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# Ancient Maya Turkey Husbandry: Testing Theories through Stable Isotope Analysis

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## Abstract

Large gaps exist in our knowledge of ancient Maya turkey husbandry and management. Among the questions still needing to be addressed are: 1) when and where was the non-local wild turkey (*Meleagris gallopavo*) introduced to and adopted by the ancient Maya, and 2) did the ancient Maya also rear captive or tame populations of the indigenous ocellated turkey (*Meleagris ocellata*)? In this paper, we assess the potential of stable isotope analysis to address these questions. We employ stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis to determine whether wild and husbanded turkeys in the Maya region can be distinguished based on their diets. Strontium isotope analysis ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) is also used to distinguish between *M. gallopavo* individuals that were imported from central/northern Mexico, and those raised on-site in the Maya lowlands. The results indicate that stable isotope analysis is a promising and underutilized method for testing theories regarding ancient Maya turkey husbandry.

**Keywords:** animal domestication, Maya, stable isotope analysis, domestic turkey (*Meleagris gallopavo*), Ocellated Turkey (*Meleagris ocellata*)

The Wild Turkey (or common turkey) (*Meleagris gallopavo*) is the only large-bodied domestic animal used by the pre-colonial Maya besides the dog (*Canis lupus familiaris*), and the only vertebrate domesticated in Mesoamerica. To better understand how managed or domesticated resources were integrated into ancient Maya subsistence, ritual and political economies, we must first understand the process and extent of Maya turkey husbandry and domestication. The subject is only recently gaining traction in Mesoamerica and the Maya world (Thornton et al 2012; Thornton and Emery, 2015; Lapham et al., this volume; Manin, Cornette and Lefèvre, this volume; Martinez Lira and Valadez, this volume) despite broad interest in the domestic dog in Mesoamerica (Blanco et al. 2006; Götz 2008; Valadez Azúa et al. 2006, 2013), and the domestic turkey in the American Southwest (e.g., Badenhorst et al. 2012; Grimstead et al. 2014; Lipe et al. 2016; McCaffery et al. 2014; McKusick 2001; Munro 2006, 2011; Newbold et al. 2012; Rawlings and Driver 2010; Speller et al. 2010). In Mesoamerica, where the timing of domestication and the possible trade of turkeys are unclear, the lack of osteological markers distinguishing domesticated from wild birds is significantly problematic. Understanding Maya turkey use is complicated by the fact that, in this area, domesticated *M. gallopavo* is found alongside the local, wild Ocellated Turkey (*Meleagris ocellata*). Although the latter is not domesticated today, ornithologists and zooarchaeologists have debated whether it too was managed by the pre-Colonial Maya through captive rearing and breeding (Hamblin 1984; Masson and Peraza Lope 2008; Pohl and Feldman 1982; Pollock and Ray 1957; Steadman 1980). Both birds are found in Maya archaeological contexts related to food and ritual, and although they are easily distinguished based on their plumage, distinguishing between them osteologically is extremely difficult especially when dealing with highly fragmented or eroded skeletal remains (Bochensky and Campbell 2006; Emery et al., this issue; Steadman 1980). For this reason, many Maya zooarchaeological studies only identify turkeys to the genus level (*Meleagris* sp.). Distinguishing domestic, captive-reared and wild individuals within a species

using zooarchaeological specimens is equally (or even more) problematic (Breitburg 1988, Munro 2006).

In view of the difficulties attendant on morphologically distinguishing the two species of Mesoamerican turkey, and also wild from domesticated/husbanded birds, we assess the potential for stable isotope and ancient DNA analysis to address these issues and elucidate the complex history of ancient Maya turkey husbandry and domestication. Specifically, we employ ancient DNA analysis to taxonomically identify individuals as *M. gallopavo* or *M. ocellata*, stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis to distinguish between wild and husbanded turkeys of either species based on their diets, and strontium isotope analysis ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) to determine if *M. gallopavo* specimens in the Maya region were imported from central Mexico shortly before death, or raised on-site in the Maya Lowlands, which would indicate early Maya experiments with turkey domestication. Isotopic studies have been used previously to document turkey domestication in the American Southwest (Grimstead et al. 2014; McCaffery et al. 2014; Rawlings and Driver 2010), but similar methods have not yet been widely applied in Mesoamerica.

This paper presents the results of our experimental use of these methods on archaeological turkey specimens from the Maya region. The results indicate that stable isotope analysis is a promising and underutilized method in Mesoamerica for identifying: (1) wild versus captive-reared turkeys, (2) the habitats where wild turkeys were hunted, and (3) whether the earliest domestic turkeys that arrived in the Maya Lowlands were reared on-site or imported from central Mexico. The isotope data also provide comparative data for archaeologists working on similar datasets from the American Southwest and elsewhere (e.g., Morris et al., this issue), and thus contribute to an overall understanding of the history of turkey use, husbandry and domestication in the ancient Americas.

## Mesoamerican Turkeys:

The South Mexican subspecies of the Wild Turkey (*M. g. gallopavo*) was first domesticated outside the Maya cultural region in central Mexico (Monteagudo et al. 2013; Speller et al. 2010). The timing of Mesoamerican Wild Turkey domestication is still unclear, but the process of domestication was likely underway by the first half part of the Preclassic or Formative period (1800 BC–AD 250) (Thornton and Emery 2015; Valadez Azúa 2003:74; Valadez Azúa and Arrellín Rosas 2000). Despite the antiquity of turkey husbandry and domestication in northern Mesoamerica, zooarchaeological evidence previously suggested that domestic turkeys were not introduced to or adopted by the ancient Maya until the Postclassic period (AD 1000–1519) (Götz 2008; Hamblin 1984). An earlier introduction, however, is suggested by the recent identification of non-local domestic turkeys at the Late Preclassic (250 BC–AD 250) Maya site of El Mirador located in Petén, Guatemala (Thornton et al. 2012). This finding indicates that some domestic turkeys reached the Maya region several centuries earlier than previously thought, although their widespread adoption and use as a subsistence resource may have occurred much later.

Understanding the adoption and use of domestic turkeys in the Maya region is further complicated by the presence of the indigenous Ocellated Turkey (*Meleagris ocellata*), which is native to Mexico's Yucatan Peninsula and northern Belize and Guatemala. Although Ocellated Turkeys have never been classified as domesticated, previous research suggests that Postclassic Maya populations reared Ocellated Turkeys in captivity alongside or instead of domestic turkeys at certain sites (Hamblin 1984; Masson and Peraza Lope 2008; Pohl and Feldman 1982; Pollock and Ray 1957). This practice would situate Ocellated Turkeys among a suite of wild taxa that was occasionally managed or reared by ancient Mesoamerican societies, as is still done today (Pohl 1977), including macaws (*Ara* sp.), parrots (Psittacidae), quail (*Colinus* sp.) rabbits (*Sylvilagus* sp.), white-tailed deer (*Odocoileus virginianus*), peccaries (Tayassuidae) and large

felids (e.g., *Puma concolor*, *Panthera onca*) (Corona Martínez 2002a, 2013; Hamblin 1984; Lapham, Feinman and Nicholas 2012; Sugiyama, Somerville and Schoeninger 2015; Valadez Azúa 1993, 2003; White et al. 2004). The extent of these practices is unclear, as is whether the captive animals were maintained for subsistence as well as for elite display and ceremonial purposes, but regardless, Ocellated Turkey husbandry would not be out of place within this cultural framework.

### **Stable Isotopes and Turkey Domestication**

Because the two species of turkey are so difficult to tease apart osteologically, and because both species may have been reared in captivity or hunted in the wild, we require an alternative method to distinguish wild from managed or husbanded turkeys in Mesoamerica. We argue that stable isotope analysis provides such a tool. We hypothesize that greater maize (*Zea mays*) consumption by domestic/captive-reared turkeys of either species will distinguish them isotopically from wild turkeys. We also predict that strontium isotopes will provide a means of determining whether the early examples of *M. gallopavo* in the Maya region represent locally reared animals, or individuals that were imported from their native range in central/northern Mexico shortly before death.

