % C_4 diet, where at least 65 % was based on C_3 protein (Froehle et al., 2012; Fig. 3B). These two models relied on data that included $\delta^{13}C_{ap}$ stable isotope results, which may be potentially problematic since bone bioapatite values have been proven to be affected by diagenesis in some contexts (Koch et al., 1997). However, the Playa del Mango, $\delta^{13}C_{en}$ values, considered to be less prone to diagenesis (Sponheimer and Lee-Thorp, 1999), led to similar conclusions regarding dietary patterns. Although $\delta^{13}C_{en-ap}$ showed an offset of -1.4 ± 0.7 ‰, comparable dietary assessments and dispersion of values suggest that $\delta^{13}C_{ap}$ results are representative of the biogenic signal of Playa del Mango individuals' bones. Variability between apatite and enamel in the same individuals may be associated with several factors, including how materials respond to sample treatments, possible diagenetic changes in $\delta^{13}C_{ap}$ and most likely, different age at the tissue formation and age-related differences in the diet.

The validity of the previous models is sustained by the fact that the MixSIAR model, combining $\delta^{15}N$ and $\delta^{13}C_{co}$, supported that Playa del Mango individuals had a diet where terrestrial animals, root cultigens and legumes (or food sources with similar stable isotope values) had a higher relative importance than marine protein and C_4 plants (Fig. 3C, Fig. 4A). It is accepted that both carbon collagen and nitrogen isotopic values mainly represent the protein source of diet, although $\delta^{13}C_{co}$ values are also influenced by macronutrients such as lipids and carbohydrates (Kellner and Schoeninger, 2007; Froehle et al., 2010; Fernandes et al., 2012). These results support that the diet of Playa del Mango individuals was highly dependent on C_3 terrestrial resources, which is further confirmed by the $\delta^{34}S$ values obtained in three individuals of Mound 1 that fit with typical values of populations under terrestrial influence (Nehlich et al., 2011). On the other hand, $\delta^{13}C_{ap}$ results are more indicative of the whole diet, which significantly correlates with its energy portion (Schwarcz, 2000; Kellner et al., 2007; Froehle et al., 2010). While C_3 protein sources such as terrestrial mammals are 85–90 % protein, maize is only 10 % (Schober and Ambrose, 1995). This may explain why the MixSIAR model predicts a higher contribution of C_4 plants such as maize, when using $\delta^{13}C_{ap}$ values (Fig. 4A vs Fig. 4B). It also confirms that stable isotope studies that only analyze carbon from collagen may underestimate the role of low protein resources in individual diets.

The presence of both C_3 and C_4 plants is apparent in the dental calculus of the individuals sampled. The C_3 plants identified included underground storage organs (USOs) belonging to the Araceae, Convolvulaceae and Marantaceae families. Starches from yams, tubers and bulbs have usually an elongate or triangular shape with a highly eccentric hila (Liu et al., 2013: 5383). This is the case for the members of the Marantaceae family identified in our sample (*Calathea* sp., including *C. allouia,* and *Maranta* sp.). They showed a combination of typical characteristics including the size of starch grains, their oval and elongate shape with slightly wavy margins, and an eccentric hilum in their thinner end combined with the projection of the extinction cross (Pagán Jiménez, 2007, Mickleburgh and Pagán Jiménez, 2012). The starch consistent with *Maranta* sp. showed oval/transovate shape, a single border, an eccentric circular hilum, and eccentric lineal fissure, with straight arms in the extinction cross. Starch grains consistent with *Calathea* sp. showed oval and transovate shapes, eccentric circular hilum, absence of fissure, border with simple dark line and size in the frequent range reported for the genus (Pagán Jiménez, 2007; Berman and Pearsall, 2020). One starch grain, identified as *Calathea allouia*, exhibited concentric B rings that are the most frequent laminate pattern for the species (Pagán Jiménez, 2007).

Starch grains identified as *Ipomoea batatas* showed their diagnostic morphological features, such as a typically polygonal shape, central and open circular hilum and three to four pressure facets (Piperno and Holst, 1998; Perry, 2004; Pagán Jiménez, 2007; Pagán Jiménez et al., 2015). The size falls within the range proposed for the species (Pagán Jiménez, 2015). The starch grain extinction crosses of 6F and 6H presented the singularity of having three thin arms and a wider quarter, a distinctive

