Carbon and nitrogen stable isotope analysis using human bones and hair from Philippine burial sites

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Abstract

In order to understand the diet of the past human population in the Philippines, we analyzed stable isotopic compositions of 61 individuals from five burial sites with different geographical locations, environmental conditions and subsistence strategies. The carbon stable isotope ratio (δ13C) and nitrogen stable isotope ratio (δ15N) of bone collagen and hair were used to estimate dietary protein resources of the population. The Batanes site is located at about 200 km north from the north coast of Luzon and about 150 km south from Taiwan with the so-called boat-shaped and jar burials (355±70 BP). Results of δ13C (-16.6±1.3‰) and δ15N (9.8±1.7‰) values for bone collagen from 21 individuals showed that dietary protein resources had more dependence on marine ecosystems than that on terrestrial resources. The isotopic values vary due to the burial types: both δ13C and δ15N values of the specimens from the boat-shaped and jar burials were higher than those from both the primer pit burial. In the Lal-lo site (N=11) of Northern Luzon which is famous for its huge shell midden (3400-1000 years ago), δ13C and δ15N values were -19±1.2‰ and 9.7±1.6‰ respectively. Results showed a diet more dependent on terrestrial resources than others, because freshwater shells indicated terrestrial values. Hair samples from the 13th century old mummies (N=3) at Kabayan in Benguet Mountain Province, northwest of Luzon had the average value of -17±1.0‰ for δ13C and 8.0±1.0‰ for δ15N reflecting a diet dependent on both terrestrial and aquatic resources. The average value of the Santa Ana site (N=8) in Manila (about 1095 AD) were -15.2±3.3‰ for δ13C and 10.4±0.7‰ for δ15N values, suggesting highest dependence on marine ecosystem among the 5 sites. Romblon site (N=18) in Central Philippines (13th-14th centuries) yielded the average δ13C value of -17.4±2.3‰ and δ15N value of 10.1±1.1‰ indicating protein resources dependent on both terrestrial and marine ecosystems. In all the sites, the δ13C and δ15N values lack gender-based difference.

Keywords: carbon stable isotope, nitrogen stable isotope, burial sites, human bones, hair sample, mummy, Philippines

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Introduction

The Philippine archipelago is composed of 7,107 islands bounded by the Pacific Ocean on the east, the South China Sea on the west and north, and the Celebes Sea on the south. To its north is the southern tip of Taiwan and ending close to northern Borneo on its south. The principal islands comprising the Philippines are the Luzon, the Visayas and the Mindanao Islands.

The Philippines prehistory can be divided into four periods based on technologically-based periodization as reflected through archaeological records. These are the Paleolithic period from about 50,000 to 10,000 BC (Ronquillo 1998), the Neolithic period 10,000 to 500 BC, the Metal age (500 BC-AD 500) and the Age of Contact (AD 500 – AD 1400).

The earliest evidence of burial practice in Philippine prehistory was in the Neolithic period (3,100 BC) where skeletal remain of a man in a fetal position was found in Duyong cave, Quezon Palawan, Southwestern Philippines (Fox, 1970). During the Metal age several burial sites were recorded. The famous site comes from Manunggul cave in Quezon Palawan, Arku cave in Cagayan Valley Northern Philippines and the Ayub cave in southern Mindanao dated about 2000 years ago (Fox 1970; Dizon et al. 1996). Both jar burials and wooden coffins were unearthed in Panhutungan site in Surigao del Norte (Dizon 1998), southern Philippines and in the Banton cave on Romblon Island (Evangelista 1966). In the Age of Contact more burial sites were recovered, for example the Santa Ana primary burial site in Manila, the primary and jar burials in Lal-lo shell middens in Cagayan Valley Northern Philippines.

Archaeological data on burial sites in the Philippines were not thoroughly studied. Diet reconstruction where mostly focused on floral and faunal analyses associated with the burials and also with the shell middens. More often than not, foods recovered were few. This posted limitation to the study on diet reconstruction for Philippine prehistory.

Carbon and nitrogen stable isotopic analysis (δ13C and δ15N) on human bone collagen is an established technique for paleodietary reconstruction of past population (van der Merwe and Vogel 1978; DeNiro and Epstein 1978; Bender et al. 1981). Collagen values provide information on the type of protein resource in their diet (Krueger and Sullivan 1984). Most isotopic studies made used of collagen since it represents the total average dietary intake of an individual. Hair is also an ideal sample for estimating ancient diets (Macko et al. 1999; Macko et al. 1999; O’Connell et al. 1999) that provide limited time scale information on diet since hair keratin reflects more recent diet limited in months. Hair contained keratin that has mechanical stability dependent on the tight packing of the α-helix and can be degraded by some parasitic and saprotrophic fungi and thermophilic anaerobic bacteria (Bockle et al. 1995; Friedrich and Antranikian 1996). The carbon isotopic values for both bone collagen and hair keratin correlate well with that of total dietary protein with the collagen enriched by about 4.5% (Ambrose and Norr 1993).

Atmospheric CO2 with δ13C value of −7.0‰ is the major source of carbon for all terrestrial plants. The plant δ13C values in turn are determined by the isotopic composition of atmospheric CO2 which is incorporated into the tissues through photosynthesis. There are three photosynthetic pathways that occur among terrestrial plants. These are the Calvin-Benson (C3), Hatch-Slack (C4) and Crassulacean Acid Metabolism (CAM) pathways. Since the photosynthetic pathways differ chemically they produce different degrees of isotopic fractionation: an average δ13C value for the C3 plants is about −19.5‰, while that for the C4 species is about −12.5‰ (O’Leary 1981; Smith and Epstein 1971; van der Merwe 1982; Vogel 1978). C3 plants include mostly the flowering plants, trees, and shrubs, and most of the temperate zone grasses. While C4 plants are represented in about ten plant families and the majority of species are xeric environment grasses, including maize, some millets, some sorghums, sugar cane and others. The CAM plants are those under arid conditions with δ13C values usually reflect their habitat.