#### *Reconstructing Turkey Diet: Stable Isotope Analysis ( $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ )*

When animals are brought under human control, their diet changes due to their feeding in a more conscripted region, or the consumption of human provided fodder. These dietary shifts may be studied through stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis since these isotopes serve as proxies for paleodiet (Ambrose and DeNiro 1986, Lee-Thorp, Sealy, and van der Merwe 1989, Schoeninger and DeNiro 1984). Isotopic shifts associated with animal husbandry and domestication have been identified previously for Old World taxa including

sheep, goats, pigs and cattle (Abarella, Dobney and Rowley-Conwy 2006; Balasse and Ambrose 2005; Makarewicz and Tuross 2012; Minagawa et al. 2005; Noe-Nygaard et al. 2005), and in Mesoamerican dogs, deer, rabbits, and captive reared predators (Somerville 2015; Sugiyama, Somerville and Schoeninger 2015; Tykot, van der Merwe and Hammond 1996; White et al. 2004). At archaeological sites in the American Southwest, stable isotope analysis has similarly documented rearing of both domestic turkeys (McCaffery et al. 2014; Rawlings and Driver 2010) and captive scarlet macaws (*Ara macao*) (Somerville, Nelson, and Knudson 2010) based on their extensive consumption of maize (*Zea mays*).

To date, stable isotopes have not been widely applied to the study of Mesoamerican turkey husbandry, but similar dietary shifts associated with human provided fodder are expected. As in the American SW, maize is the most important agricultural crop grown in Mesoamerica. High maize consumption is detectable in both humans and animals within the region because maize utilizes the C4 or Hatch-Slack photosynthetic pathway, resulting in higher (less negative)  $\delta^{13}\text{C}$  (average  $\delta^{13}\text{C} = -12.5\text{‰}$ ) than most other plants including trees, shrubs, root crops and forbs, which utilize the C3 or Calvin-Benson photosynthetic pathway, (average  $\delta^{13}\text{C} = -27\text{‰}$ ) (Smith and Epstein 1971; van der Merwe, 1982). As the agricultural staple, maize was likely provided directly or indirectly (via household waste) to animals raised within human settlements. This is confirmed through ethnographic data (Götz and Garcia Paz, this volume), as well as isotopic evidence for C4-based diets in archaeological Maya dogs and the occasional captive-reared white-tailed deer (White et al. 2001; 2004). Similarly, at the non-Maya site of Teotihuacan in central Mexico, isotopic analysis indicates C4-based diets for captive-reared turkeys (Morales Puente et al. 2012), rabbits (Somerville 2015), eagles, pumas and wolves (Sugiyama, Somerville and Schoeninger 2015). Domestic or captive-reared turkeys in the Maya region are therefore expected to exhibit elevated  $\delta^{13}\text{C}$  indicative of significant maize consumption.

In the wild, both species of Mesoamerican turkey (*M. gallopavo* and *M. ocellata*) have a varied, omnivorous diet including fruits, flowers, seeds, nuts, insects, terrestrial gastropods, small lizards, and the leaves of shrubs, forbs and grasses (Hurst 1992; Leopold 1959; Williams, Baur and Eichholz 2010; Márquez Olivas et al. 2005). The majority of foods consumed by wild turkeys are C3 plants (e.g., fruits, shrubs, nuts, flowers and some grasses), but they also consume maize when it is available on the landscape (McRoberts 2014; Leopold 1948; Stearns 2010). Other C4 plants potentially consumed include wild or domestic amaranth (*Amaranthus* sp.), and various species of tropical grasses (e.g., *Paspalum conjugatum*). Consumption of CAM (Crassulacean Acid Metabolism) plants could also elevate wild turkey  $\delta^{13}\text{C}$  (Edwards and Walker 1983). CAM plants available to turkeys include bromeliads and cacti (e.g., *Opuntia* sp., *Yucca* sp.), but none of these are known to contribute significantly to the diet of wild Mesoamerican turkeys (Baur 2008; Leopold 1959; McRoberts 2014; Márquez Olivas et al. 2005). Although wild foraging turkeys in Mesoamerica may consume C4 and CAM plants that elevate the  $\delta^{13}\text{C}$  recorded in their skeletal tissues, the overall expectation is that domestic and captive-reared turkeys will exhibit higher  $\delta^{13}\text{C}$  than their wild counterparts due to their greater maize consumption and less varied diet. In contrast, wild turkeys should show diets consisting of pure C3 or mixed C3/C4 resources indicative of feeding in forested or mixed forest and agricultural field habitats.

The  $\delta^{13}\text{C}$  in Mesoamerican turkeys may also vary due to the “canopy effect”. In dense forested environments,  $^{13}\text{C}$ -depleted carbon dioxide ( $\text{CO}_2$ ) released through plant respiration is recycled at or near the forest floor, resulting in plant  $\delta^{13}\text{C}$  as low as -35‰ in the forest understory (van der Merwe and Medina 1991). Turkeys feed primarily on the ground and obtain much of their food by scratching in leaf litter (Williams, Baur and Eichholz 2010). We would thus expect to observe lower (more negative)  $\delta^{13}\text{C}$  in turkeys feeding in forested versus more open or disturbed habitats. Combined with the fact that drier, more open or anthropogenic habitats also



tend to contain more of the C4 or CAM plants available to turkeys, this further suggests that  $\delta^{13}\text{C}$  in wild turkeys may range from very low in forest-hunted turkeys to intermediate in turkeys feeding primarily along forest edges, or in more open/anthropogenic habitats. In addition to distinguishing between wild and captive turkeys, stable carbon isotope analysis may therefore be able to also identify the habitats where animals were acquired via hunting.

Regardless of habitat, the diverse diet of individual turkeys also may vary according to age, sex and season. For example, poults (0-12 weeks old) ingest large quantities of insects to obtain the protein needed to support early muscle and feather development (Flake et al. 2006). Prior to laying eggs, female turkeys may also consume large quantities of terrestrial snails to build up calcium reserves for egg shell formation (Beasom and Patee 1978). These types of specialized feeding patterns could alter the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of turkey diets, specifically resulting in elevated  $\delta^{15}\text{N}$  associated with increased animal protein consumption. Bulk bone sample  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  represent average dietary intake with slower turnover rates than blood, soft tissues or feathers (Hobson and Clark 1992; Vander Zanden et al. 2015), but the turnover rate in avian bone is poorly documented. Turnover may be as fast as ~200 days as observed in Japanese quail, or possibly much slower (Hobson and Clark 1992). Turnover rates may also occur at faster rates during sub-adult growth (Hedges et al. 2007; Hobson and Clark 1992), indicating that isotopic values in young birds could preferentially reflect the diet consumed during growth and development.

In contrast, more prolonged or permanent dietary variation between captive and wild turkeys could also result in different  $\delta^{15}\text{N}$  due to variation in relative carnivory or trophic level, consumption of human waste, or rearing conditions that promote protein or water stress. Trophic level effects associated with the transfer of nitrogen up the food chain-result in a 3-4‰ increase with each trophic step from producers to consumers (DeNiro and Epstein 1981; Schoeninger and DeNiro 1984). It is difficult to predict whether wild or captive turkeys would have differed in



imported from northern Mesoamerica shortly before or after death, and those raised on-site in the Maya lowlands. Strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) analysis provides a method for distinguishing between imported and locally-reared animals. Within Mesoamerica, the method has been used successfully to identify human migration (Buikstra et al. 2003; Price, Manzanilla, and Middleton 2000; Price 2006; Price et al. 2008; Price et al. 2010; White, Price, and Longstaffe 2007; Wright 2005a, 2005b), and animal trade (Thornton 2011). Since turkey domestication and rearing was widespread in northern Mesoamerica by the start of the Classic Period (Thornton and Emery, 2015; Valadez Azúa 2003) small numbers of domestic turkeys could have been imported into the Maya region from central Mexico, along with other trade goods arriving from this area such as green Pachuca obsidian and ceramic vessels (Golitzko and Feinman 2015; Sharer 2003).

Strontium isotope ratios can be used to distinguish between turkeys reared in the Maya lowlands and northern Mesoamerica (e.g., the central Mexican highlands) because the two regions have non-overlapping  $^{87}\text{Sr}/^{86}\text{Sr}$  based on the underlying geology. The Maya lowlands are underlain by limestone bedrock with strontium values that range from 0.7080–0.7092 ( $\bar{x}$  = 0.7088) in the northern lowlands of Mexico's Yucatan Peninsula, and from 0.7070–0.7085 ( $\bar{x}$  = 0.7077) in the southern/central lowlands of Belize and Guatemala (Hodell et al. 2004; Thornton 2011). In contrast,  $^{87}\text{Sr}/^{86}\text{Sr}$  in central Mexico range from 0.7046–0.7055 (Price, Manzanilla, and Middleton 2000; Price et al. 2008; White, Price, and Longstaffe 2007). Although central Mexico, where *M. gallopavo* is thought to have been first domesticated, is readily distinguishable from the Maya lowlands, similar  $^{87}\text{Sr}/^{86}\text{Sr}$  (0.7038–0.7053) are found in the Maya highlands of Guatemala and along the Pacific coast (Hodell et al. 2004; Price et al. 2008). Regardless of whether the exact origin of non-local turkeys can be determined, strontium isotope ratios can indicate whether turkeys were being husbanded onsite in the Maya lowlands, or obtained through long-distance trade with outside regions where they are indigenous.