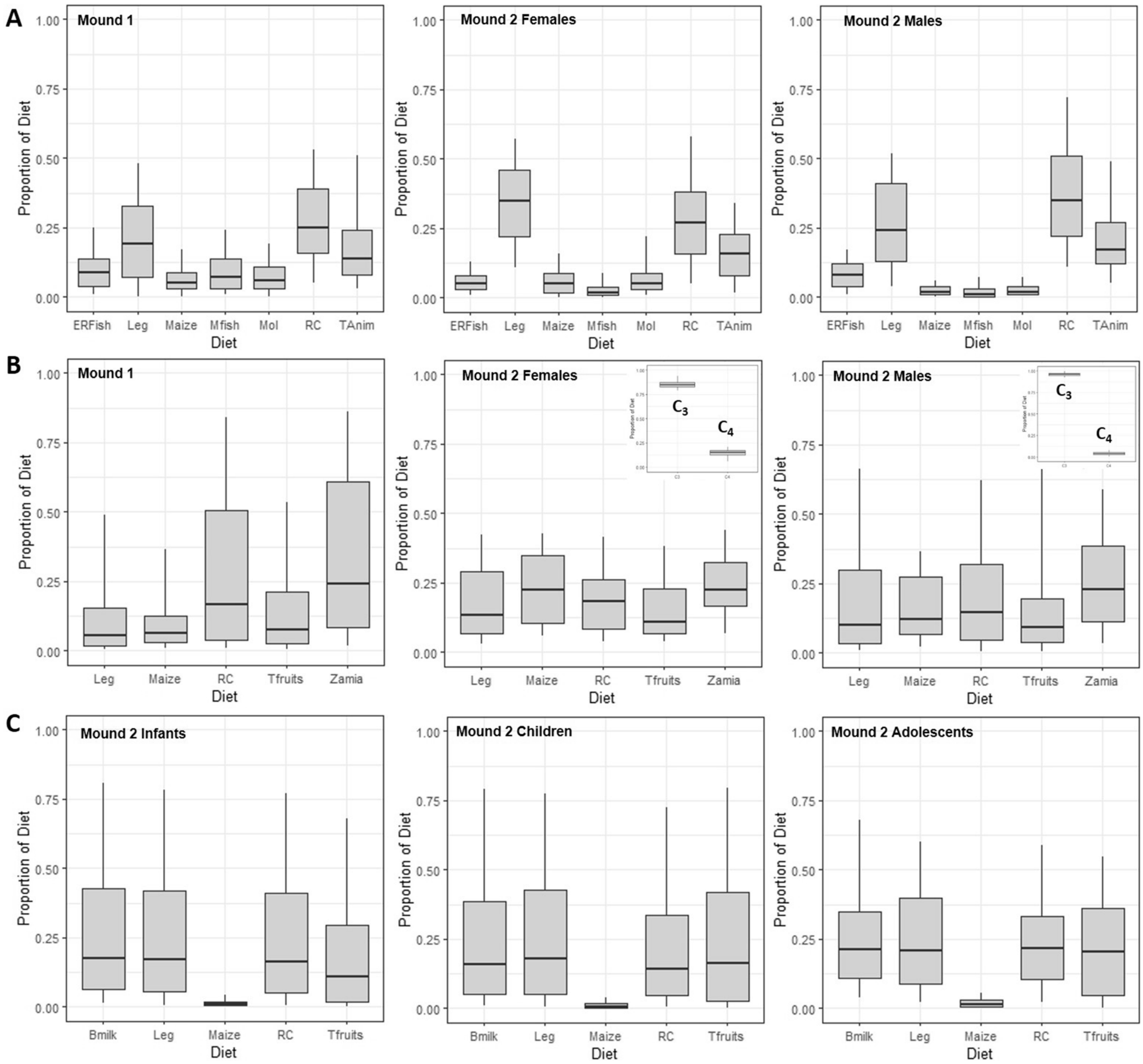

Fig. 4. MixSIAR mixing model results. $\delta^{13}C_{co}$, $\delta^{15}N$ (Row A), $\delta^{13}C_{ap}$ (Row B), $\delta^{13}C_{en}$ (Row C) percent median estimates and their associated high (HCI) and low (LCI) 95 % credibility intervals (CI) for potential diet protein sources for Mound 1, Mound 2 females and males (Row A and B), infants, children and juveniles (Row C) exhumed at Playa del Mango. ERFish: estuarine/riverine fish; Leg: legumes; Mfish: marine fish; Mol: molluscs; RC: root cultigens; TAnim: terrestrial animals; TFruits: terrestrial fruits; Bmilk: breastmilk.

Table 5
MixSIAR model ($\delta^{13}C_{ap}$) percent estimates for potential diet carbohydrate sources for Playa del Mango individuals. The shaded columns indicate the median estimates (highest potential diet protein sources in bold).

Food Sources[a]	Mound 1					Mound 2 Females					Mound 2 Males				
	5 %	**25 %**	**50 %**	**75 %**	**95 %**	**5 %**	**25 %**	**50 %**	**75 %**	**95 %**	**5 %**	**25 %**	**50 %**	**75 %**	**95 %**
RC	0.9	4.1	**16.5**	50.5	84.2	4.1	8.6	**18.3**	26.4	41.5	0.5	4.8	**14.5**	31.9	62.3
Leg	0.6	2	5.4	15.5	48.7	2.9	6.8	13.6	29.2	42.4	1.1	3.6	9.9	30.1	66.4
TFruits	0.6	2.7	7.8	21.2	53.4	3.8	6.8	11.1	22.7	38	0.7	3.7	9.3	19.5	66.2
Zamia	1.7	8.5	**24.1**	60.7	86.2	6.9	16.8	**22.5**	32.5	43.9	3.4	11.4	**22.7**	38.7	58.8
Maize	0.9	2.9	6.3	12.7	36.7	5.8	10.4	**22.6**	35	42.9	2.4	6.6	12.1	27.3	36.7
C_3	–	–	–	–	–	79.0	82.7	**85.0**	87.4	93.9	92.1	94.5	**95.9**	97.4	99.2
C_4	–	–	–	–	–	6.1	12.6	15.0	17.3	21.0	0.8	2.6	4.1	5.5	7.9

[a] RC: root cultigens, Leg: legumes, TFruits: tropical fruits, C_3: C_3 plants, and C_4: C_4 plants.

Table 6
MixSIAR model ($\delta^{13}C_{en}$) percent estimates for potential diet protein sources for Playa del Mango individuals. The shaded columns indicate the median estimates (highest potential diet protein sources in bold).