Marine phytoplankton approximates the C3 cycle but obtains their carbon from dissolved oceanic carbon which has isotope ratios of about 0‰ (vs PDB; Pee Dee Belemnite) their values average is about −19.5‰ (Sackett et al. 1965; Degen et al. 1968; Brown et al. 1972; Chisholm and Koike 1996). Previous studies have used stable isotope measurements that are enriched in marine food webs and compared with terrestrial food webs, tracing relative contribution of marine versus terrestrial
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Atmospheric N₂ is the main source of nitrogen in the food web (Ambrose 1991). There are different ways in which nitrogen enters the biological domain. The δ¹⁵N value is different between the plants or bacteria that fix nitrogen directly from the air (nitrogen-fixers) and bacterial nodules (azotobacter) and terrestrial plants that incorporate nitrogen source from root as nitrate in the soil from the decomposed organic material (Schoeninger and Moore 1992). Food derived from marine ecosystem such as coral reefs and salt marshes is similar in the δ¹⁵N value with those of terrestrial plants and animals (Schoeninger and Deniro 1984).

Trophic enrichment of δ¹³C is small — mostly within 1% for each trophic level (DeNiro and Epstein 1981; Minagawa and Wada 1984; Hobson and Welch 1992, Vander Zanden and Rasmussen 1999) while that of δ¹⁵N is usually large, by 3 to 4% (DeNiro and Epstein 1981; Minagawa and Wada 1984). Since the ratio is sensitive to reflect the trophic level, the nitrogen isotope has been used as a food web indicator for animals in various ecosystems (Schoeninger and DeNiro 1984; Wada et al. 1987; Fry 1988; Hobson and Welch 1992). Nitrogen fractionation values for carnivores is lower (3.2 ± 0.4‰) compared with herbivores which has a value of 2.5% ± 2.5‰ (Vander Zanden and Rasmussen 2001).

Considering the δ¹³C and δ¹⁵N composition of bone collagen from human skeletal remains, information on the consumption of C₃, C₄ and CAM plants as well as marine versus terrestrial food groups can be obtained (O'Leary 1981; van der Merwe 1982; Schoeninger and DeNiro 1984). Consequently, the stable isotope analyses cannot identify the diet species, however, it can indicate the primary producer and the trophic level in their ecosystem.

The δ¹³C and δ¹⁵N analyses in Southeast Asia and the Pacific are limited. In the Marianas Islands, Western Pacific, human skeletal remains from Guam, Rota and Saipan were analyzed for δ¹³C and δ¹⁵N using bone collagen and of carbonate in apatite tooth (Ambrose et al. 1997). In Southeast Asia, δ¹³C and δ¹⁵N analysis was done in Peninsular Malaysia and Sarawak using human skeletal remains from Niah Cave by Krigbaum (2003) and in Thailand on the skeletal remains from the Ban Chiang site by King and Knorr (2006). Isotopic analysis in the Philippines has not been done until Mihara et al. (2005) who measured bone collagen δ¹³C and δ¹⁵N of some human remains from Lal-lo midden sites.

In this paper, δ¹³C and δ¹⁵N analysis were applied to estimate paleodiet using the bone collagen obtained from 58 individuals and hair samples from three mummies in the Philippines. Features of these sites were described and individual information such as burial types, sex and age were shown in appendix.

Materials

A total of 61 individuals were sampled for the δ¹³C and δ¹⁵N analysis from five burial sites were considered in this study (Fig.1). Bones were collected in most cases with exception with Kabayan site where hair was collected from the mummies. In general, the five sites were categorized into sex groups, male (M), female (F) and unidentified (UI). The specimens were also categorized into age groups. These are juvenile (> 9 years), adolescence (> 17 years), young adult (< 18 but > 30 years), adult (< 60 years) and mature (> 60)

Information pertaining to the archaeological site, diet

Fig. 1. Map showing the five burial sites in this study
resources and burial description are presented below.

**Batanes site**

**Site description:** Batanes, a group of islands and islets, is located in the northernmost part of the Philippines. The Batanes Group of Islands is surrounded by Pacific Ocean to the east, South China Sea to the west, Bashi Channel to the North the Balintang Channel to the South, the Luzon Strait is between Taiwan and Luzon, and connects the Philippine Sea to the South China Sea. Evidence of the earliest habitation in Batanes was believed to be over 2000 years ago based from preliminary archaeological undertakings done by Kumamoto in 1983 and by a team of geologists headed by Maryannick Richard in 1986. The evidence came from the excavated red-slipped pottery, burnt wood and pottery sherd found under the ash layer deposit caused by the eruption of Mount Iraya in 325 B.C. (Hornedo 2000). The National Museum of the Philippines started the Batanes Archaeological Project in 1994 (Dizon 1995) that resulted in discovering the “castle-like habitation” known as “idjang” and the “stone boat shape burial markers” made of stone arranged in the shape of a boat typical for Batanes. The smallest grave marker discovered was measured 2.3 m in length and 90 cm in width, and the largest was about 5 m in length and 3.5 m in width (Dizon 1998). The $^{14}$C date acquired for boat shape burial was 355 ± 70 BP or 1595 AD that showed an over 300 years of burial activity in the area. On the other hand the recovered jar burials was of primary type where the complete remains of the dead person were buried inside the jar. No radio-carbon date was obtained for this. But it was believed to have been practiced by the Ivatans (natives of Batanes) between 100 BC to 300 AD and was noted to have been practiced until the late 18th century (Hornedo 2000).