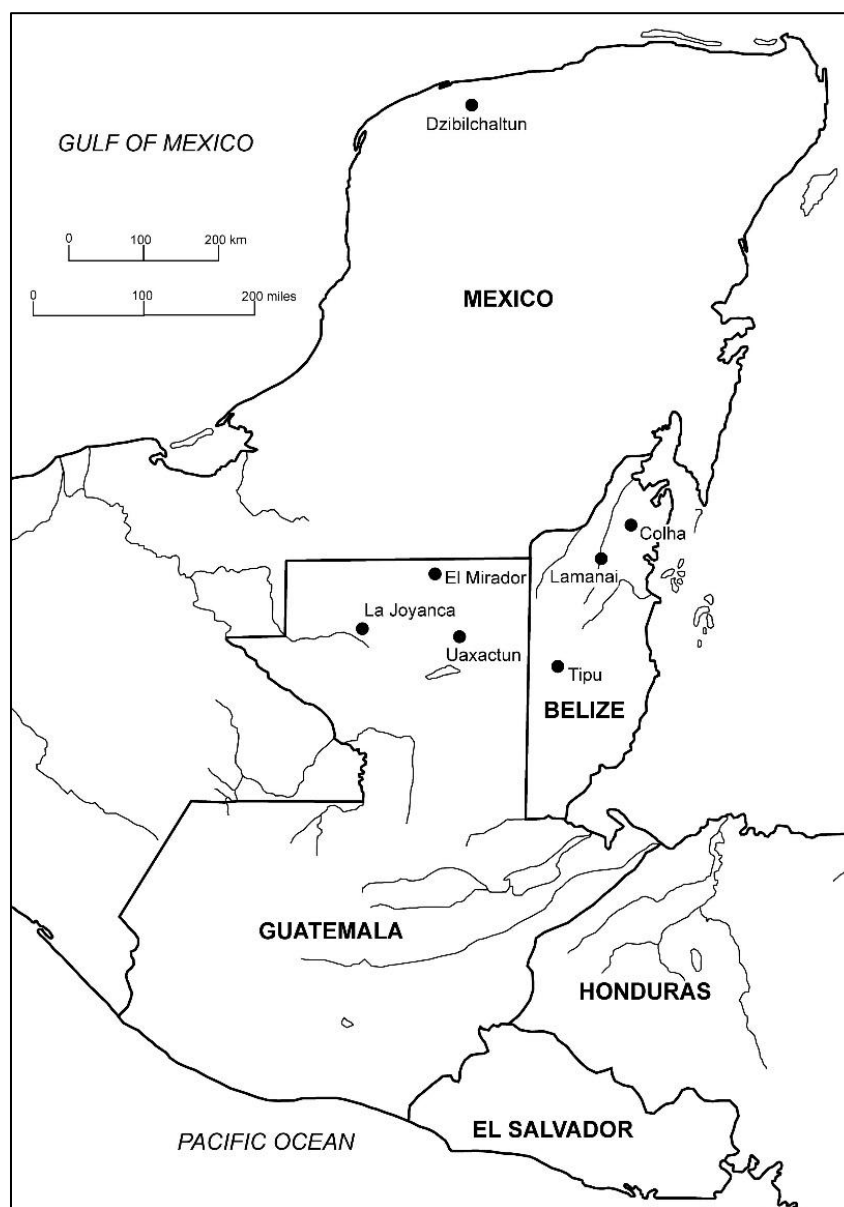
The preferred skeletal material for strontium isotope analysis is tooth enamel due to potential problems with diagenesis in bone, which is more porous (Hoppe, Koch, and Furutani 2003, Price et al. 1992, Sillen and Sealy 1995). Testing tooth enamel, however, is not an option for avian specimens. Standard chemical pretreatment procedures may eliminate or reduce potential Sr contamination in bone apatite, but the results of such procedures are not always effective or consistent (Budd et al. 2000; Hoppe, Koch and Furutani 2003). Despite the potential for sample contamination through intrusive Sr, the large difference in  $^{87}\text{Sr}/^{86}\text{Sr}$  between the Maya lowlands and the Mexican highlands means that importation from Central Mexico may be detectable even if some level of diagenetic alteration has occurred. Partial replacement of in-situ strontium would result in intermediate  $^{87}\text{Sr}/^{86}\text{Sr}$  (Hoppe, Koch and Furutani 2003) falling between those found in the southern Maya lowlands (0.7070-0.7085) and central Mexico (0.7046–0.7055) (Hodell et al. 2004; Price et al. 2010). Thus, in any situation short of complete replacement, importation of turkeys from central Mexico or some other isotopically similar highland area could be detectable.

## **Materials and Methods**

### *Study Sites and Samples*

Archaeological turkeys were selected from six lowland Maya sites, which provide both temporal and geographic coverage of the Maya lowlands (Figure 1). El Mirador, located in north-central Petén, Guatemala, represents one of the largest Preclassic centers built within the Maya lowlands. Late Preclassic *M. gallopavo* specimens identified at El Mirador are currently the oldest known examples of this species within the Maya region (Thornton et al. 2012). The samples were recovered along with other faunal remains between 1980 and 1982 during excavations led by Bruce Dahlin and Ray Matheny, and identified by Thornton and Emery at the

Florida Museum of Natural History (FLMNH). The turkey bones were associated with Late Preclassic ceramics in well-sealed, undisturbed contexts (Hansen 1990), and AMS radiocarbon ages from animal bones found in close association with the turkey remains confirm that the deposits are Preclassic (cal 327 BC–AD 54) (Thornton et al. 2012).



**Figure 1:** Map of Maya cultural area showing study sites. Map by Thornton.

The site of La Joyanca is also located in northern Petén, Guatemala, but is situated southwest of El Mirador along the Rio San Pedro Martir. Excavations led by Charlotte Arnauld (Arnauld et al. 2013) indicate that major occupation of the site primarily dates to the Classic Period (~AD 600-950). Identification of the La Joyanca faunal assemblage is in progress at the Florida Museum of Natural History under the supervision of Kitty Emery. Turkey bones selected for use in this study were excavated from two elite patio groups surrounding the site's main plaza.

Turkey samples from the Belizean sites of Lamanai, Tipu and Colha date to the later Postclassic and early Colonial periods (AD 1100-1520+). The fauna from Lamanai and Tipu originate from excavations led by Elizabeth Graham and David Pendergast (Graham 1991; Pendergast 1991), with original zooarchaeological analysis completed by Emery (1990, 1999). Faunal remains from both Lamanai and Tipu come from middens and structures located within and around the site's monumental core. Turkey bones from Colha were originally identified and curated by Elizabeth Wing at the Florida Museum of Natural History. The Colha remains originate from excavations at the site led by Norman Hammond (Hammond 1973).

The site of Dzibilchaltun is located in the drier northern Maya lowlands of Mexico's Yucatan Peninsula. Dzibilchaltun has an extremely long occupation history ranging from the Preclassic through the arrival of Spanish conquistadors. The turkey specimens we analyzed were excavated under the direction of E. Wyllys Andrews IV between 1956 and 1966. Original faunal identifications were completed by Elizabeth Wing and David Steadman at FLMNH (Wing and Steadman 1980:327). Turkey bones in our sample were excavated within the site's monumental core and include specimens recovered from Postclassic middens, two Late/Terminal Classic tombs, the 40m deep Cenote Xlakah, and unstratified contexts of unknown date. Bones recovered from the bottom of the ritual cenote are difficult to date securely, but they were found in

association with artifacts dating to the Late and Terminal Classic (Wing and Steadman 1980:326).

For each site, we controlled for sampling multiple bones from the same individual by restricting our sample whenever possible to a single skeletal element from a particular side of the body (e.g., all right tarsometatarsi). When skeletal elements were non-repeating at a site, we relied on element age and size comparisons, and selection of samples from temporally or spatially discrete proveniences. Although there is potential for food sharing across contemporary contexts, and transport of bones before or after primary deposition, we feel that extensive scattering of a single turkey carcass is unlikely, and that our age and size comparisons prevented redundant sampling of single individuals.

Age assessments were based on categories outlined by McCusick (1986:19). Sub-adult turkeys (<12 months) of both species were included in the sample to assess potential age-related dietary differences that could potentially confound species distinctions. A confounding factor in this study, however, is our inability to assign age classes to all specimens, and to accurately distinguish young adult (12-24 months) from adult (>24 months) turkeys. Age assessments were primarily limited by taphonomic factors such as specimen fragmentation and erosion.