Food	Infancy					Childhood					Adolescence				
Sources[a]	5 %	25 %	50 %	75 %	95 %	5 %	25 %	50 %	75 %	95 %	5 %	25 %	50 %	75 %	95 %
RC	0.8	5.2	**16.3**	41.2	76.8	0.5	4.7	**14.1**	33.6	72.5	2.2	10.6	**21.6**	33.4	59
Leg	0.8	5.4	**17.3**	41.8	78.3	0.7	5.2	**17.9**	42.7	77.2	2.2	9	**20.9**	39.7	60.2
TFruits	0	1.9	11.1	29.6	68	0	2.7	**16.1**	41.8	79.4	0.1	4.6	**20.3**	36.1	54.7
Maize	0.1	0.4	0.9	1.9	4.5	0.1	0.3	0.8	1.7	4.1	0.2	0.7	1.5	3	5.4
BMilk	1.3	6.4	**17.5**	42.7	80.7	1.1	5	**15.8**	38.7	79	4	11	**21.4**	35	67.9

[a] RC: root cultigens, Leg: legumes, TFruits: tropical fruits, BMilk: breastmilk

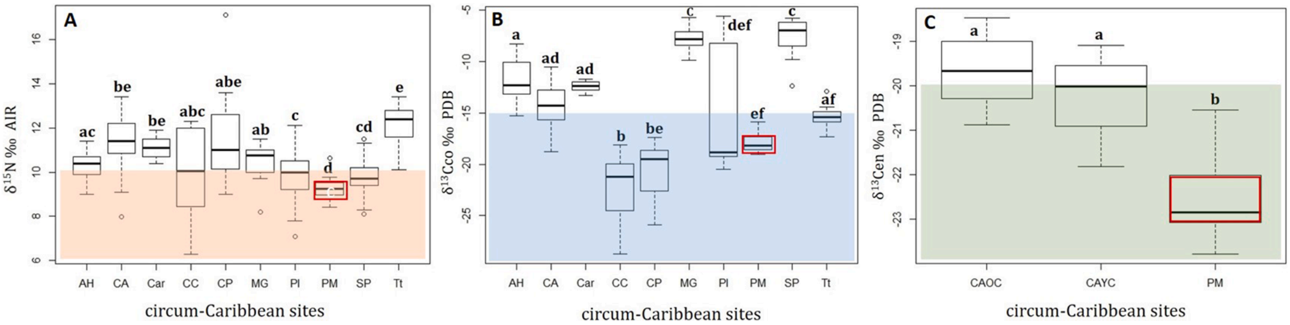

Fig. 5. $\delta^{15}N$, $\delta^{13}C_{co}$, $\delta^{13}C_{en}$ isotopic variations between individuals from different populations from the Caribbean. CA: Canímar Abajo; CAOC: Canímar Abajo, older Cemetery; CAYC: Canímar Abajo, younger Cemetery; CC: Cueva Calero; CP: Cueva del Perico I; PM: Playa del Mango (Cuba); PI: Paso del Indio (Puerto Rico); Tt: Tutu (Virgin Islands); Car: Carriacou (Grenada); AH: Altun Ha; MG: Marco González: SP: San Pedro (Belize). Different letters represent statistically significant differences. Shadow areas represent isotopic values for populations with higher dependance on terrestrial (A), C3 based resources (B and C). Values plotted in A and B (collagen) are unadjusted.

Table 7
Ubiquity and frequency of starch granules found in the dental calculus of Playa del Mango individuals.

Starch Counts per Individual	Individual ID						
	PM_E-1	**PM_E-2**	**PM_E-3**	**PM_E-6**	**PM_E-11**	**Total**	**Ubiquity**
Araceae (*Xanthosoma* sp.)			1			1	1/5
Convolvulaceae (*Ipomoea batatas*)		1			3	4	2/5
Marantaceae (*Calathea* sp., *Maranta* sp.)	4					4	1/5
Poaceae (*Zea mays*)	7	1	2		1	11	**4/5**
Not Identified	3			1*		4	
Total	14	2	3	1	4	24	
Proportion C_3:C_4							**1:1**

* This plant does not have any trait typical from maize.

and almost unique characteristic observed in sweet potato (Pagán Jiménez et al., 2015; Chinique de Armas et al., 2015). In addition, all starches securely and potentially classified as sweet potatoes showed a partially depressed extinction cross. The three grains reported here have a combination of/or diagnostic traits typical from sweet potatoes and resemble the 'purple variety' studied by Pagán Jiménez (2015: 54-55).

One starch grain was potentially identified as *Xanthosoma* sp. based on its irregular and rectangular shape, linear fissure (type A), double-border (optic effect), pressure facets, and mainly straight lines. These characteristics have been described for the genus by Pagán Jiménez (2007). One of the grains classified as sweet potatoes (6F) showed some features that can be present in *Xanthosoma* sp. and *Manihot esculenta* (manioc) (Pagán Jiménez, 2007; Ciofalo et al., 2019; Berman and Pearsall, 2020). Both plants were available for the communities of Playa del Mango, as they were recently identified on an artifact from the site (Rodríguez Suárez et al., 2020). However, the partially depressed extinction cross indicates that this grain is likely from sweet potatoes. Some of the USOs identified in this paper may be from taxonomically related plants. Future taxonomic studies of USOs in Cuba will contribute to increase the accuracy of starch grain identifications.