From 1995-2001, the University of the Philippines Anthropology Field School (UPAFS) under the supervision of Dr. Francisco Datar, had continuously performed several excavations in a burial site in Itbud, Uyugan, Batanes exposing about 22 individuals. The burial site was located near the seashore. Sand was the main composition of the soil and this affected the preservation condition of the bones. Two types of burial practices were revealed, these are the primary burials where the body was directly buried on the ground and primary jar burials. In both ways, the individual was placed in: a fetal position. Associated with the burials are grave goods such as beads, pot sherds, marine shells where the most common type was the Turbo spp. The site was relatively dated between 16th–17th centuries.

**Diet resources:** Ethnographic account about Batanes in the 17th–18th centuries (Hornedo 2000) depicted the Ivatan (natives of Batanes) was already engaged in farming and fishing. Yam, sweet potato, bananas and sugarcane were basically grown but not rice or corn. Domestication of goats and pigs was also employed. In the 19th century, corn, millet, cottons and beans were cultivated in the island.

Faunal analysis done by Szabo et al. (2003) in some archaeological sites in Batanes, such as in Naidi and Sunget Top Terrace, the earliest site with $^{14}$C dating of 2630±30 (standardized with ANU 11693) revealed the presence of labrid, diodontid and scarid fishes and domesticated pig bones. Mavuyok a Ahchip 900 BP), a shell midden site showed variety of marine (56%), land (37%) and freshwater (7%) shells consumed. While mammal remains were scarce, but one pig incisor and two goat bones were identified.

**Human burials analyzed:** A total of 21 individuals (07 HM21–08HM69) were sampled (Appendix-1) of whom, seven individuals were identified as male, seven were female and seven individuals have unidentified sex due to missing morphological characteristics. A total of 18 human remains recovered from Itbud, Uyugan (07 HM21–08HM58; 08HM61–08HM66 and 08HM69) site were not in a good preservation condition and most of the bones were broken or fragmented due to disturbances caused by the sand, roots encrustations and intrusions aside from the soil matrix. Despite the condition, the students from the Anthropology Department of the University of the Philippines have tried to fit together the broken parts and done a detailed description and analysis.

The boat-shape burials (08HM67 and 08HM67) were recovered from western coast of Ivuhos Island. The area was a rolling hill covered with pandan vegetation. The Chuhangin stone boat-shaped burial site (Grave No.1) contained a juvenile individual who was
about three years old female at the time of her death (Bautista 1998). While the Mayahao Locality 1 Beach Ridge boat-shape burial contained mature individuals. The bones were well preserved.

The jar burial is of primary type. Sand was also put inside the jar. Bones were mostly in bad preservation and majority of the bone structures was fragmented (Bautista 1998). Two individuals were buried in the jar, 08HM59 and 08HM60. They were recovered from Itbud, Uyugan Batanes site.

Lal-lo site

Site description: Lal-lo, Cagayan Valley in Northern Philippines is known for its huge and extensive shell midden deposit which is considered to be the biggest shell midden deposit in the Philippines and probably in Southeast Asia (Ogawa 2000). Cagayan River, the largest river in the Philippines, runs through the area and flows out to Luzon Strait that connects the Philippines Sea to the South China Sea. Interestingly, these shell middens are mainly composed of freshwater bivalve shells locally known as “kabibe” (Batissa sp.). The midden is found usually along the riverbanks, inland and limestone hilly formation.

In 1986, an extensive archaeological exploration was conducted along the lower reaches of Cagayan River in Lal-lo municipality by the Archaeology Division of the National Museum of the Philippines and Japanese archaeologists (Aoyagi et al. 1993). The exploration resulted into identification of 21 shell midden sites. From 1995 to 2005, archaeological undertaking continued resulting into establishment of local chronology based on typological analysis of potteries (Ogawa et al. 2006; Tanaka 2002) and 14C dates (Mihara et al 2002; Mihara 2006). Four pottery assemblages were established. These are: Non-decorated Red-Slipped Pottery assemblage from 3400-3000 cal BP; Decorated Red-slipped pottery assemblage with 14C dating of 2925-2460 cal BP; Decorated Black pottery assemblage with period ranging from 2000-1500 cal BP and Non-decorated black pottery assemblage from 1500-1000 cal BP (Mihara et al. 2002; Mihara 2006).

Mihara (2006) in his doctoral thesis has done isotopic analysis of some of the skeletal remains recovered from the other shell midden sites. The seven individuals from his study were included in this paper.

Diet resources: Aside from the riverine shells and fish, faunal analysis from the different shell middens (Garong 2006) revealed pig (Sus sp.) and deer (Cervus sp.) bones and were abundantly found in almost all levels/layers of all the shell middens. Deer antlers were mostly modified. Fish bones especially the jaws belongs to Family Labriidae (marine fish) were recovered. Bovid bones were mostly carabao. Nagsabaran shell midden site in Alaguia, Lal-lo was excavated by a team from the Archaeology Division of the Philippines and Taiwanese Archaeologists from Academia Sinica, Taiwan that recovered a carabao skull in the silty clay layer below the shell layer. The skull was associated with red-slip pottery, adze and shell bead. It was found with some teeth scattered within the square suggesting that the carabao was probably butchered. The 14C date of the silty clay layer showed a 2240±270 BP (Tsang and Santiago 2001). Also, they recovered a pig mandible in the same clay layer but from a different square. The mandible was associated with red-slipped pottery midden and has a 14C date of 3390±130 BP. The dating of the pig mandible corresponds to the Non-decorated Red pottery assemblage.

Human burials analyzed: In this paper, the burial site were categorized based on the typological analysis of pottery. For Lal-lo site, 11 individuals (08HM28-08 HM31; 08HM04, 00HM07, 00HM04, 01HM24, 03 HM01, 03HM07 and 03HM08) were sampled (Appendix2). Three of them were identified as male, one was female and the eight samples have unidentified sex. Majority of the skeletal remains from Lal-lo site were found in an open primary pit burial recovered from the shell midden deposit above silty clay layer. The bones were well preserved. There were also burials recovered from the shell layer. However, the burials unearthed from shell layer were mostly disturbed, incomplete and fragmented.