Fifteen modern Ocellated Turkeys hunted near the modern community of Uaxactun, Petén, Guatemala were also sampled to provide baseline data for wild turkey diet. The sample included both male and female individuals collected by Erick Baur (a specialist in the modern galliforms and particularly the Ocellated Turkey) as part of a community-based sustainable hunting program within the Maya Biosphere Reserve (Baur et al. 2012; Williams, Baur and Eichholz 2010) and curated at the Florida Museum of Natural History. The area of capture is composed of a mixture of secondary or regenerating forest, and swidden agricultural fields. Turkeys were hunted 3–33km from human settlements in forested, disturbed and agricultural

habitats. All modern birds were clearly identified as Ocellated Turkeys by Baur based on external morphology while alive, and shortly following death.

### *Taxonomic Identification of Archaeological Specimens via Ancient DNA*

All archaeological turkey specimens in this study were identified through ancient DNA (aDNA) analysis given the difficulties of identification using osteology (see Emery et al., this volume) to ensure that our tests of isotopic data were linked to accurate identifications. Genetic analyses of the archaeological turkey specimen were conducted in the dedicated ancient DNA Laboratory at the University of York following strict contamination control protocols. A section of turkey bone was removed using a sterilized saw blade. The sub-sample was rinsed in HPLC water, irradiated under UV light for 30 minutes on two sides, and ground into powder. Approximately 50–150mg of bone powder was combined with 1 ml lysis buffer (0.5M EDTA, pH 8.0, 0.5 mg/ml proteinase K) and incubated overnight at 37°C. Following the protocol recommended in Gamba et al. (2015), the partially digested bone sample was centrifuged to consolidate the pellet and the supernatant removed; an additional 1.5ml of lysis buffer was added to the remaining pellet, and incubated for two days at 50°C until the pellet was completely demineralized. DNA extraction used only the supernatant from the second digestion followed a silica-spin column method proposed in Yang et al. (1998) as modified in Speller et al. (2010), with DNA eluted in 50ul of EB buffer. PCR amplifications targeted a 139bp fragment of the turkey mtDNA D-loop using published primers TK-F205/TK-R405 (Thornton et al. 2012) with PCR reactions and annealing conditions as described in Speller et al. 2010. This region is ideal for species identification as there are over 15 single nucleotide polymorphisms (SNPs) distinguishing the two species within a relatively short locus. Successfully amplified PCR products were sequenced using forward and/or reverse primers at Eurofins Genomics. Obtained sequences were edited and primer sequences removed using Chromas Pro (<http://technelysium.com.au/>), and multiple alignments were conducted through BioEdit (Hall,



1999). Following the removal of primer sequences, sequence were assigned to either *M. gallopavo* or *M. ocellata* through multiple alignments with reference sequences obtained through GenBank.

#### *Stable Isotope Analysis of Archaeological and Modern Specimens*

Sample preparation and isotopic analysis was conducted at the University of Florida in laboratory facilities house in the Departments of Anthropology and Geological Sciences. Bone collagen and apatite samples were prepared using standard procedures (Ambrose 1990; Koch et al. 1997) with slight modifications. Bone samples were manually cleaned and sonicated in distilled water to remove visible dirt and debris. After cleaning, archaeological bone samples were gently crushed with a mortar and pestle. Modern bone samples were resistant to manual breakage and were therefore ground using a Spex 6700 freezer/mill. Lipids were also removed from modern bone samples using a Dionex Accelerated Solvent Extractor (ASE). Samples were infused with petroleum ether, heated to 100°C, soaked for 5 min, rinsed, and dried with compressed nitrogen. The removal of lipids from modern samples was a necessary because residual lipids may skew bone collagen  $\delta^{13}\text{C}$  by up to 7‰ (Evershed et al. 1995; Liden et al. 1995). Archaeological samples were not subjected to lipid treatment because lipids are largely absent or highly degraded in ancient samples.

Bone collagen was obtained by demineralizing ~0.25–0.5g of crushed cortical bone in 0.2M hydrochloric acid (HCl), followed by treatment with 0.125M sodium hydroxide (NaOH) to remove organic contaminants. Samples were then solubilized with  $10^{-3}\text{M}$  HCl at 90°C and centrifuged to remove particulate contaminants. Bone apatite was obtained by soaking ~0.05g of finely crushed cortical bone in a 2% sodium hypochlorite (NaClO) solution for 16 hours to dissolve sample organics. After being rinsed to neutral, non-biogenic carbonates were removed by soaking the samples for four hours in 0.1M acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ).

Stable isotope ratios were measured on a Finnigan-MAT DeltaPlus isotope ratio mass spectrometer. All collagen samples were also combusted in a Carlo Erba NA1500 CNS elemental analyzer to obtain C/N ratios as a measure of sample integrity (Ambrose 1990). To maintain analytical accuracy, isotope data were accepted only when C/N ratios fell between 2.8 and 3.8 (Ambrose and DeNiro 1986) and when yield was greater than 1% of dry weight. Carbon ratios preserved in bone apatite were analyzed on a Micromass PRISM Series II isotope ratio mass spectrometer. Accuracy of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were confirmed through repeated ( $n>10$ ) analysis of international laboratory standards (NBS-19 and USGS-40). Reproducibility was better than  $\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}_{\text{ap}}$ ,  $\delta^{13}\text{C}_{\text{coll}}$  and  $\delta^{15}\text{N}$ .

Stable isotope ratios for carbon and nitrogen are reported in delta notation ( $\delta$ ) as parts per thousand (‰, per mil) which constitutes the difference of the sample from a standard reference material as outlined in the following equation:

$$\delta (\text{‰}) = [(R_{\text{sample}}) / (R_{\text{standard}}) - 1] \times 1000$$

where R is the ratio of the heavier isotope to the lighter isotope. The established standard for stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) is Vienna Pee Dee Belemnite (v-PDB) and for stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) is atmospheric air (AIR). All modern  $\delta^{13}\text{C}$  are reported with a +1.5‰ correction factor to account for modern enrichment of atmospheric  $^{12}\text{C}$  caused by the burning of fossil fuels (Friedli et al. 1986; Marino and McElroy 1991). By adjusting the modern samples by +1.5‰, modern values are directly comparable to those obtained from archaeological samples.

Bones selected for strontium isotope analysis were cleaned and subsampled using a fine-tipped dental drill. Sample pretreatment and strontium isolation was then conducted in a class 1000 clean lab. Samples were pretreated for 30 minutes in a 5% acetic acid solution to remove post-depositional contaminants and rinsed to neutral with 4x distilled water. This method is generally considered to be an effective means of removing contaminants (e.g., Nielsen-Marsh and Hedges 2000; Price et al. 1992; Sillen and Sealy 1995). After pretreatment, the samples were

transferred to sterile Teflon beakers and hot-digested in 3ml of 50% nitric acid ( $\text{HNO}_3$  optima). Samples were then loaded into cation exchange columns packed with strontium-selective crown ether resin to isolate strontium from other ions. Sample  $^{87}\text{Sr}/^{86}\text{Sr}$  was measured with at Micromass Sector 54 thermal ionization mass spectrometer (TIMS). Multiple samples of the strontium standard NBS-987 were run to confirm instrument accuracy. External precision of analysis was  $\pm 0.00002$  (2 sigma absolute) based on 314 analyses of NBS-987. Strontium isotope ratios are reported as an absolute value against the established standard NBS-987. Strontium isotope analysis was only applied to the three Late Preclassic turkeys from El Mirador that current pre-date most other examples of this species in the Maya lowlands (Thornton et al. 2012; Thornton and Emery 2015).

## Results and Discussion

### *Carbon and Nitrogen Isotopes: Species Diet Comparisons*

All 40 archaeological specimens successfully identified to the species level via aDNA yielded enough collagen for testing, and acceptable C:N ratios ranging from 3.2–3.4 (Table 1). In all but two samples (#2697, 3184) morphological identifications were consistent with the aDNA results. Based on the aDNA identifications, the isotopic sample is composed of 27 *M. gallopavo* and 13 *M. ocellata* archaeological specimens<sup>1</sup>, for a total assemblage of 55 birds including the modern *M. ocellata*.

Isotope data for our tests of 15 modern Ocellated Turkeys reflect typical C3-based terrestrial diets (average  $\delta^{13}\text{C}_{\text{co}} = -20.2\%$ ; average  $\delta^{15}\text{N} = 5.8\%$ ) (Table 1, Figure 2). Slightly higher  $\delta^{13}\text{C}_{\text{co}}$  are observed in modern Ocellated Turkeys hunted in agricultural, heavily disturbed (regenerating), and scrub habitats ( $n=7$ , avg.  $\delta^{13}\text{C}_{\text{co}} = -19.95$ ) than in forested areas ( $n=8$ , avg.