Starch grains consistent with maize were identified in the dental calculus of the 80 % of the individuals sampled. Maize granules differ in size and shape from the wild teosinte and other native grasses of America (Holst et al., 2007; Piperno et al., 2009; Musaubach et al., 2013). While Teosinte exhibits rounded grains up to approximately 10 μm in size (Piperno et al., 2009: 5020), maize starch grains are typically facetted and larger (8–25 μm) with a number of pressure facets (Holst et al., 2007). The maize hilum typically has radial or stellate fissures, although other types of fissures have been identified (Holst et al., 2007: 17609; Mickleburgh and Pagán Jiménez, 2012: Appendix B). Starch grains in wild grasses are typically smaller (Holst et al., 2007). Some grains exhibited features observed in several maize varieties but because they may be potentially similar to other Poaceae starch grains, they were kept as tentative. Grain 7B was kept as tentative because the size is out of the typical range for grains where diagnostic traits are still visible. The presence of maize in the diet of Playa del Mango individuals is not

Table 8

Description of the starches from Playa del Mango individuals showed in Fig. 6 (ID 6A-L) and Fig. 7 (ID 7A-L). Starches with non-visible traits were not included to this table.

ID	Grain Shape	Size		Extinction cross shape	Hilum Position	Lamellae	Fissure	Border	Pressure facets	Potential Identification
		Diameter	L/W							
6A	Oval		25.0/20.0	Eccentric, Straight	Eccentric	Absent/not observable	Eccentric Lineal A	Single (dark)	Absent	Marantaceae *Maranta* sp.
6B	Oval		38.5/30.0	Eccentric, Wavy	Eccentric	Absent/not observable	Eccentric Linear H	Double extern: light; intern: dark	Absent	Marantaceae
6C	Oval		29.5/16.2	Eccentric, Wavy	Eccentric	Absent/not observable	Absent	Single (dark)	Absent	Marantaceae *Calathea* sp.
6D	Oval		30.0/20.0	Eccentric, Wavy	Eccentric	Concentric regular rings B	Absent	Single (dark)	Absent	Marantaceae *Calathea allouia*
6E	Rectangular		35.0/20.0	Central, Straight	Central	Absent	Eccentric Linear B/A	Double (dark)	Absent	Araceae cf. Xanthosoma
6F	Truncate		14.1/ 12.1	Central, Straight	Eccentric	Absent	Central Linear A	Double extern: dark; intern: light	Present	Convolvulaceae cf. *Ipomoea batatas*
6G	Polygonal		11.2/9.9	Central, Straight	Central	Absent	Absent	Double (dark)”	Present	Convolvulaceae cf. *Ipomoea batatas*
6H	Polygonal		12.0/11.3	Central, Straight	Central	Absent	Absent	Double (dark)”	Present	Convolvulaceae cf. *Ipomoea batatas*
6I	Polygonal		10.9/9.1	Central, Wavy	Central	Absent	Central, Linear Y	Double	Present	Convolvulaceae *Ipomoea batatas*
6 J	Bell		10.9/8.2	Eccentric, Wavy	Eccentric	Absent	Absent	Simple (dark)	Absent	Not identified
6 K	Circular	9.1		Central, Straight	Absent	Absent	Absent	Simple (dark)	Absent	Not identified
6L	Oval (the central one)		27.8/24.4	Central, Straight	Absent	Absent	Absent	Simple (dark)	Absent	Not identified
7A	Polygonal		27.1/20.0	Central, Straight	Central	Absent	Central Linear E	Double (dark)	Absent	Poaceae *Zea mays*
7B	Oval irregular		41.3/35.0	Central, Straight	Central	Absent	Central Asymmetric	Double (dark)	Absent	Poaceae *Zea mays*
7C	Circular Irregular	18.3		Central, Straight	Central	Absent	Absent	Double (dark)	Absent	Poaceae cf. *Zea mays*
7D	Oval Irregular		13.3/10.9	Central, Straight	Central	Absent	Central Linear H	Double (dark)	Absent	Poaceae *Zea mays*
7E	Pentagonal		15.3/17.1	Central, Straight	Central	Absent	Absent	Double (dark)	Absent	Poaceae *Zea mays*
7F	Oval		11.3/9.3	Central, Straight	Central	Absent	Absent	Double (dark)	Absent	Poaceae cf. Zea mays
7G	Oval		9.6/8.0	Central, Straight	Central	Absent	Absent	Double (dark)	Absent	Poaceae cf. *Zea mays*
7H	Circular	18.8		Central, Straight	Central	Absent	Absent	Double (dark)	Absent	Poaceae cf. *Zea mays*
7I	Circular Irregular		13.0/12.1	Central, Straight	Central	Absent	Absent	Double extern: dark; intern: light	Absent	Poaceae cf. *Zea mays*
7 J	Polygonal		14.0/13.0	Central, Straight	Central	Absent	Absent	Double	Present	Poaceae cf. *Zea mays*
7 K	Polygonal		10.0/8.0	Central, Wavy	Central	Absent	Linear A	Simple (dark)	Present	cf. Poaceae
7L	Oval irregular		10.0/6.3	Central, Straight	Absent	Absent	Absent	Simple (dark)	Present	Not identified

*PM_E-1 (6A – 6D, 6 J-K, 7A-7G, 7L), PM_E-2 (6I, 7 K), PM_E-3 (6E, 7H, 7 J), PM_E-6 (6L), PM_E-11 (6F-6H, 7I), NI17 (6L).