Non-decorated red-slipped pottery (3400-3000 cal BP): Along the eastern side of the Cagayan River, Clemente Irigayen Property Site located in Barangay Sta. Maria revealed many important findings. After excavating six 2 x 2 m squares, the shell midden deposit were not
extensive however, both black pottery and red-slip pottery were found on this site. Layer II of the site consisted of shell deposit and black pottery sherds while Layer III silty clay yielded non-decorated red-slipped pottery sherds (de la Torre 2000). The red pottery assemblage was dated from 3400-3000 cal BP and was associated with primary burial. 08HM28 was a complete male adult skeleton recovered from clay layer.

The Pasqua site at the Fabrica shell midden had a thick shell deposit, however the whole site was disturbed since the shells were being collected for feeds for chicken. Salvage archaeology was done in 2000 and 2001 (de la Torre 2002; Garong, 2002). Red slip pottery sherd and one human mandible (01HM24) was found from layer 1 on the slope (Garong 2002). Calibrated 14C age ranged from 3380 to 3210 BP (Mihara 2006). In Barangay Nagsabaran, from the silty clay layer above the shell midden, 08HM30, a female individual was recovered.

Decorated black pottery (2000-1500 cal BP): Shell midden deposit was also found in two sites in Camalaniugan, next town after Lal-lo. These were the Leon Ibe and Cortez sites. Both sites were associated with the decorated black pottery phase. Cortez site has a shell midden deposit of almost 2 m thick (Garong 2002). The layer 1 contained skeletal remains but were merely disturbed. The remains consisted of scapula and ribs only. 03HM07 was collected from this burial. In Leon Ibe property, human remains were found in layer 3 which was a shell layer (Garong 2006). The burial was incomplete and contained mixed skull and phalanges (03HM08) belonging to a young individual and teeth of an adult individual.

Non-decorated black pottery (1500-1000 cal BP): Two shell midden sites were excavated in Barangay Catayuan (Garong et al. 2000). Both sites are located in the riverbank side. Fausto Sison site has a shell deposit of almost 2 m thick. However, due to some problems encountered, the excavation was limited at exposing 50 cms deep from the surface only. Burial #1 (00HM07) was found associated with carnelian beads (17th-18th centuries) together with black pottery sherds within the midden. Only bone fragments were collected for analysis.

The Conciso site has a 2-m-thick shell midden deposit containing black pottery. One primary burial was recovered from grid N3E2 associated with the shell layer. Burial #3 (08HM04) could be a complete skeletal remains but due to time constraints, complete retrieval was not achieved. However, the stature suggested an adolescence individual. Burial #1 (08HM31) was found in grid N0E2 below the shell midden deposit and was a complete male skeleton (Garong 2001).

On the western side of the Cagayan River in Catugan, San Lorenzo III, Siriban site was excavated by Tanaka (1998). The site revealed black pottery assemblage with 14C date of 1500-1000 cal BP (Ogawa 2006; Mihara et al. 2002; Mihara 2006). Both 08HM29 and 01HM01 remains were recovered in an open pit burial in the clay layer above the shell midden deposit. Another shell midden was the Dumbrique property excavated in 1997 (Tanaka, 1997). From layer 2 at a 20 to 60 cm depth, human femur (08HM04), humerus and mandible were found without a burial pit.

Kabayan site

Site description: The Kabayan site is located in Benguet province in mountain range in the northern central part of the Philippines. Kabayan is like a bowl-like valley that was surrounded by mountains. It is about 7,000 feet in altitude. The Philippine mummies that can only be found in Kabayan was noted and recognized as early as in 1947. Twenty one caves have been mapped and assessed to contain wooden coffins that have human remains of both mummified and skeletal remains. The mummies were well preserved considering the climate of the area. They were all in fetal or flexed position. Traces of tattoos were visible especially for adult burials, while hairs were still present. The caves were considered sacred by the locals. The mummification in Kabayan was believed to have been in existence since the 13th century (Merino 1989) but was cease as early as Spanish Period (1521) due to conflict in beliefs/religion as well as sanitary and health issues.

Since 1968, the National Museum of the Philippines has been continuously mapping and documenting the different caves and rockshelters that yield mummies and skeletal remains. Conservation on the other hand, is in a continuous endeavor up to now by the Conserva-
Diet resources: Both hunting and gathering were noted as the main source of subsistence in early times. Hunting wild animals included deer, wild boar, civet cats, birds and fowl. While food gathering involved mostly root crops. Kabayan is famous for its farming producing the red rice. No archaeological undertakings were done or recorded.

Human burials analyzed: For this study, three mummies (07HM01, 07HM33 and 08HM34) were considered (Appendix-3). The hair samples were collected from Timbac 2 (N = 1) and Kangal (N = 1) caves and from the mummy displayed in the National Museum branch office (N = 1) in Poblacion; two males and one female. There were several mummies interred in Timbac 2 where 07HM01 hair sample was taken and were all inside the wooden coffins and well preserved. Mummy from Kangal cave (08HM34) was deteriorating due to the highly unstable conditions of the cave, insect and rat infestations. The mummy displayed in the museum was in good preservation condition (07HM33).

Santa Ana site

Site description: Santa Ana site is situated on the southeast of Manila, the capital of the Philippines. It is located at the southeast banks of the Pasig River. The whole river and most portions of its tributaries lie entirely within Metro Manila. During the Spanish period the location of the site was ideal for trading activity. The site is only 300 m away from the river which was an important transport route at that time.

