

Isotopic Evidence of Weaning in Hunter-Gatherers From the Late Holocene in Lake Salitroso, Patagonia, Argentina

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KEY WORDS breastfeeding; stable isotope analysis; collagen; Patagonia; hunter-gatherers

ABSTRACT Objectives: The timing and duration of breastfeeding and weaning in past hunter-gatherer populations are discussed based on the results of stable carbon and nitrogen isotope analyses undertaken on a Late Holocene skeletal sample from Lake Salitroso in the Patagonian steppe (Argentina). Research in Lake Salitroso is part of a regional project that studies the relationship between hunter-gatherer societies, their organizational systems and the environmental changes during the last 3,000 years in Patagonia.

Methods: The sample included 52 individuals: 33 subadults and 19 adults of both sexes. They were recovered from 24 burial stone structures, locally called *chenques*, with dates ranging from ca. 800 BP to 350 BP. Ribs were selected for collagen extraction and measurement of ¹³C/¹²C and ¹⁵N/¹⁴N ratios.

Results: A $\delta^{13}\text{C}$ mean value of $-18.7\text{‰} \pm 0.5\text{‰}$ was obtained, with a range between -19.8‰ and -17.1‰ whereas $\delta^{15}\text{N}$ recorded a mean of $11.9\text{‰} \pm 1.1\text{‰}$ with a range between 10.1‰ and 14.8‰ . $\delta^{15}\text{N}$ data showed an age-related pattern with particularly marked difference between values for subadults under the age of 4 and older individuals. As opposed to $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ showed little variation with age.

Conclusions: An early incorporation of supplementary solid food between 0.75 and 2 years of age and a late cessation of breastfeeding at about 5–6 years of age were inferred. This suggests that among this Patagonian hunter-gatherer population weaning was a gradual and lengthy process. These results are consistent with the patterns observed in cross-cultural studies and archaeological samples of hunter-gatherer groups. *Am J Phys Anthropol* 158:105–115, 2015. © 2015 Wiley Periodicals, Inc.

The last few decades have seen a growing interest in the anthropological study of breastfeeding and weaning in past and contemporary societies. Such interest lies not only in understanding the cross-cultural variability of breastfeeding and weaning practices, but also in the well-established biological and cultural relationships between these processes and other demographic aspects of populations such as their natural fertility, infant morbidity and mortality and child growth patterns (Hewlett, 1991; Stuart-Macadam and Dettwyler, 1995; Fouts et al., 2001; Fouts et al., 2005; Konner, 2005; Sellen, 2007). For modern hunter-gatherers, the available information on breastfeeding and weaning practices comes both from long-term studies of contemporary groups (e.g., Howell, 1979; Hewlett, 1991; Blurton Jones, 1993; Hill and Hurtado, 1996; Fouts et al., 2001, 2005; Konner, 2005; Marlowe, 2005) and from information gathered from cross-cultural studies that used these and other ethnohistorical data to evaluate different hypotheses about breastfeeding and weaning in small-scale societies (e.g., Short, 1984; Kelly, 1995; Sellen, 2001, 2007; Sellen and Smay, 2001; Fouts et al., 2005). These studies have highlighted the high inter- and intra-group variability of the fac-

tors that condition weaning¹, its duration, rate (gradual or abrupt), and the age at which the main

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¹Building on the work by Katzenberg et al. (1996), we refer to weaning as a process—as opposed to an event—consisting on the introduction of other foods while gradually reducing dependence on breast milk, and differentiate this from complete weaning, that is, the cessation of breastfeeding.

milestones that characterize it take place: the first incorporation of liquids, the first incorporation of solid foods and complete weaning (e.g., Fouts et al., 2001, 2005; Konner, 2005; Sellen, 2007).

Inter and intra-population variability in the duration of breastfeeding can be due to a variety of biological, environmental, socio-economic, and cultural factors. However, from a cross-cultural perspective, one of the factors most frequently mentioned is the availability of appropriate foods for weaning in relation to differences in the subsistence strategies of societies. This hypothesis predicts that the weaning process will take place earlier in life in agricultural and pastoral societies than in hunter-gatherer societies because the former have greater availability of soft and easily digestible foods suitable for weaning, such as animal milk, cereals, etc. (e.g., Buikstra et al., 1986; Sellen and Smay, 2001; Schurr and Powell, 2005). Sellen and Smay (2001) evaluated this hypothesis by comparing the average age of weaning milestones in contemporary and societies of the recent-past with different subsistence strategies and identified two aspects that characterize the weaning process in hunter-gatherer groups. First, and contrary to the prediction of the aforementioned hypothesis, foragers present the earliest ages for the onset of weaning. In this way, it follows that hunter-gatherers do not lack food resources for weaning (Sellen and Smay, 2001). Second, statistical differences for the complete weaning age were identified among societies with different types of subsistence, with hunter-gatherers showing the oldest ages (Sellen and Smay, 2001).