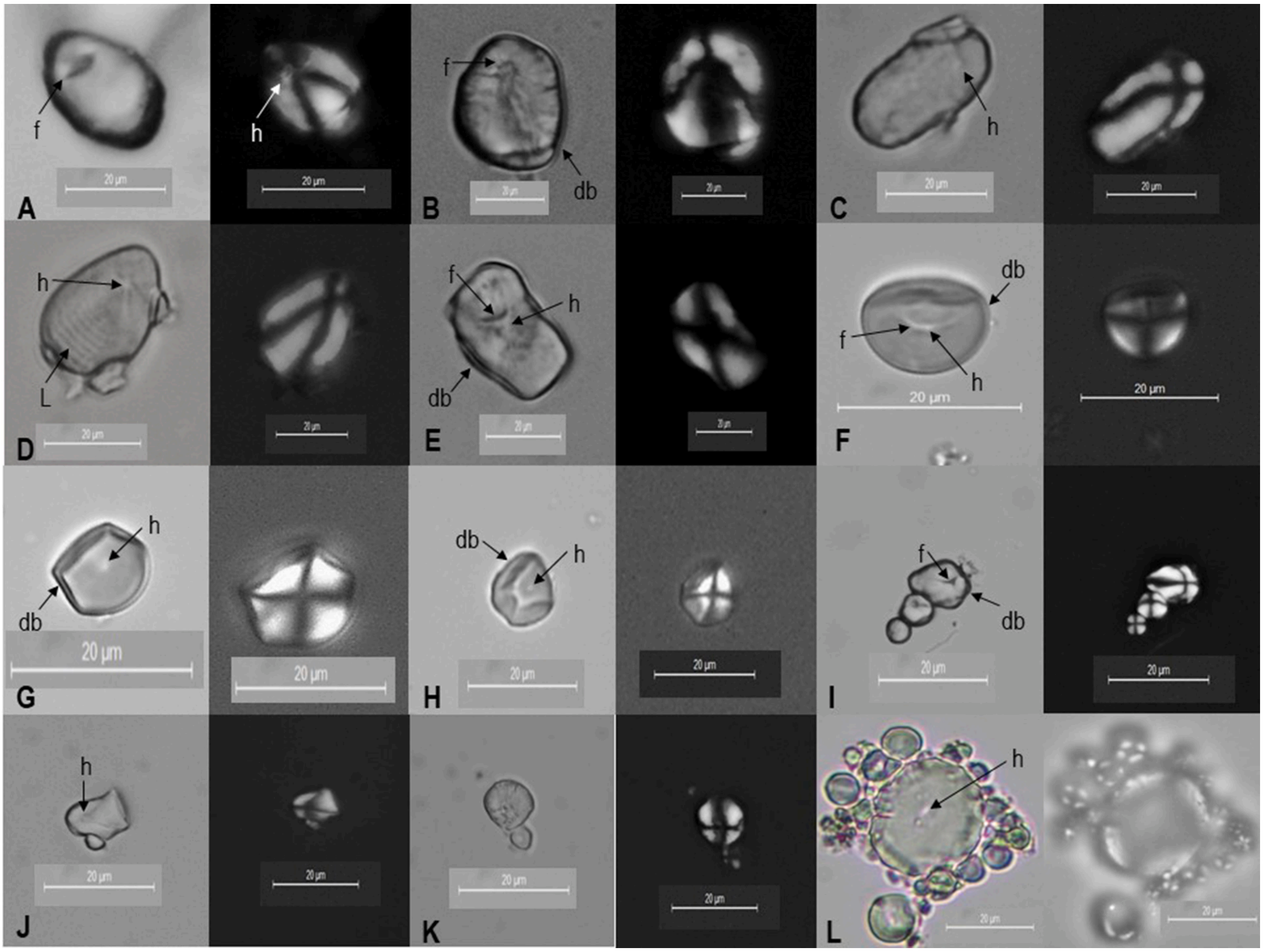

Fig. 6. Starch grains consistent with C_3 plants found in the dental calculus of Playa del Mango individuals. A: *Maranta* sp.; B: Marantaceae; C: *Calathea* sp.; D: *Calathea allouia*; E: cf. *Xanthosoma* sp.; F, G, H, I: cf. *Ipomoea batatas*; J, K, L: Not identified. f: fissure; db: double border; h: hilum. Scale 20 µm.

surprising, as the earliest direct evidence (from dental calculus in a dated individual) of maize consumption in the Greater Antilles was already recorded at the archaeological site of Canímar Abajo in west Cuba (E-105: cal. BC 990–800; Chinique de Armas et al., 2019). These results indicate that maize was also being consumed in east Cuba at cal. 48 – 235 CE (2σ) (dental calculus of the individual E-1) and possibly earlier, at cal. BC 116 –75 AD (2σ) (dental calculus of E-3).

Starches consistent with maize were observed in higher frequency and ubiquity (80 %) than any other single plant identified in our sample. However, the carbon stable isotope results, the estimated carbon isotopic composition of diet and its energy portion (~-21 ‰), as well as the three models, strongly suggest that C_4 plants, while consumed, had a smaller contribution to diet than C_3 plants (especially for males). Factors controlling rate and intensity of calculus formation are variable and not well understood (Lieverse, 1999)). In addition, starch granule identification depends largely on preservation. Recovered starch granules can appear warped in shape, swollen, globular, and in some cases cracked (Barton and Torrence, 2015). Pre-depositional factors, such as the methods used for food processing, can also affect starch granule features, including erosion of extinction cross features and an enlargement of granules (Crowther, 2012). All of these factors can cause over/under representation of different starches in the dental calculus.