The site was actually a churchyard. The church itself was built in 1578 by Spanish missionary, thus named as Santa Ana church. Stratigraphic profile of the site revealed that signature of Spanish refuse occurs in the upper layer but not in the lower layer, and shell midden consisted of brackish shells, animal bones, pottery, trade wares ceramics and iron slag (Fox et al. 1977). The site was believed to have been occupied, as early as 11th century based on the trade ware ceramics that were mostly blue-and-white and late Sung Dynasty porcelain. The period from 12th–13th century AD was the beginning of an extensive trading with Chinese who brought with them metals, cloth and highly fired porcelains and stonewares. The site has a \(^{14}C\) dating of 1095 A.D.

Diet resources: In the 16th century, the main staple food crop was rice though root crops such as taro, yam sweet potato were also common but considered second choice (Scott 1994). Both hunting of deer and wild pig and fishing utilizing both coastal and inland waters were exploited and employed.

Faunal remains recovered from the pre-Spanish layer included a thick shell midden deposit containing brackish water shells mostly oyster, bones of pig, deer and water buffalo (Fox et al. 1977).

Human burials analyzed: A total of 71 human graves were recovered from this site (Fox et al. 1977). The remains were buried in extended position with funerary offerings. The skeletal remains from this site had been long curated in museum. The storage condition was indeed poor that affected the poor preservation of the skeletal remains. Also, most of the skeletal remains were not complete that posted problem for sex identification. The analyzed eight individuals (08HM11-08HM13, 08HM16-08HM18 and 08HM22-08HM23) from Santa Ana site (Appendix-4) consisted of male (N = 3), female (N = 2) and unidentified sex (N = 3).

Romblon site

Site description: Romblon is an island province that forms part of the Southern Tagalog islands and part of the Visayan islands of the Philippines which is 346 km south of Manila. The province consists of numerous islands situated in the Sibuyan Sea. The cave burial site was actually located in Banton which is known for its rocky island surrounded by bodies of water.

Romblon site was discovered in 1937 but it was only in 1966 that the National Museum started an expedition to recover the remains from this reported secondary burial cave using wooden coffin (Evangelista 1966). A total of thirty-four human remains were retrieved. The site was dated late 13th or 14th century based on the associated Chinese and Siamese jars, gold ornaments,
carnelian beads, turtle shell combs, coconut shells and bamboo flute (Evangelista 1966). The specimens were presently stored in the Archaeology Division's storage room of the National Museum.

**Diet resources:** Staple foods for the Central Philippine island in the 16th century were rice, millet, taro yams, sago palm (lumbia) and bananas (Scott 1994). Root crops were common all year round. Bananas and plantains (cooking banana) were also staple foods and like rice, yams when still unripe were being boiled. They also hunted deer, civet cat and pig. Fishing was most commonly done since the Visayan waters abound with fish. Seafood was the main source of protein in their diet. However, they also domesticated animals such as pig.

**Human burials analyzed:** The human remains were well preserved when they were recovered from the site. However, the skeletal remains were mixed and piled together inside the coffin (Evangelista 1966). One reason for this disturbance could be attributed to the fact that the site was looted by antique collectors prior to the retrieval of the National Museum. The skulls were all deformed and were believed to be deliberately done. Eighteen individuals (08 HM 01-08 HM 10 and 08HM13-08HM20), male (N = 7); female (N = 2) and unidentified sex (N = 9) from Romblon site were considered for this study (Appendix-5).

**Methods**

For this study, bone and hair samples were used for \( \delta^{13}C \) and \( \delta^{15}N \) measurements.

**Preparation of bone samples for collagen extraction:** Surface of each bone sample was cleaned. Using a dental drill, 200 mg powdered sample was extracted and cleaned by sonication in double distilled water (DDW). Then, alkaline treatment with 0.1N NaOH to remove soil acids followed. This step was repeated until the solutions became colorless. The samples were then washed with DDW and placed in cellulose dialysis tubes for acid treatment to remove inorganic carbon. Each sample tube was placed in a beaker containing 0.1 N HCl with a stirrer (Horai et al. 1989). The HCl solutions were changed daily for 5 days to achieve complete dissolution. The organic residues were centrifuged to pellets, and rinsed with DDW three times. The samples were again washed with 0.01N NaOH and then rinsed with DDW and centrifuged to pellets. The pellets were frozen and then freeze-dried over night using an Eyela freeze dryer (Tokyo-Rika, FDU 506). Aliquots of 0.8 ± 0.05 mg of each sample were placed in tin capsules (two capsules per sample) for isotopic measurement.

**Preparation of hair samples:** About 40 strands of hair from each mummy were sorted out from root to tip and for the skin tissue, and the hair sample was cut into small pieces (less than 1 × 1 mm). The samples were cleaned by sonication in double distilled water (DDW). Lipid removal follows by soaking it with 5-10 times volume of lipid extraction solution: chloroform methanol 2 : 1, then subject the sample for ultrasonication process for about five minutes. After removing the supernatant liquid, this process was repeated until the solution became colorless and clear. After complete drying in the draft chamber for a couple of hours, the samples were cut by 1 cm interval since hair grows at a rate of about 1 cm/month (O'Connell et al. 1999). Aliquots of 0.8±0.05 mg of each sample were placed in tin capsules (two capsules per sample) for isotopic measurement.

**\( \delta^{13}C \) and \( \delta^{15}N \) measurements**

The \( \delta^{13}C \) and \( \delta^{15}N \) values were analyzed twice for each sample using a continuous-flow mass-spectrometer (ANCA-mass 20-20, Europa Scientific Instruments, UK). Repeated measurements of a running standard (glycine) produced measurement errors of less than 0.1 % for \( \delta^{13}C \) and 0.3% for \( \delta^{15}N \). If the difference in results between the two analyses per sample was larger than the expected error, that sample was re-measured.