In recent years, within the framework of bioarchaeological research, several studies have shown the utility of stable nitrogen and carbon isotope analyses of different tissues to discuss human paleodiets. Nitrogen isotope analyses on subadult individuals -or on tissues that were formed at subadult ages- have been especially useful to discuss issues related to breastfeeding and weaning (e.g., Fogel et al., 1989; Katzenberg et al., 1996; Schurr, 1997, 1998; Dupras et al., 2001; Richards et al., 2002; Schurr and Powell, 2005; Fuller et al., 2006; Turner et al., 2007; Jay et al., 2008; Prowse et al., 2008; Waters-Rist et al., 2011; Bourbou et al., 2013; Burt, 2013; Burt and Garvie-Lok, 2013; Eerkens and Bartelink, 2013). Combined with other lines of bioarchaeological analysis, these studies have allowed researchers to address other issues such as changes in the fertility patterns in populations from different social and ecological contexts, due to the contraceptive effects of breastfeeding (e.g., Schurr, 1997; Schurr and Powell, 2005; Clayton et al., 2006; Waters-Rist et al., 2011); the issue of the "weanling dilemma"² (e.g., Katzenberg et al., 1996; Schurr, 1997); or the analysis of the diversity of subadult diets during and after weaning (Dupras et al., 2001; Turner et al., 2007). There are only a few of studies performed on samples from hunter-gatherer populations (e.g., Fogel et al., 1989; Weber et al., 2002; Schurr and Powell, 2005; Clayton et al., 2006; Waster-Rist et al., 2011; Eerkens and Bartelink, 2013). The demographic properties, mobility patterns and mortuary behavior of forager societies usually result in a sparse and low den-

²The weanling's dilemma refers to the health trade-off faced by the infant of suffering either growth or nutritional deficits due to prolonged exclusive breastfeeding when it no longer satisfies the infant's nutritional needs or to be exposed to alternative foods contaminated with diarrheal pathogens in contexts of poor hygiene (e.g., Rowland et al., 1978; Katzenberg et al., 1996).

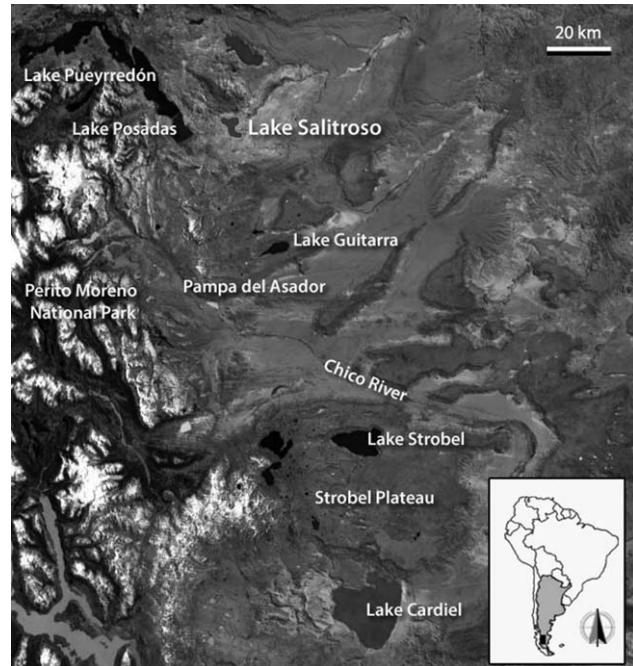


Fig. 1. Satellite Image of Northwestern and Central Santa Cruz (Patagonia, Argentina).

sity bioarchaeological record, which makes it difficult to obtain skeletal samples large enough to evaluate hypotheses at a population level (Paine and Harpending, 1996). Additionally, most archaeological studies for which isotope data are available to discuss weaning have been carried out on samples that come either from hunter-gatherers in transition to agriculture (e.g., Fogel et al., 1989; Schurr and Powell, 2005) or comprise very long periods of time, sometimes ranging over a thousand years or more (e.g., Weber et al., 2002; Clayton et al., 2013).