The results of this paper support that the amount of starch grains, their lack, or abundance, cannot directly indicate the frequency of plants consumed within a population, especially when the sample is small (Weslowski et al., 2010; Leonard et al. 2015; Radini et al., 2016; Mariotti Lippi et al., 2017). This is important in understanding the role of some cultigens, such as maize, in the dietary practices of indigenous populations of the precolonial Antilles. In the case of Playa del Mango individuals, regardless of its ubiquity, it is likely that maize (and other C_4 and CAM plants) provided a minor dietary contribution relative to C_3 plants, especially in comparison to the USOs.

The absence of beans among the identified starch grains is also notable, especially considering the resistance of their starch grains to cooking (Rodríguez Suárez et al., 2016) and their ubiquity in other sites in the region (Chinique de Armas et al., 2015; 2019; Pagán Jiménez et al., 2019). In addition, starch grains of beans were recently found in artifacts from Playa del Mango (González Herrera et al., 2019; Rodríguez Suárez et al., 2020). The absence of frequently consumed plants in the dental calculus of a living population of known diet was reported by Leonard et al. (2015). The absence of beans in this study may be the result of a stochastic process due to the small sample size. Experimental analysis testing the response of beans to different cooking techniques, and the analysis of dental calculus of more individuals from Playa del Mango, may help to better understand the unexpected lack of beans in comparison to other plant taxa.

It is important to emphasise that some sites in the Caribbean were in use over a long period of time (e.g., Canímar Abajo; Roksandic et al., 2015), and that individual dietary practices could have been variably influenced by several factors, including but not limited to, environmental constraints and cultural perceptions. Assuming that a population behaved as a cohort, and using average values, may be misleading when trying to reconstruct biological and cultural aspects of a given population. In the case of Playa del Mango, most individuals were inhumed in a

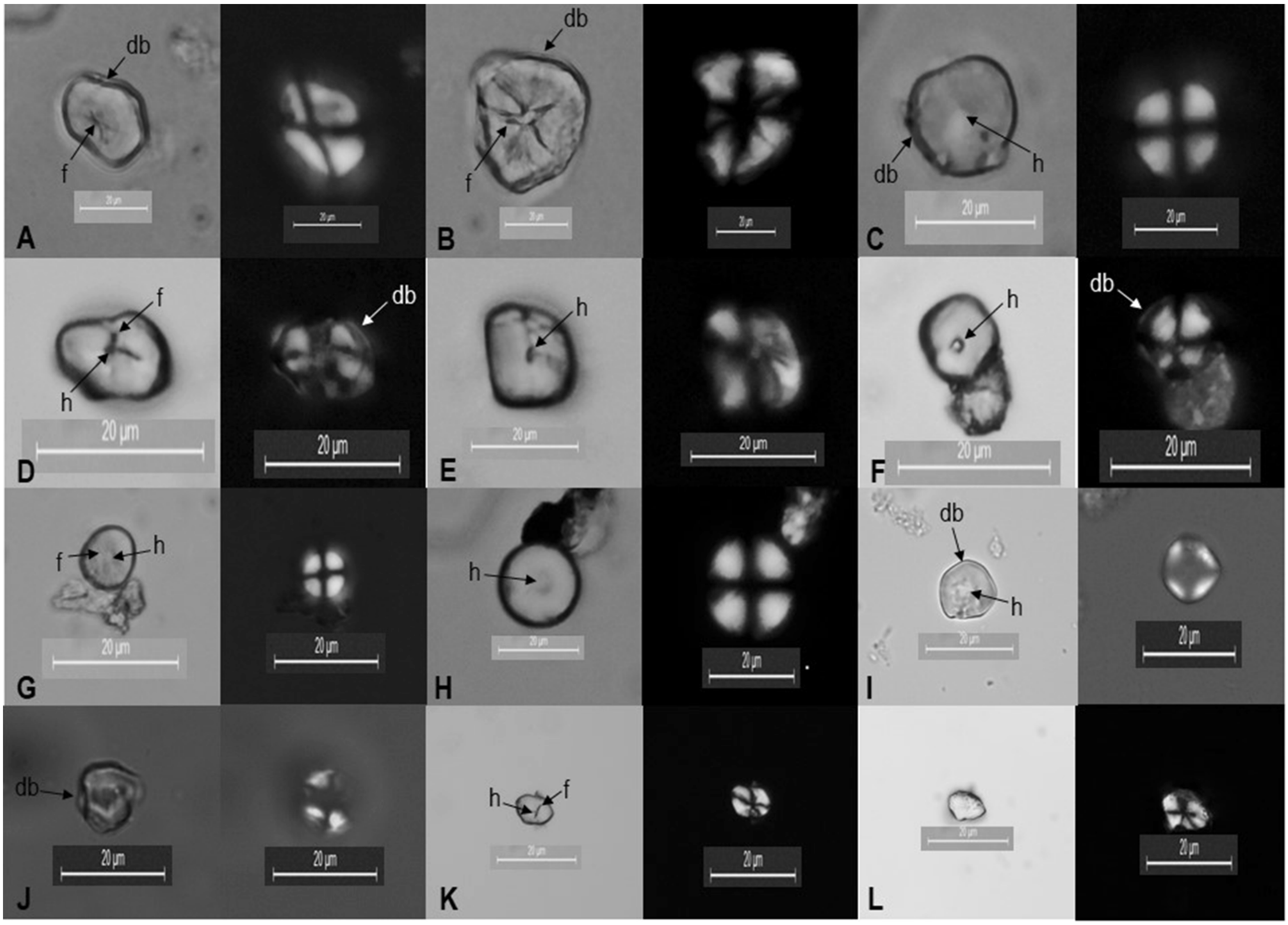

Fig. 7. Starch grains consistent with C_4 plants found in the dental calculus of Playa del Mango individuals. A, B, D, E: *Zea mays*; C, F, G, H, I, J: cf. *Zea mays*; K: cf. Poaceae; L: Not identified. f: fissure; db: double border; h: hilum. Scale 20 μm.