<sup>1</sup> The 40 archaeological specimens with successful DNA identifications were selected from a larger dataset which included multiple failed PCR amplifications. The overall success rate for DNA amplifications from the assemblage as a whole was ~75%.

$\delta^{13}\text{C}_{\text{co}} = -20.33$ ), but the difference was not statistically significant (Student's t-test:  $t(8)=2.31$  ,  $p=0.32$ ). Thus we can state that, even in areas near human settlements and agricultural fields, wild turkeys in the Maya lowlands consume a diet consisting of primarily C3 resources.

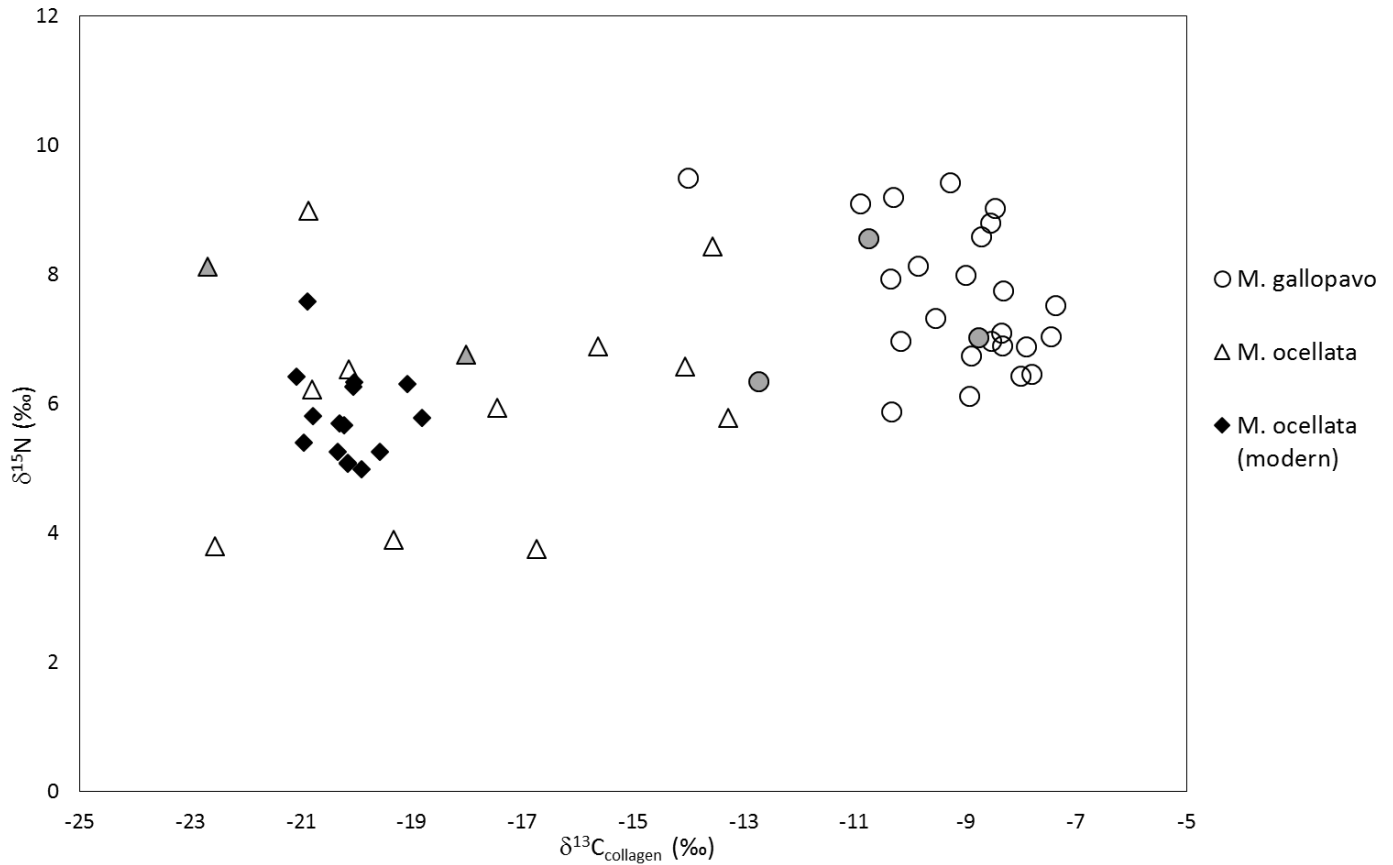
The archaeological Ocellated Turkeys of this test exhibited significantly higher average  $\delta^{13}\text{C}_{\text{co}}$  (avg.: -18.1‰; Student's t-test:  $t(13)=2.16$ ,  $p<0.05$ ) than the modern Ocellated Turkeys (Table 1, Figure 2). The difference in mean  $\delta^{13}\text{C}_{\text{co}}$  primarily results from the greater upper range of values observed in the archaeological sample ( $\delta^{13}\text{C}_{\text{co}}$ : -13.3 to -22.7‰). While eight of the archaeological Ocellated Turkeys consumed predominantly C3-based diets similar to modern Ocellated Turkeys, five archaeological specimens have intermediate  $\delta^{13}\text{C}_{\text{co}}$  (-17 to -13‰) indicative of a mixed diet containing both C3 and C4 resources. This pattern is also observable in  $\delta^{13}\text{C}$  obtained from bone apatite ( $\delta^{13}\text{C}_{\text{ap}}$ ) (Figure 3) although the results are considered less reliable than  $\delta^{13}\text{C}$  from bone collagen due to greater potential for diagenesis (Lee-Thorp and Sponheimer 2003). The archaeological Ocellated Turkeys with elevated  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{13}\text{C}_{\text{ap}}$  are not restricted to any particular site or region, and instead occur alongside Ocellated Turkeys with C3-based diets. This suggests that elevated  $\delta^{13}\text{C}$  does not result simply from natural regional variation in vegetation between the wetter southern and drier northern Maya Lowlands, and instead reflect differences in individual turkey diet. The archaeological Ocellated Turkey specimens also exhibit greater variation in  $\delta^{15}\text{N}$  than their modern counterparts, but the difference is not statistically significant (Student's t-test:  $t(15)=2.13$ ,  $p=0.35$ ) (Table 1, Figure 2).