The aim of this study is to discuss the weaning process in foraging populations and test the aforementioned assumption of an early onset of weaning and a late complete cessation of breastfeeding (Sellen and Smay, 2001) based on the results of stable carbon and nitrogen isotope analyses performed on a skeletal sample of hunter-gatherers from Southern Patagonia (Fig. 1). This sample belongs to a small population whose residential mobility was markedly reduced during the Late Holocene (Goñi, 2010) due to regional environmental droughts (Stine, 1994) that led to changes in the organization and land use of these societies. The sample includes individuals ranging from ~36 weeks to older adults (50+) and both sexes, recovered from burial stone structures from the Lake Salitroso basin in the province of Santa Cruz (Argentine Patagonia). These burials have radiocarbon dates ranging from about 800 BP to 350 BP (García Guraieb, 2010; García Guraieb et al., 2015; Goñi, 2010). This narrow temporal and spatial distribution, together with the high number of subadult individuals, is uncommon for skeletal collections of hunter-gatherers populations and, therefore, present a unique opportunity to study breastfeeding and weaning in foraging societies. In addition, the fact that this population lived in a high latitude area, where plant resources frequently used for supplementary infant feeding are scarce and mainly seasonal, makes this case particularly useful to discuss the influence

of the availability of alternative foods on the onset age of weaning (Sellen and Smay, 2001). Thus, the case presented here consists of one of the few available samples of foragers worldwide and represents a relevant comparative case to analyze the onset and length of the weaning process among hunter-gatherer populations from the past.

Studies of breastfeeding and weaning through stable isotope analysis

Nitrogen and carbon stable isotopes from different biogenic tissues record the diet of an organism. Stable isotopes are incorporated into the tissues with an isotopic enrichment that can be defined as the difference in the isotope ratio between the substrate, ingested food, and the product, i.e., consumer tissues (Schoeninger, 1995). Because breastfeeding and weaning imply changes in the diet of the individual, it is possible to study these processes by analyzing the isotopic composition of different tissues such as bones and teeth. It is important to point out that there is a lag between the new diet and the incorporation of its isotopic composition in the tissues (Williams et al., 2005). This lag can be divided into two stages. The first one, called equilibrium rate, is the time needed for the body protein pool to reflect the composition of the new diet. The second stage is the time it takes for the new tissue to reflect the composition of the protein pool of the new diet. The bone modeling and remodeling rates will determine the time it takes for the new bone composition to appear in the analysis (Schurr, 1997; Williams et al., 2005).

Thus, it is possible to consider an isotopic model that comprises four categories of individuals based on their relative isotopic composition of the predominant diet. The first category comprises unborn or perinatal individuals whose tissues show similar isotopic composition as their mothers (Schurr, 1997; Bourbou et al., 2013). The second category of individuals refers to exclusively breastfed infants whose tissues will show a progressive isotopic enrichment in ^{15}N as the new diet, consisting of breast milk, is incorporated into their tissues (Fogel et al., 1989; Schurr, 1997). The third category is nonexclusive breastfed infants whose tissues will present an isotope composition that will become more depleted in ^{15}N with age since the supplementary foods usually have lower $\delta^{15}\text{N}$ values than breast milk (Schurr, 1997; Fuller et al., 2006). Finally, weaned individuals will show isotope compositions increasingly depleted in ^{15}N until they present values similar to those of adults, assuming that mother and weaned individuals have similar diets (Schurr, 1997; Fuller et al., 2006). Furthermore, a tendency has been observed where this last group presents less positive isotope compositions than adults (Schurr, 1997; Richards et al., 2002). Two hypotheses have been presented to account for this pattern. One suggests that supplementary foods used at weaning are different from those consumed by adult individuals (Schurr, 1997; Richards et al., 2002; Nitsch et al., 2011) whereas the other suggests that high growth rates recorded at this age could be associated to the differential isotopic enrichment seen in children compared to adult individuals (Schurr, 1997; Millard, 2000).