relative short period of time (Chinique de Armas et al., 2020), making assumptions based on averages less problematic. However, we performed comparisons between segments of population in order to understand potential intra-site variations.

The number of individuals from Mound 1 available for this study was small in comparison to Mound 2, thereby making conclusions about their diets more contingent. The main potential difference that it is possible to observe between Mound 1 and 2 individuals is a possible higher reliance on marine/riverine resources in the Mound 1 population. However, the general values observed, and the statistical analysis, suggested that similar dietary traditions were in place throughout Playa del Mango populations for over 300 years (Chinique de Armas et al., 2020), with some idiosyncratic variation.

In general terms, the higher variability found in $\delta^{13}C_{ap}$ and $\delta^{13}C_{en}$ values, in comparison to $\delta^{13}C_{co}$, suggest that in terms of carbon, the energy portion of diet was more isotopically variable than the protein sources exploited, a pattern that has been previously noted for the Caribbean region more broadly (Laffoon et al., 2016:176). This is further confirmed by the statistically significant differences found between males and females for the carbon isotope composition of their whole diet and its energy portion, while the carbon isotope composition of proteins had similar averages for both groups. These results suggest a differential consumption of plants (which are richer in carbohydrates) between the females and males buried in the periphery of the Mound 2. The MixSIAR model, when using $\delta^{13}C_{ap}$, predicted that maize was more important in female diets than in male diets (Fig. 4: Row B) which could be at the origin of the previously observed difference. Even when the carbon coming from proteins ($\delta^{13}C_{protein}$) showed similar average values, the MixSIAR model (Fig. 4A) predicted that root cultigens were more important in male diets. Since the prediction is based on both $\delta^{13}C_{co}$ and $\delta^{15}N$ values, it is likely that a lower consumption of C_4 resources by males is influencing this tendency. However, this differential consumption of maize is not evident in the starch analysis results since the three male individuals sampled showed starch grains consistent with maize. This emphasizes the need to rely on a combination of methods to reach a more balanced reconstruction of dietary practices in the past. We suggest that when identifying dietary differences between individuals and populations, starch analysis needs to be combined with biochemical methods, such as stable isotope analysis.

In addition, the statistically significant differences observed in $\delta^{15}N$ between females and males may be affecting the MixSIAR model predictions. In the Playa del Mango case, female individuals showed lower nitrogen stable isotope values than males, which may explain why the contribution of riverine/estuarine and terrestrial animals have similar mode values in males. The difference in nitrogen values may suggest that further differences existed between female and male diets with regard to the dietary protein, with male diets more dependent on resources from higher trophic levels (e.g., estuarine/riverine) than females. Unfortunately, stable carbon and nitrogen values of food sources from different ecosystems (e.g., terrestrial/riverine) overlap (Pestle, 2010; 2013), making it difficult to ascertain potential variations in diet. In addition, some physiological factors can increase or decrease nitrogen isotope values in humans, including nutritional stress, disease (Katzenberg and Lovell, 1999; Fuller et al., 2004; Mekota et al., 2006; Olsen et al., 2014), and pregnancy (Fuller et al., 2004; Nitsch et al., 2010). Although it is not possible to explain at this point the observed differences in diet between females and males at Playa del Mango site, it is likely that gender roles in obtaining and preparing food sources could be contributing to the variations in diet.

Further differences were observed between the collagen values of non-adults in comparison with adults. The younger infant (E-8), had similar lower nitrogen isotope values to E-9, a female individual close to whom the baby was buried. Low nitrogen values at early ages can arise when breastmilk isotopic signals have not been completely fixed in bone collagen tissues (Chinique de Armas et al., 2017). In contrast, the older infant E-6 exhibited higher nitrogen values than the female average of the population. The δ^{15}N values for infants with an exclusive consumption of breastmilk are ~ 2–3 ‰ higher than the values of their mothers, due to the trophic level effect (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Bocherens and Drucker, 2003). The observed enrichment of 3 ‰ may indicate that breast milk was the most important source of protein for E-6, which is further supported by the MixSIAR model. As explained before, the δ^{15}N values of infants may decrease because of growth and development (Water-Rist and Katzenberg, 2010; Reitsema and Muir, 2015) or increase because of nutritional stress (Fuller et al., 2004; Warinner and Tuross, 2010). Given that this data relies on individuals that did not survive past the age at which they are studied, it is possible that some of them may have been affected by one or more of these physiological factors (Wood et al., 1992), thus not representing the actual weaning trajectory of their source population.