**Table 1:** Stable isotope data from archaeological and modern turkeys from the Maya region

Lab #	Site	Chronology or Location	Age (months)	Element	$\delta^{13}\text{C}_{\text{ap}}$ (‰) <sup>a</sup>	$\delta^{13}\text{C}_{\text{co}}$ (‰) <sup>a</sup>	$\delta^{15}\text{N}$ (‰)	Wt %C	Wt %N	C:N	Collagen yield (%wt)	<sup>87</sup> Sr/ <sup>86</sup> Sr	mtDNA identif. <sup>b</sup>
Archaeological ( <i>M. gallopavo</i> ):													
2696	El Mirador	Late Preclassic	~12 mos.	tarsometa.	-3.8	-10.9	9.1	32.2	11.0	3.4	2.4	0.70792	- <sup>b</sup>
2701	El Mirador	Late Preclassic	-	ulna	-3.9	-10.4	7.9	36.1	12.7	3.3	3.1	0.70790	- <sup>b</sup>
2702	El Mirador	Late Preclassic	12+ mos.	ulna	-6.2	-14.0	9.5	35.0	12.2	3.3	2.1	0.70790	- <sup>b</sup>
2367	Dzibilchaltun	No date	-	humerus	-0.6	-8.7	8.6	45.0	16.0	3.3	12.5	-	MG
8	Dzibilchaltun	No date	-	carpometa.	-1.8	-8.6	8.8	43.8	16.0	3.2	13.3	-	MG
12	Dzibilchaltun	No date	12+ mos.	femur	-2.1	-10.3	9.2	42.3	15.1	3.3	10.4	-	MG
2384*	Dzibilchaltun	Postclassic	3-6 mos.	femur	-8.1	-12.7	6.3	46.4	16.3	3.3	17.8	-	MG
2400	Dzibilchaltun	Postclassic	12+ mos.	femur	-2.4	-9.9	8.1	47.5	16.9	3.3	12.8	-	MG
71*	Lamanai	Postclassic/Colonial	3-6 mos.	tibiotars.	-6.6	-8.5	7.0	37.9	13.3	3.3	2.6	-	MG
86	Lamanai	Colonial	12+ mos.	tarsometa	-5.0	-9.3	9.4	14.2	5.0	3.3	9.9	-	MG
98	Lamanai	Colonial	12+ mos.	tarsometa	-4.4	-9.0	8.0	38.9	13.6	3.3	8.0	-	MG
122	Lamanai	Postclassic/Colonial	12+ mos.	tarsometa	-5.1	-8.8	7.0	36.9	13.2	3.3	3.4	-	MG
129	Lamanai	Postclassic/Colonial	12+ mos.	tarsometa	-5.6	-8.3	6.9	39.7	14.1	3.3	9.5	-	MG
195	Lamanai	Postclassic/Colonial	12+ mos.	tarsometa	-5.4	-8.5	9.0	38.5	13.6	3.3	11.4	-	MG
200	Lamanai	Postclassic/Colonial	12+ mos.	tarsometa	-4.8	-8.3	7.8	40.4	14.4	3.3	13.8	-	MG
256	Lamanai	Postclassic/Colonial	12+ mos.	tarsometa	-6.1	-7.5	7.1	38.2	13.3	3.4	6.9	-	MG
259	Lamanai	Postclassic/Colonial	12+ mos.	tarsometa	-4.8	-10.2	7.0	41.4	14.5	3.3	12.4	-	MG
2753	Tipu	Postclassic	-	tarsometa	-4.2	-8.9	6.7	41.5	14.7	3.3	18.0	-	MG
2851	Tipu	Colonial	12+ mos.	tarsometa	-5.1	-10.3	5.9	40.3	14.0	3.4	17.4	-	MG
2853	Tipu	Colonial	~9+ mos.	tarsometa	-4.6	-8.9	6.1	39.9	13.9	3.4	18.0	-	MG
3152	Tipu	Colonial	12+ mos.	tarsometa	-4.4	-9.5	7.3	41.2	14.5	3.3	16.4	-	MG
3184	Tipu	Colonial	12+ mos.	tarsometa	-5.0	-7.8	6.5	41.1	14.6	3.3	15.8	-	MG
2510	Colha	Postclassic	-	humerus	-3.6	-8.4	7.1	40.3	14.4	3.3	13.9	-	MG
2525*	Colha	Postclassic	3-6 mos.	tibiotars.	-7.8	-10.7	8.6	39.5	13.6	3.4	4.3	-	MG
2529	Colha	Postclassic	-	ulna	-6.5	-7.9	6.9	41.1	14.8	3.3	10.1	-	MG
2538	Colha	Postclassic	-	ulna	-5.7	-8	6.4	41.2	14.7	3.3	7.9	-	MG
2541	Colha	Postclassic	12+mos.	coracoid	-6.4	-7.4	7.5	41.5	14.7	3.3	6.4	-	MG
Mean:					-4.8	-9.3	7.6						
Archaeological ( <i>M. ocellata</i> ):													
2697	El Mirador	Late Preclassic	-	femur	-12.7	-20.1	6.5	41.5	14.8	3.3	20.9	-	MOC
13	Dzibilchaltun	Late/Terminal Classic	12+ mos.	ulna	-5.8	-13.6	8.4	45.2	16.1	3.3	12.6	-	MOC
2371	Dzibilchaltun	Terminal Classic	12+ mos.	ulna	-12.0	-20.9	9.0	43.8	15.3	3.3	14.1	-	MOC
2388*	Dzibilchaltun	Postclassic	6-11 mos.	coracoid	-13.1	-22.7	8.1	48.2	17.0	3.3	19.2	-	MOC
2381	Dzibilchaltun	Postclassic	-	tarsometa.	-13.4	-20.8	6.2	47.1	16.4	3.4	17.1	-	MOC
2380*	Dzibilchaltun	Postclassic	3-5 mos.	coracoid	-10.4	-18.0	6.8	47.5	16.5	3.4	16.3	-	MOC
2402	Dzibilchaltun	Classic?	12+ mos.	humerus	-7.2	-15.6	6.9	48.0	17.1	3.3	12.8	-	MOC
2428	La Joyanca	Classic	12+ mos.	ulna	-12.7	-17.5	5.9	42.0	14.9	3.3	12.7	-	MOC
2433	La Joyanca	Classic	12+ mos.	carpometa.	-13.1	-19.3	3.9	39.0	13.8	3.3	9.8	-	MOC
2434	La Joyanca	Classic	-	humerus	-7.3	-16.8	3.8	40.4	14.6	3.2	7.3	-	MOC
450	Lamanai	Early Postclassic	12+ mos.	tarsometa.	-6.4	-13.3	5.8	40.3	14.2	3.3	11.0	-	MOC
458	Lamanai	Early Postclassic	-	carpometa.	-7.4	-14.1	6.6	38.2	13.1	3.4	2.1	-	MOC
2757	Tipu	Postclassic	-	coracoid	-12.1	-22.6	3.8	40.6	14.6	3.3	13.5	-	MOC
Mean:					-10.3	-18.1	6.3						
Modern ( <i>M. ocellata</i> ) <sup>a,c</sup> :													
41686	Guatemala	active milpa	12+ mos.	tarsometa.	-12.2	-19.6	5.3	42.5	15.0	3.3	23.9	-	-
41667	Guatemala	regenerating milpa	12+ mos.	tarsometa.	-13.7	-21.1	6.4	40.8	14.4	3.3	29.4	0.70781	-
41668	Guatemala	regenerating milpa	12+ mos.	tarsometa.	-13.7	-20.9	7.6	43.8	15.7	3.3	27.3	-	-
41674	Guatemala	regenerating milpa	12+ mos.	tarsometa.	-11.2	-18.8	5.8	39.3	14.1	3.2	29.0	0.70799	-
41688	Guatemala	regenerating milpa	12+ mos.	tarsometa.	-13.5	-20.2	5.7	41.0	14.3	3.3	9.6	-	-
41719	Guatemala	regenerating milpa	12+ mos.	tarsometa.	-15.0	-20.1	6.3	42.0	14.7	3.3	22.4	-	-
41665	Guatemala	scrub habitat	12+ mos.	tarsometa.	-12.2	-19.1	6.3	38.3	13.8	3.2	21.1	0.70786	-
41670	Guatemala	upland forest	12+ mos.	tarsometa.	-14.2	-20.9	5.4	41.5	15.0	3.2	27.1	0.70793	-
41673	Guatemala	upland forest	12+ mos.	tarsometa.	-12.7	-20.2	5.1	42.1	15.3	3.2	26.8	-	-
41676	Guatemala	upland forest	12+ mos.	tarsometa.	-13.5	-19.9	5.0	42.3	14.9	3.3	24.0	-	-
41696	Guatemala	upland forest	12+ mos.	tarsometa.	-14.8	-20.3	5.7	41.7	14.8	3.3	23.2	-	-
41702	Guatemala	upland forest	12+ mos.	tarsometa.	-14.3	-20.3	5.3	42.5	15.0	3.3	23.9	-	-
41712	Guatemala	upland forest	12+ mos.	tarsometa.	-12.2	-20.8	5.8	42.6	15.0	3.3	24.7	-	-
41713	Guatemala	upland forest	12+ mos.	tarsometa.	-13.4	-20.1	6.3	42.4	15.1	3.3	24.1	-	-
41717	Guatemala	upland forest	12+ mos.	tarsometa.	-14.1	-20.2	5.1	41.4	14.6	3.3	23.3	-	-
Mean:					-13.4	-20.2	5.8						

<sup>a</sup> Modern  $\delta^{13}\text{C}$  values have been adjusted by +1.5‰ to make them comparable to archaeological  $\delta^{13}\text{C}$  (Marino and McElroy 1991)<sup>b</sup> MG = *M. gallopavo*, MOC = *M. ocellata*, - = not tested; El Mirador aDNA identification (n=3) from Thornton et al. (2012).<sup>c</sup> Modern sample numbers refer to FLMNH-Ornithology catalog numbers.

\* subadult (&lt; 12 month of age)



**Figure 2:**  $\delta^{13}\text{C}_{\text{collagen}}$  and  $\delta^{15}\text{N}$  for archaeological (open/gray symbols) and modern (black symbols) turkeys from the Maya region. Gray shading denotes sub-adult archaeological turkeys.