Analytical results are expressed as delta values, in parts per mil (%), as follows:

\[
\delta(\%O) = \frac{[R_{\text{sample}}/R_{\text{standard}}-1]}{1000}
\]

where R is \((^{13}C/^{12}C)\) or \((^{15}N/^{14}N)\). Conventional standards for carbon and nitrogen are PDB (Pee Dee Belemnite) and the atmospheric N\(_2\), respectively.
Results

%C and %N Values

C/N which is the ratio of %C and %N was obtained from 13C and 15N counts by the ANCA-MASS. Extracted collagen samples from the 59 individual had reasonable carbon and nitrogen contents, yielding a C/N ratio between 2.8-3.1, which is comparable with the ratio observed from modern hair samples (3.0-3.8; O'Connel 1996). The reasonable range of the obtained C/N ratio showed that the collagen was uncontaminated and that no further gelatination treatment is not needed.

$\delta^{13}C$ and $\delta^{15}N$ values for the bone collagen samples

The average and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values for each of the five burial sites are shown in Fig. 2. Data separated based on sex, age, burial type, locality and period were shown for Batanes, La I-lo, Santa Ana and Romblon sites (Fig. 3).

Batanes site: The average and standard deviation (1s) of the collagen $\delta^{13}C$ and $\delta^{15}N$ values obtained from the Batanes site ($N=21$) are $-16.6 \pm 1.3\%o$ and $9.8 \pm 1.7\%o$, respectively. For sex categories (Fig. 3A), males ($N=7$) have $\delta^{13}C$ values of $-16.6 \pm 1.3\%o$ in average, ranging from $-19.0$ to $-14.3\%o$ and $\delta^{15}N$ values of $9.4 \pm 1.1\%o$ in average, ranging from $14.5$ to $7.6\%o$. For female samples ($N=7$), $\delta^{13}C$ was $-16.7 \pm 1.3\%o$ in average ranging from $-18.5$ to $-14.7\%o$, and $\delta^{15}N$ was $9.9 \pm 1.4\%o$ in average, ranging from $11.7$ to $8.0\%o$. Individuals with unidentified sex ($N=7$) recorded $\delta^{13}C$ and $\delta^{15}N$ values that were $-16.4 \pm 1.6\%o$ with a range from $-18.1$ to $-14.4\%o$ and $9.5 \pm 2.2\%o$ with a range from $11.8$ to $6.6\%o$, respectively. No significant difference was found among the sex categories. However, the difference in $\delta^{13}C$ values was recognized using the burial categories. Individuals buried in primary position ($N=17$) were $-16.9 \pm 1.3\%o$ (ranging from $-19.1$ to $-14.4\%o$) while individuals with boat shaped burial ($N=2$) were $-14.5 \pm 0.3\%o$ (ranging from $-14.3$ to $-14.1\%o$). Probability using t-test was 0.0203 ($p<0.05$) showing a significant difference between the two burial categories. Individual buried using jars ($N=2$) had no significant difference with the other two categories. The difference in $\delta^{15}N$ values among the three categories was not statistically significant ($p>0.05$). For the age categories, both $\delta^{13}C$ and $\delta^{15}N$ values yielded no significant difference.

Lai-lo site: The $\delta^{13}C$ values for Lai-lo site ($N=11$) were higher than other burial sites (Fig. 2). The values were between $-21.3$ to $-18.4\%o$ with an average of $-19.4 \pm 1.2\%o$. The $\delta^{15}N$ values were between $10.8$ to $6.8\%o$, yielding the average value of $9.6 \pm 1.6\%o$. For male individuals ($N=3$), $\delta^{13}C$ value was $-19.5 \pm 1.5\%o$ in average, ranging from $-21.3$ to $-18.5\%o$ and $\delta^{15}N$ value was $8.9 \pm 1.9\%o$ in average, ranging from $10.5$ to $6.8\%o$. A female sample ($N=1$) recorded $\delta^{13}C$ value of $-18.4\%o$ and $\delta^{15}N$ value of $10.8\%o$. Individuals with unidentified sex ($N=7$) showed $\delta^{13}C$ value of $-19.0 \pm 1.0\%o$ in average and ranging from $-20.0$ to $-17.7\%o$, and $\delta^{15}N$ value of $10.0 \pm 1.0\%o$ in average and ranging between $11.0$ to $9.2\%o$. Comparison between male and Ul sex revealed no significant difference in both $\delta^{13}C$ and $\delta^{15}N$ values. Correlation among the different localities of shell midden burial sites also showed no significant difference in the average collagen $\delta^{13}C$ and $\delta^{15}N$ values; Catayuan ($-19.5 \pm 0.7\%o; 9.0 \pm 1.6\%o$), Dugo ($-19.0 \pm 1\%o; 10\%o$) and San Lorenzo ($-19.9 \pm 1.9\%o; 10.2 \pm 1.1\%o$).
A. Batanes site

B. Lal-lo site

C. Santa Ana site

D. Romblon site

Fig. 3. Cross-plotting of $\delta^{13}$C and $\delta^{15}$N values by categories. (A) Batanes site by sex, burial type and age: (B) Lal-lo site by sex and shell midden site; (C) Santa Ana and (D) Romblon sites by sex.

Kabayan site: Hair from three mummies was analyzed for seasonal change reflecting the diet during the entire hair growth until the death of the individual (Fig 4). Results of the $\delta^{13}$C and $\delta^{15}$N values average was $-17.0 \pm 1\%$ and $8.0 \pm 1\%$ respectively. Male mummies ($N=2$) recorded $\delta^{13}$C values of $-17.0 \pm 1\%$ in average and ranging from $-17.3$ to $-15.7\%$, and $\delta^{15}$N value of $9.0 \pm 2\%$ in average and ranging from $9.7$ to $7.5\%$. For a female mummy ($N=1$) the average $\delta^{13}$C and $\delta^{15}$N values were $-17.2\%$ and $8.3\%$, respectively and showed some temporal change in $\delta^{13}$C values. The two male mummies showed higher values of $\delta^{15}$N.