A series of parameters must be determined in order to interpret the isotopic ratios obtained from bone collagen in terms of breastfeeding and weaning. First, the isotope composition of female adults must be characterized. It is assumed that female adults of the same site would be the subadults' mothers (Waters-Rist et al., 2011); therefore, their isotopic ratios are used as a reference to interpret subadult diets. In addition, it is important to determine

the degree of internal variation of the female diets, as that variation is expected to be mirrored in the breastfeeding individuals (Waters-Rist et al., 2011). Second, the onset of weaning -the introduction of supplementary food- must be established. Several methods have been used to isotopically determine the end of exclusive breastfeeding. The most employed ones consider either the highest recorded value, the second highest recorded value, the age period with the highest average or the use of regressions to calculate the isotope age curve (Schurr and Powell, 2005). Finally, complete weaning (i.e., cessation of breastfeeding) needs to be determined. This can be defined as the age at which the isotopic compositions of juvenile diets are no different from or are similar to adult diets, assuming that mother and weaned individuals have similar diets (Schurr, 1997; Fuller et al., 2006). If children do not share the same diets as adults, complete weaning can be established when isotopic values stabilize at a new level (Burt, 2013).

This model was developed mainly for $\delta^{15}\text{N}$ (e.g., Schurr, 1997). However, it can also be used for $\delta^{13}\text{C}$ (Dupras et al., 2001; Richards et al., 2002; Fuller et al., 2006; Jay et al., 2008). Fuller et al. (2006) suggested that $\delta^{13}\text{C}$ would offer complementary information to that given by $\delta^{15}\text{N}$ to understand breastfeeding and weaning, because $\delta^{13}\text{C}$ would record the presence of alternative foods earlier than $\delta^{15}\text{N}$. Thus, the latter would be a good indicator of the completion of weaning while the former would be better to determine its onset. This would explain some archaeological cases where $\delta^{13}\text{C}$ records little variability whereas there is an increase in $\delta^{15}\text{N}$ of subadult individuals (Fuller et al., 2006; Jay et al., 2008; Nitsch et al., 2011). This pattern would suggest the death of those individuals when they had started the weaning process but were still being breastfed.

Archaeology of Lake Salitroso

Research in the Lake Salitroso region is part of a project that studies the relationship between hunter-gatherer societies, their organizational systems and the environmental change during the last 3,000 years in southern Patagonia (Goñi et al., 2002; Goñi, 2010). The study area comprises the northwestern sector of the Province of Santa Cruz (Fig. 1). It is a landscape of tectonic, glacial and lake basins separated by Miocene basaltic plateaus (Fig. 1) and bounded to the west by the Andes Mountains. The current climate is cold-temperate and the prevailing winds are from the west (Oliva et al., 2001). Annual mean rainfall ranges from 1,000 to 150 mm, falls mainly in winter and presents a marked west-east gradient. This gradient is responsible for the change in vegetation distribution, which is characterized by subantarctic forests of *Nothofagus* to the west and the grassland and low shrub steppes to the east (Oliva et al., 2001). Altitude is another relevant variable in landscape: the low basins such as those of Lakes Pueyrredón, Posadas and Salitroso are between 100 and 400 m above sea level (asl) and are surrounded by areas which are over 900 m asl, such as the Perito Moreno National Park (PMNP) basins and the Pampa del Asador and Strobel plateaus (Fig. 1).

The main animal resource in the region is the guanaco (*Lama guanicoe*) which is the most frequently represented species in the zooarchaeological record, followed by the choique (*Pterocnemia pennata*) and other minor species such as rodents, armadillos, lizards and a variety of birds which appear in lower frequencies. Regarding plant resources, their supply is low and they present a