The archaeological *M. gallopavo* specimens have much higher  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{13}\text{C}_{\text{ap}}$ , averaging -9.3 and -4.8‰, respectively than archaeological *M. ocellata* (Table 1, Figure 2). These values indicate significant consumption of C4 foods, which largely distinguishes *M. gallopavo* from both modern and archaeological Ocellated Turkeys. The isotopic separation between the two species, however, is stronger in bone collagen (Figure 2) than it is in bone apatite (Figure 3). Two archaeological *M. gallopavo* (#2702, 2384) have lower  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{13}\text{C}_{\text{ap}}$  that approach or partially overlap the highest Ocellated Turkey values, and reflect a more mixed diet of C3 and C4 resources. The *M. gallopavo* with the lowest  $\delta^{13}\text{C}_{\text{co}}$  is an adult (12+ mos.)

turkey from Late Preclassic deposits at El Mirador. The other two contemporary *M. gallopavo* from the site show elevated  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{13}\text{C}_{\text{ap}}$  indicating greater consumption of C4 foods. No difference is observed in *M. gallopavo* from Postclassic versus Colonial contexts, suggesting that potential changes in domestic turkey diet resulting from European Colonialism cannot account for the overall pattern of  $\delta^{13}\text{C}$  species separation (Table 2). Instead, domestic turkeys in the Maya lowlands appear to have been maize provisioned from their earlier introduction through Colonial times.

**Table 2: Mean Stable Isotope Values of Archaeological *Meleagris gallopavo* by Time Period**

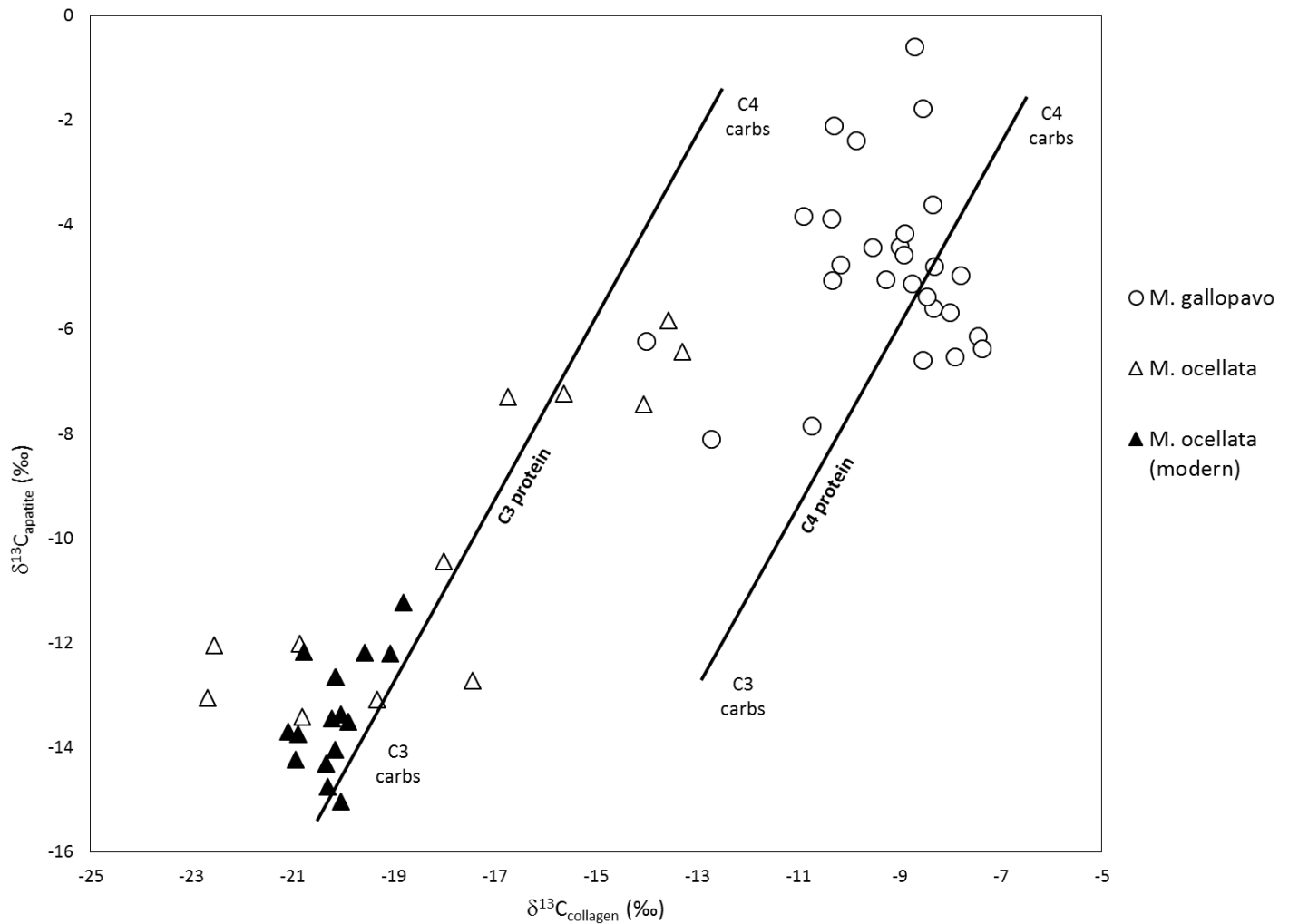
Time Period (# samples)	$\delta^{13}\text{C}_{\text{ap}}$	$\delta^{13}\text{C}_{\text{co}}$	$\delta^{15}\text{N}$	Sites Included
Preclassic (n=3)	-4.6	-11.8	8.8	El Mirador
Postclassic (n=8)	-5.6	-9.2	7.2	Dzibilchaltun, Tipu, Colha
Colonial (n=6)	-4.8	-9.1	7.2	Lamanai, Tipu

All three sub-adult *M. gallopavo* (Table 1) fall at the lower  $\delta^{13}\text{C}_{\text{ap}}$  range for this species (Figure 3), but only one of the sub-adults also has a lower  $\delta^{13}\text{C}_{\text{co}}$  that does not cluster as well with the other adult and un-aged *M. gallopavo* (Figure 2). The two sub-adult Ocellated Turkeys (Table 1) do not vary in a consistent pattern from the other C3 resource eating *M. ocellata*. Average to slightly higher  $\delta^{15}\text{N}$  are observed in the sub-adults of both species, but a consistent age-based pattern is not apparent. Therefore, although age-based dietary differences have the potential to affect individual turkey  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , the effect appears to be weak and it does not obscure dietary differences observed between the two species. Our sample size, however, is very small due to our inability to assign age classes to all specimens. The potential for lower  $\delta^{13}\text{C}$  in subadult *M. gallopavo*, especially in bone apatite, warrants further investigation in future studies as this may reflect decreased maize consumption during the periods of early growth and development. For domestic flocks where culling may have occurred shortly after the birds reached adult size (10-12 months old), the potential for age-related isotopic effects could be significant.





$\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{13}\text{C}_{\text{ap}}$  are highly correlated ( $r^2=0.92$ ; 0.86 archaeological samples only). When plotted against each other (Figure 4), and compared with dietary models generated by Kellner and Schoeninger (2007), it is evident that most *M. gallopavo* fall towards the upper end of the C4-protein line, indicating primarily C4 sources for both dietary protein and non-protein. Although other C4 plants cannot be ruled out, it is very likely that domestic turkeys in the Maya Lowlands fed extensively on maize based on modern ethnographic data (Gotz and García Paz, this volume), and evidence of maize consumption in other captive or domestic animals in Mesoamerica (e.g., Sugiyama, Somerville and Schoeninger 2015; Tykot, van der Merwe and Hammond 1996; White et al. 2001). In terms of dietary protein, many of the insects, land snails and small lizards consumed by domestic turkeys may have had elevated  $\delta^{13}\text{C}$  based on their feeding within houses, house lots, and nearby middens and latrines which likely contained substantial amounts of the dietary staple maize. Turkeys could also have fed directly on human/animal waste, which is as an additional potential source of C4-based protein. Alternately, higher  $\delta^{13}\text{C}_{\text{co}}$  in domestic turkeys could result from a low protein diet. Under typical conditions, bone collagen preferentially reflects the  $\delta^{13}\text{C}$  composition of dietary protein, but when animal diets are protein deficient, more carbon from non-protein sources are used for collagen synthesis (Ambrose and Norr 1993). Regardless, the heavier  $\delta^{13}\text{C}_{\text{co}}$  of domestic turkeys, either alone or plotted against  $\delta^{13}\text{C}_{\text{ap}}$ , distinguishes them in most cases from Ocellated Turkeys.