The MixSIAR model using non-adult $\delta^{13}C_{ap}$ values were considered to be non-representative of the population because only two individuals (with notable differences in diet) were available, and because $\delta^{13}C_{ap}$ in non-adults is even more prone to diagenesis. Far more reliable are the results obtained for $\delta^{13}C_{en}$ from adults who survived the weaning process as they represent dietary evidence for an individual at different stages of development. These results supported a higher consumption of C_3-based resources during growth and development (Fig. 4: Row C). The contribution of milk did not change significantly from infancy to adolescence which suggests that the weaning process started long before 3–4 years (when the tissue developed during infancy mineralized) and the food sources consumed during childhood and adolescence had similar carbon stable isotope values as breast milk (C_3-based resources). According to the model, the importance of maize in the diet of infants, children and juveniles was low and comparable to the values observed for males (Fig. 4: Row C). The results of dental calculus from the only non-adult with teeth available for Playa del Mango (E-6) shows very few starch grains. Of these, none of the observed starches had characteristics consistent with maize. This absence of maize in both the non-adults' dental calculus (Mickleburgh and Pagán Jiménez, 2012) and in stable isotope values (Chinique de Armas et al., 2017, 2022; Chinique de Armas and Pestle, 2018) had already been reported before in the Caribbean. Future studies including both stable isotope and starch analysis in the dental calculus of non-adults may help to understand the apparent low consumption of maize in some Caribbean precolonial populations. Furthermore, as no important variations in $\delta^{13}C_{en}$ were found between tissues formed at different ages, or between males and females during infancy or adolescence are in evidence, it is reasonable to suggest that differences in diet between females and males started at adulthood, suggesting that different diets may be related to gender roles within the population.

The stable isotope results presented here support our initial findings that cultural variability in foodways was greater in the precolonial Caribbean than previously acknowledged (Chinique de Armas et al., 2016). The values of δ^{15}N, $\delta^{13}C_{co}$ and $\delta^{13}C_{ap}$ for Playa del Mango individual were statistically significantly different from the individuals from other coeval sites from Cuba such as Canímar Abajo (Chinique de Armas et al., 2015), Cueva Calero, Cueva del Perico I and Guayabo Blanco (Chinique de Armas et al., 2016). On average, δ^{15}N values at Playa del Mango are the lowest ones reported for the Caribbean, only similar to the ones observed in some more terrestrial based individuals from Cueva Calero (western Cuba) and Paso del Indio (Puerto Rico; see Pestle, 2010). Their $\delta^{13}C_{co}$ values indicated that they had a more C_3-based diet with some contribution of C_4 plants, similar to the one observed in some 'Ceramic Age' sites such as Paso del Indio (Pestle, 2010) or the Tutu site (Norr, 2002; Pestle and Laffoon, 2018), where both C_3 and C_4 plants were consumed. In comparison to Canímar Abajo, the juveniles from Playa del Mango had a diet more dependent on C_3 resources (Chinique de Armas and Laffoon forthcoming). As the early component of the younger cemetery of Canímar Abajo was in use at the same time as the Playa del Mango burial area, these findings support previous observations that 'Archaic Age'-related populations, with different dietary traditions (Chinique de Armas et al., 2016), and possibly also of different geographic ancestries (Nägele et al., 2020), coexisted in Cuba in precolonial times.

5. Conclusions

The three isotope mixing models used in this study indicate that Playa del Mango individuals had a diet where C_3-based resources, such as terrestrial animals and C_3 plants, were predominant. In contrast, similar proportions of C_3 and C_4 plant starch grains were found. This cautions against sole reliance on starch analysis in understanding dietary practices and their variation among individuals and/or populations, especially when sample sizes are small. While stable isotope data provide information about the relative proportions of different food sources in an individual's diet, starch analysis has the potential to identify (and bring into the food web calculations) some of the actual (starchy) plants consumed. The combined use of different models based on stable isotope and starch analysis provides a better resolution for reconstructing dietary practices than studies where these approaches are used separately.

Although the MixSIAR model indicated that Playa del Mango populations had a diet mainly based on C_3 terrestrial resources, such as terrestrial animals and root cultigens, the consumption of C_4 plants was apparent in both the stable isotope values and in the dental calculus of some individuals. The identification of *Zea mays* was confirmed by at least cal. 48 – 235 CE (2σ). Other plants such as *Ipomoea batatas*, *Calathea* spp., *Maranta* sp. and possible *Xanthosoma* sp. were present at the same time and possibly earlier at BC 116–75 AD. A differential consumption of C_4 plants, was observed between female and male individuals buried in the peripheral area of Mound 2. The similarities in stable isotope values of tooth enamel formed at different age ranges (from infancy to adolescence) indicated that these differences are the results of differential consumption during adulthood and could be linked to gendered patterns in food access, preparation and/or consumption. Our results support a better predictive power of $\delta^{13}C_{ap}$ over $\delta^{13}C_{co}$ values in identifying the consumption of resources low in protein such as maize.

The differences in stable isotope analysis between Playa del Mango and other Caribbean populations, including other 'Archaic Age'-associated groups from western Cuba, support the notion that populations with different dietary traditions were co-existing in the Caribbean in precolonial times. In the case of Cuba, differences in diet between Playa del Mango individuals and other contemporaneous groups from western Cuba such as Canímar Abajo, offer further support to the finding that populations with different dietary traditions inhabited the main island at the same time. Future studies combining environmental, biological (including DNA analysis), and artifact analyses will allow a better understanding of the biological and cultural variations of early indigenous groups in the insular Caribbean.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are contained in the manuscript

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