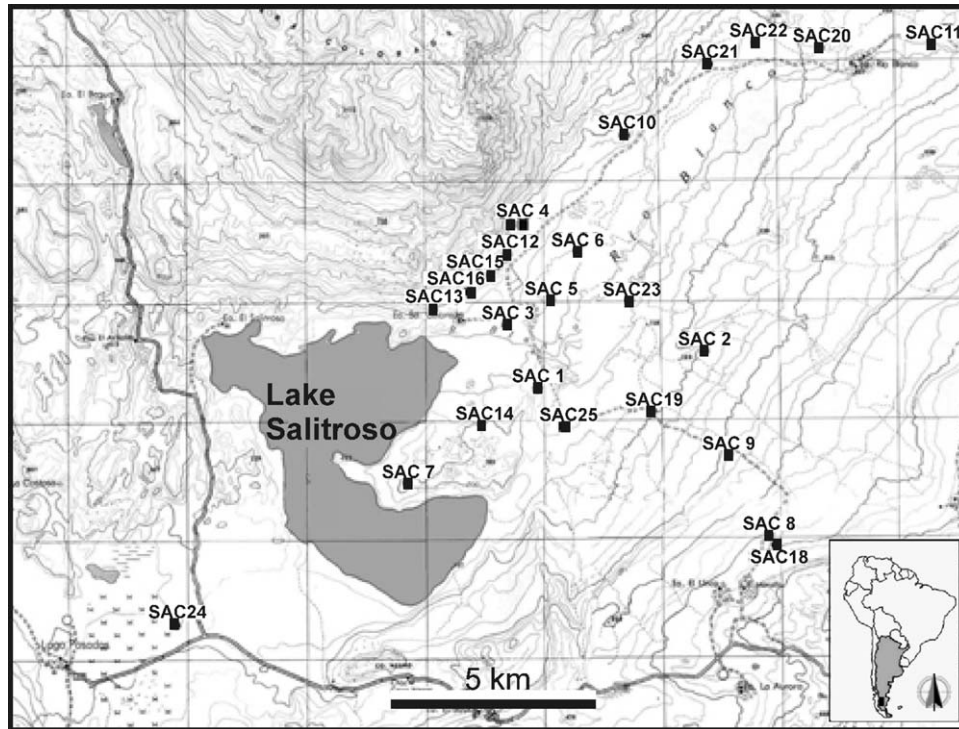


Fig. 2. Spatial distribution of burials and archaeological sites in Lake Salitroso.

marked seasonality, being available mostly during spring and summer (Ochoa and Ladio, 2011). However, the ethnohistorical and ethnographic sources suggest their relatively frequent consumption by human populations in Patagonia (Viganti, 1941; Martinic, 1995; Ochoa and Ladio, 2011).

Paleoclimatic records show a tendency towards conditions of lower relative humidity during the Late Holocene in southern Patagonia (Stine, 1994). In consequence, areas such as low basins that during the Early and Middle Holocene were covered by water were now available for human occupation (Goñi, 2010). During the last 1,000 years of the Late Holocene, arid conditions would have intensified (Stine, 1994). Water, which is a critical resource in Patagonia, would have been restricted to permanent water courses and low altitude basins, thus creating new scenarios for human populations in the region. Goñi et al. (Goñi et al., 2002; Goñi, 2010) have proposed that during this period, human groups would have developed a more restricted residential mobility, clustering their residential bases in low altitude basins with comparatively more benign conditions for human occupation (food and water resources, shelter, firewood, etc.) such as Lake Salitroso basin (Goñi et al., 2002; Goñi, 2010). This residential use of low basins would have been complemented by a seasonal and logistic use of high areas such as the lake basins from the western forests (Perito Moreno National Park) and nearby plateaus (Pampa del Asador) (Fig. 1) (Goñi et al., 2002; Goñi, 2010).

Throughout more than 20 years of research in the area, this model has been evaluated through multiple lines of evidence (Goñi et al., 2002; Goñi, 2010). The bioarchaeological record from Lake Salitroso has played a key role in the research since it represents the highest

concentration of burials corresponding to hunter-gatherer populations known in the region (Fig. 2). Thus, the study of different aspects of these burials and the recovered skeletal sample has contributed greatly in the discussion of different hypotheses derived from the model. Among these studies, we can mention recent paleodemographic and paleopathological analyses (García Guraieb, 2010; García Guraieb et al., 2015), as well as ancient mtDNA (Moraga et al., 2009), morphometrics and discrete traits (Perez et al., 2004; Bernal, 2008), taphonomy (Barrientos et al., 2007), paleodiet studies (Tessone et al., 2009) and the analysis of different aspects of mortuary contexts (Cassiodoro and García Guraieb, 2009; García Guraieb, 2010; Cassiodoro, 2011).