Santa Ana site: The collagen $\delta^{13}$C value recorded the
ing from -2

individuals (N=3) and male (N=2) and unidentified sex individuals (N=3) on both \( \delta^{13}C \) and \( \delta^{15}N \) values.

**Rommol site:** The collagen isotopic data were clustered around -18% in \( \delta^{13}C \) and 10% in \( \delta^{15}N \), except for one outlier (07HM19). The average values are -17.4 \( \pm 2.3\% \) in \( \delta^{13}C \) and 10.1 \( \pm 1.1\% \) in \( \delta^{15}N \). Sex category (Fig. 3D) showed no significant difference between male (N=3) and female (N=2) and unidentified sex individuals (N=3) on both \( \delta^{13}C \) and \( \delta^{15}N \) values.

**Discussion**

The recognized large variation in \( \delta^{13}C \) values ranging from -21.3 to -8.4% suggests variation in dietary resources. The \( \delta^{13}C \) values as well as \( \delta^{15}N \) values are highly variable among plants and animals. Terrestrial \( C_3 \) plants analyzed from Batanes site showed \( \delta^{13}C \) value range from -32.2 to -25.2% and \( \delta^{15}N \) value range from 10.6 to -0.7%. For \( C_3 \) plants consisting of corn, sugarcane and millet, \( \delta^{13}C \) and \( \delta^{15}N \) values ranged were between -12.6 to -11.2% and 3.2 to -2.0%, respectively. The \( \delta^{13}C \) and \( \delta^{15}N \) values of medium size marine fish range from -16.1 to -16.4% and from 12.1 to 6.3%, and those of shellfish are -14.7 to -7.1% and 8.3 to 2.9% for shellfish. One terrestrial herbivore was analyzed and showed \( \delta^{13}C \) and \( \delta^{15}N \) values of -25.1% and 6.8%, respectively. River mollusks from Cagayan River were analyzed by Mihara (2006) and yielded \( \delta^{13}C \) and \( \delta^{15}N \) values range between -30.2 to -27.0% and 8.1 to 6.0%, respectively. Likewise, river fish \( \delta^{13}C \) value ranges between -27.4 to -18.3%, and the \( \delta^{15}N \) values were from -13.9 to 5.6%.

In general, \( C_3 \) plants dominate the Philippine staple food such as root crops like taro and yams in earlier periods and later on rice, however, millets (\( C_4 \)) were also cultivated in the Central Philippines in the 16th century. While sweet potato which was first introduced by the Spaniards in the 16th century and corn that was introduced in the 17th century (Scott 1994) became also staple food.

The wide range of \( \delta^{15}N \) values from 14.1 to 7.5% reflects variation in food; e.g. relative importance of terrestrial versus aquatic resources. Hunting was common especially in the mountainous area while fishing and shellfish gathering are normally employed in areas where ocean or river is available. The variation is normally insignificant in terms of sex difference, as in the case of the \( \delta^{13}C \) and \( \delta^{15}N \) analysis of the bone collagen and apatite from the human remains (N=33) of Ban Chiang site northeast Thailand about 4000-1800 cal BP, showing that the \( \delta^{13}C \) values obtained from collagen showed ranged from -20.5 to 18.0% reflecting \( C_3 \) plants (King and Norr 2006). On the other hand, this case study showed that \( \delta^{15}N \) values suggested increased consumption of aquatic resources or carnivorous terrestrial animals over time.
From the archaeological finding, marine fish bones and shellfish were believed to have been exploited although the numbers are small. Terrestrial snail also appeared in some sites and was believed to have also played a major role in protein resources in Batanes (Szabo et al. 2003). However, the results of δ¹³C (−16.6±1.3‰) and δ¹⁵N (9.8±1.7‰) measurement were inconsistent with pure terrestrial protein resources.

The Lal-lo site δ¹³C and δ¹⁵N values average (−19.4±1.2‰ and 9.6±1.6‰) showed a diet more dependent on terrestrial resources. The Cagayan river runs through Lal-lo municipality and since prehistoric time to present, the river was the source of protein for the inhabitants. Considering the presence of deer and wild pig bones from archaeological sites (Tanaka 1997; de la Torre 2000; Garong 2000, 2001, 2002 and 2006), it is evident that hunting wild animals also contributed to the diet of the individuals. Recent food items (freshwater fish and mollusks) measured for δ¹³C and δ¹⁵N from Lal-lo (Mihara 2006) reflects the values obtained from the collagen.

The δ¹³C values average (−17.0±1‰) of the mummies from Kabayan site showed dependence on both terrestrial C₃ plant and aquatic ecosystems. Considering the topographical location, animals from river and streams were the possible sources of protein. The hair samples showed lower average δ¹⁵N values (8.0±1‰) compared with the other four sites. It is assumed that a low-meat diet would produce lower δ¹⁵N values in the body proteins and meat diet would produce higher δ¹⁵N values (Schoeninger and De Niro 1984). C₃ plants or vegetables could have possibly influenced the diet.

In general, the δ¹³C (−15.2±3.3‰) value of Sta. Ana site showed protein resources more dependent on aquatic ecosystem. However, three individuals showed lower δ¹³C values between −11.5 to −11.1‰ that was different from other individuals depending on C₃ plant ecosystem. The shell midden unearthed from the excavation was mainly brackish shellfish which were believed to have been collected from the nearby river or bodies of water. The Pasig River that runs through the site is actually a tidal estuary and played a major role as one of the trade route during the Age of Contact in Philippine prehistory between AD500 - AD1400. It is difficult to know exactly the type of the plant and to identify C₃ plants, since no plant remains were found in the excavation aside from recent food items.