Comparison of  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{13}\text{C}_{\text{ap}}$  also informs our interpretation of the five archaeological Ocellated Turkeys with intermediate, or elevated  $\delta^{13}\text{C}$ . These individuals fall either on or slightly closer to the C3-protein line, indicating that they consumed a mix of C3 and C4 plants, but that a larger amount of their protein was C3-based in comparison to domestic turkeys (Figure 4). Although these individuals consumed a substantial amount of C4-plants (likely maize), we would expect their isotopic signatures to overlap more with *M. gallopavo* if they were also bred and reared in captivity from birth alongside domestic turkeys. One possible explanation for the observed pattern is that select Ocellated Turkeys were captured from the wild and reared in captivity for a portion of their lives, resulting in a mid-life dietary shift. Unfortunately, a lack of well-documented turnover or equilibrium rates for bird bone collagen and apatite precludes a determination of how long wild turkeys previously foraging on C3 resources would need to be reared on maize to result in the observed intermediate  $\delta^{13}\text{C}$ . It would, however, likely take at least a few months for a shift to occur based on known turnover rates in the much smaller-bodied Japanese quail (Hobson and Clark 1992).

An alternate-explanation is that the intermediate group of archaeological Ocellated Turkeys includes wild individuals that fed in forested habitats, as well as in agricultural fields, or on maize bait provided by hunters. Isotopic data from other species including white-tailed deer (*Odocoileus virginianus*) and peccary (Tayassuidae) in the Maya region support this interpretation. While the majority of deer and peccary from Maya sites have C3-based diets, substantial consumption of C4 plants has been reported for select individuals with  $\delta^{13}\text{C}_{\text{co}}$  ranging from approximately -17.5 to -7.3‰ (Emery, Wright and Schwarcz 2000; Tykot et al. 1996; White et al. 2004; Wright 2006: 192). Although deer and peccary with very high  $\delta^{13}\text{C}_{\text{co}}$  (-13 to -7 ‰) could indicate captive rearing, the majority of mixed C3/C4 feeding deer and peccary have only slightly elevated  $\delta^{13}\text{C}_{\text{co}}$  ranging from -15 to -18‰, which could result from occasional feeding in maize agricultural fields. We did not observe any birds with intermediate  $\delta^{13}\text{C}$  in our

sample of modern Ocellated Turkeys from Petén, Guatemala, despite the inclusion of individuals that would be classified as garden-hunted (i.e., killed while feeding within and around the edges of active or regenerating agricultural fields). The low  $\delta^{13}\text{C}_{\text{co}}$  observed in our modern Ocellated Turkeys may be due to the large amount of forest still present in the region where they were hunted despite the presence of *milpas* and other anthropogenic habitats. As others have suggested, population levels and the degree of deforestation may have been higher in some regions of the Maya lowlands during ancient times (Cook et al. 2012).

The combined  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results document the utility of stable isotope analysis to identify wild vs. captive-reared and/or domestic turkeys based on dietary differences. Isotopic variation among archaeological Ocellated Turkeys from pure C3 to mixed C3/C4 feeders further suggests that the method is able to identify whether wild Ocellated Turkeys were hunted from forested or more open/anthropogenic habitats. Expanded testing along these lines is needed to determine whether the elevated  $\delta^{13}\text{C}$  in bone collagen and apatite of Ocellated Turkeys and other common prey species could alternately indicate some degree of human provisioning, management, or confinement.

#### *Strontium Isotopes: Non-local Imports vs. On-site Rearing*

All three *M. gallopavo* from Late Preclassic deposits at El Mirador yielded  $^{87}\text{Sr}/^{86}\text{Sr}$  (0.7078-0.7079) matching those previously reported from archaeological fauna at El Mirador (Thornton 2011). Similar values were also found in our sample of modern Ocellated Turkeys hunted near Uaxactun, Petén, Guatemala (Table 1). Diagenetic tests (e.g., Ca/P ratios, crystallinity indices) have not yet been conducted, but the current results do not support the idea that the non-local *M. gallopavo* individuals were imported from central Mexico shortly before death. Our results are limited by the fact that complete replacement of the in-situ strontium could result in local values being exhibited in a non-local specimen (Budd et al. 2000), but, in any

situation short of complete replacement, importation of turkeys from central Mexico or some other isotopically similar highland locale would have been detected. A final caveat is that although we can tentatively conclude that the early *M. gallopavo* found at El Mirador were reared outside of central Mexico, we cannot rule out their origin from another non-local region within the southern Maya lowlands, or possibly even Oaxaca (Price et al. 2010) that exhibits similar  $^{87}\text{Sr}/^{86}\text{Sr}$  to those found at El Mirador.

The potential for sample contamination via diagenesis severely hampers our ability to apply strontium isotope analysis to distinguish between long-distance turkey trade and on-site rearing within the Maya lowlands since bone apatite is the only skeletal tissue available for analysis. Diagenesis tests such as Ca/P ratios or crystallinity indices may be employed, but these methods may not detect all sources of diagenetic contamination (Budd et al. 2000). Another potential route may be to conduct multi-proxy isotopic analyses that combine strontium and oxygen ( $\delta^{18}\text{O}_{\text{phosphate}}$ ) isotopes. Bone phosphate  $\delta^{18}\text{O}$  is more resistant though not impervious to diagenetic alteration (Sharp, Atudorei and Furrer 2000), but the combined proxies would provide stronger evidence than a single data point. The costs of such analyses, and the amount of bone required to run multiple isotopic tests, however, may be prohibitive. Researchers must therefore consider whether generating valuable, but potentially unreliable data is worth the cost of expensive laboratory testing.

## **Conclusions**

Stable isotope analysis has the potential to yield substantial insights into the history of turkey husbandry and domestication in Mesoamerica. The most promising application is the use of stable carbon and nitrogen isotopes to distinguish between wild and captive-reared/domestic individuals. Morphometric features for such distinctions are often lacking, and both species may have been raised in captivity. Stable isotope analysis thus provides an additional and

underutilized tool that both complements and expands the capabilities of other currently employed methods including skeletal morphology, osteometrics and aDNA analysis.

The isotope data document that *M. gallopavo* introduced to the Maya region consumed a diet heavy in C4 resources, which contrasts with Ocellated turkeys that consumed primarily C3 or mixture of both C3 and C4 foods. Isotopic separation between the species likely reflects domestic turkeys' greater access to a C4-based food web within human settlements including human-provided maize fodder, household and human/animal waste, and various invertebrates feeding within the same anthropogenic environment. If further testing confirms isotopic separation of the two species, stable carbon and nitrogen isotopes may be used to distinguish between wild and husbanded turkeys in Maya faunal assemblages. Ancient DNA analysis would still be required to identify the species of turkeys with intermediate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , but stable isotope analysis could serve as a means of identifying and narrowing down the number of individuals that would need to be subjected to costly aDNA analyses.

We also tentatively conclude that the lower and wider range of  $\delta^{13}\text{C}_{\text{coll/ap}}$  observed in archaeological Ocellated Turkeys can best be explained by variation in hunting habitat (i.e., forest vs. field) rather than captive rearing of select individuals, but this suggestion requires further investigation. Expanded isotopic testing of archaeological turkeys, and wild modern turkeys from Mesoamerica may be able to resolve this question. Local extirpation of wild turkeys from many parts of Mesoamerica makes it difficult to obtain wild hunted turkeys from a variety of habitats ranging from heavily forested to highly anthropogenic, but modern animals may provide the best evidence for the range of  $\delta^{13}\text{C}$  expected in garden-hunted animals. The question of whether Ocellated Turkeys and other prey species such as deer were managed or reared by the ancient Maya is non-trivial as it has major implications for the degree of control ancient populations maintained over their environment and subsistence resources.

Our strontium isotope analysis of the earliest known introduced *M. gallopavo* in the Maya region suggests that they were reared locally for an extended period of time, rather than being imported from central Mexico shortly before death. This statement is based on a number of assumptions regarding a lack of extensive diagenetic alteration of the samples' original  $^{87}\text{Sr}/^{86}\text{Sr}$  signature, and where non-local turkeys likely originated. More testing is required to confirm this statement, but we report the results to support our methodological claim that stable isotope analysis may be the only way to identify whether introduced domestic turkeys represent Maya investment in turkey rearing, or occasional non-local imports. Despite the limitations of this approach, expanded isotopic sourcing of Mesoamerican turkeys may be the only way to address this question.

In sum, this study demonstrates the utility of stable isotope analysis in studying Mesoamerican turkey husbandry, but combined, multi-proxy studies are likely the most powerful methodological approach. Coupled isotopic and aDNA studies provide a means to identify species presence and diet, which is difficult to impossible to determine from morphometric traits alone. Skeletal morphology and metrics, however, provide useful information about the effects of domestication on species biology. Stable isotope analysis of Mesoamerican turkeys should therefore be conducted alongside complementary morphometric, and whenever possible, genetic analyses to generate the most complete record of turkey acquisition, husbandry and use.

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