In Rombon site, the δ¹³C and δ¹⁵N values (−17.4±2.3‰ and 10.1±1.1‰ in average) are concentrated in a small range and do not show so much variations. Generally, the diet reflects dependence on both terrestrial resources and marine ecosystems. Both sea and at the same time, hunting and possible domestication of animals might have been introduced at that time. But there was one male outlier that showed lower δ¹³C and δ¹⁵N value of −8.4‰ and 14.1‰ suggesting a diet dependent on coral fish, of which δ¹³C value was about −13‰ or seaweeds with δ¹³C value between −13.2 to −4.0‰ (Fry et al. 1982).

The earliest evidence of protein resources in the Philippines was from Palawan in Tabon cave (47,000 ± 11,000 BP) where shellfish, fish and bats remains were recovered (Ronquillo 1998). Towards the end of the Neolithic period with the introduction of rice cultivation, it became evident that domestication of crops and animals were being employed. The five sites used in this study were mostly in the Age of Contact period and even later. The results of the δ¹³C and δ¹⁵N analysis from the 61 individuals had largely terrestrial plant-based diet and consumed both terrestrial and aquatic protein that could be either marine or riverine, depending on the topographical location and environment. Exception are the Lal-lo site that suggest protein resources more on terrestrial-based ecosystem and the Santa Ana site that showed large reliance on marine-based ecosystem. Higher δ¹⁵N value for some individuals suggests consumption of large amounts of marine fish. It is difficult to determine whether there was an increase in animal domestication since most of the sites relied mostly on aquatic resources aside from not so much faunal remains.

References


Ambrose, S.H., Butler, B.M., Hanson, D.B., Hunter-


Vander Zanden, M.J. (1999). Primary consumer δ15N and δ13C and the trophic position of aquatic con-
Carbon and nitrogen stable isotope analysis using human bones and hair from Philippine burial sites


### Appendix 1. List of human remains from Batanes site

<table>
<thead>
<tr>
<th>Lab. No.</th>
<th>Accession No. and/or Site Code</th>
<th>Locality</th>
<th>Burial Type</th>
<th>Period</th>
<th>Grid</th>
<th>Feature</th>
<th>Sex</th>
<th>Age</th>
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<th>$%\text{N}$</th>
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Appendix 2. List of human remains from Lal-lo site

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<td>03HM01*</td>
<td>II-96-U2-10452</td>
<td>San Lorenzo</td>
<td>Jar burial</td>
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<td>-</td>
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<td>juvenile</td>
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<tr>
<td>03HM07*</td>
<td>II-80-J-8211</td>
<td>Cortez site</td>
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<td>S₁₂W₁₂</td>
<td>-</td>
<td>UI</td>
<td>UI</td>
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<td>Leon Ibe</td>
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<td>-</td>
<td>UI</td>
<td>UI</td>
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* Mihara 2006

Appendix 3. List of human remains from Kabayan site

<table>
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<th>Sex</th>
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<th>Tissue</th>
<th>%C</th>
<th>%N</th>
<th>C/N</th>
<th>δ¹³C</th>
<th>δ¹⁵N</th>
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<td>07HM01</td>
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Appendix 4. List of human remains from Santa Ana site

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<th>Sex</th>
<th>Age</th>
<th>%C</th>
<th>%N</th>
<th>C/N</th>
<th>δ¹³C</th>
<th>δ¹⁵N</th>
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<tbody>
<tr>
<td>08HM11</td>
<td>Box SA66 7</td>
<td>Old Lamayan road  primary pit burial associated with Sung, Yuan/early Ming dynasties tradewares</td>
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<td>Adult</td>
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<td>BL G-R-23</td>
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Appendix 5. List of human remains from Romblon site

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<th>Age</th>
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<th>%N</th>
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<th>δ¹³C</th>
<th>δ¹⁵N</th>
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Appendix

フィリピン埋葬遺跡出土人骨コラーゲンを用いた炭素・窒素安定同位体分析
A. GARONG1, F. DATAR2, W. RONQUILLO3, 小池 裕子1

要 旨
フィリピンにおける古代人の食性を調べるため、それぞれ異った地理的な位置・環境条件・生活戦略をもつ5箇所の遺跡から出土した61個体の炭素・窒素安定同位体比（δ13C and δ15N）を分析した。Batanes諸島はLuzon島北端から約200km、台湾から150kmに位置し、いわゆる船型埋葬と塼棺埋葬（355±70 B.P.）が知られている。21個体の人骨コラーゲン分析の結果、δ13C値は−16.6±1.3%、δ15N値は9.8±1.7%で、食料中の蛋白源や海洋生態系に依存していることを示した。同位体比は埋葬形式で異なり、船型埋葬と塼棺埋葬の人骨のδ13C・δ15Nはともに土坑墓のものよりも高かった。一方、Luzon島北部に位置するLal-lo貝塚遺跡群（1000 BP）から出土した11個体では、δ13C値が−19.4±1.2%、δ15N値が9.6±1.6%で、より陸上生態系に依存することを示し、貝塚を構成する淡水貝が陸上生態系に属する値をもつことが示唆された。北西Luzon島のBenguet山地にあるKabayan遺跡は13世紀のミイラを産出する遺跡で、3体のミイラの毛髪はδ13C値が−17.0±1.0%、δ15N値が8.0±1.0%で、陸上と内水面生態系の両者を利用していたことを示唆した。マニラ郊外のSanta Ana遺跡（about 1095 AD）の8個体では、δ13C値が−15.2±3.3%、δ15N値が10.4±0.7%で、5つの遺跡群、最も海洋生態系に依存していた。フィリピン中央部のRomblon島遺跡（13th-14th centuries）の18体の骨コラーゲンはδ13C値が−17.4±2.3%、δ15N値が10.1±1.1%で陸上と内水面生態系の両者を利用していたことを示唆した。またこれらの5遺跡において、δ13C・δ15N値の性差はいずれも見られなかった。

キーワード：炭素安定同位体、窒素安定同位体、埋葬遺跡、人骨、ミイラ、骨コラーゲン、毛髪、フィリピン

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