The study presented here is centered on the recovered individuals from the so-called Late *Chenques* which are the most numerous burials recorded at Lake Salitroso basin (SAC) (Fig. 2). Their chronology ranges from about 800 to 350 years BP (García Guraieb, 2010). These are 25 artificial stone structures, approximately 5 m in diameter and 0.5 m tall, which contained single, double and mostly multiple burials adding up to a total of 71 individuals (García Guraieb, 2010; Goñi, 2010). Contrary to what has been recorded for earlier burial sites at the basin (12 structures with mostly single primary burials that range discontinuously from 2600 to 1200 years BP; García Guraieb, 2010) the group of Late *Chenques* is larger in size and more temporally restricted, thus making it more adequate to evaluate population patterns. In fact, only the skeletal sample collected from this group of burials ($n = 71$) shows a continuous age distribution, with a large representation of subadults (56.5% of the individuals are younger than 20 years of age), and an even sex distribution of adults and

TABLE 1. Sex and age, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results with measures of collagen quality for individuals from Salitroso lake (Late Chenques, ca. 800–350 yrBP)

Sample	Laboratory No.	Age (years)	Age midpoint	Sex	^{14}C date (years BP)	Skeletal element	% Collagen yield	C/N ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
SAC 1-1-2	EILAB 196009	NN–PN	0	nd	nd	Rib	22	3.2	–18.8	12.7
SAC 10-1-7	EILAB 196032	NN–PN	0	nd	nd	Rib	16	3.3	–18.5	13.5
SAC 1-2-6	AIE 24612	NN–PN	0	nd	nd	Fibula	15	3.5	–17.7	14.2
SAC 2-4-2	AIE 24618	0.5–1	0.7	nd	nd	Rib	21	3.4	–18.4	13.1
SAC 10-4-2	EILAB 196033	0.5–1	0.75	nd	nd	Rib	24	3.3	–19.6	14.4
SAC 1-5-B	AIE 24625	0.7–1.3	1	nd	nd	Rib	24	3.3	–18.6	14.1
SAC 1-2-4	EILAB 98124	1–2	1.5	nd	nd	Rib	nd	3.2	–17.8	13.6
SAC 1-2-3	EILAB 196014	1–3	2	nd	nd	Rib	<1	4.1	–19.1	12.8
SAC 1-2-5	EILAB 196015	1.4–2.6	2	nd	nd	Rib	7	3.1	–17.9	14.8
SAC 2-4-1	EiLAB 196022	1.4–2.6	2	nd	486 ± 43	Rib	17	3.2	–18.5	12.9
SAC 1-2-1	EILAB 196012	2–4	3	nd	418 ± 40	Rib	23	3.2	–18.2	13.2
SAC 10-1-5	EILAB 196030	3–5	4	nd	nd	Rib	14	3.3	–18.4	13.4
SAC 1-6-1	EILAB 196017	3.7–6.3	5	nd	756 ± 32	Rib	24	3.1	–19.0	12.2
SAC 1-6-4	EILAB 196020	4–8	6	nd	nd	Rib	11	3.2	–18.7	11.6
SAC 8-3-1	AIE 24613	4–8	6	nd	nd	Rib	22	3.2	–18.8	11.7
SAC 1-1-A	EILAB 98115	4.5–8.5	6.5	nd	nd	Rib	nd	3.3	–19.8	11.1
SAC 22-2-1	EILAB 196036	5–9	7	nd	432 ± 43	Rib	21	3.3	–18.8	11.0
SAC 1-1-4	EILAB 98118	5–9	7	nd	nd	Rib	nd	3.2	–18.3	11.1
SAC 1-1-5	EILAB 196011	6.5–8.5	7.5	nd	nd	Rib	23	3.2	–19.2	10.2
SAC 1-5-1	EILAB 196016	7–10	8.5	nd	nd	Rib	20	3.2	–17.1	11.9
SAC 1-4-1	AIE 24614	7.5–9.5	8.5	nd	424 ± 39	Cranium	10	3.4	–19.3	13.5
SAC 10-1-2	EILAB 196031	7–11	9	nd	nd	Rib	24	3.2	–19.2	11.7
SAC 8-3-2	AIE 24619	7.5–12.5	10	nd	435 ± 46	Rib	24	3.3	–19.7	10.2
SAC 2-5-3	EiLAB 196023	8.5–13.5	11	nd	nd	Rib	19	3.2	–18.4	11.1
SAC 10-1-4	EILAB 98123	8.5–13.5	11	nd	687 ± 43	Rib	nd	3.2	–19.5	11.2
SAC 8-3-3	EILAB 196029	12–14	13	I	nd	Rib	22	3.1	–19.0	11.3
SAC 1-1-1	EILAB 98117	13–15	15	I	nd	Rib	nd	3.3	–18.7	12.7
SAC 10-2-1	AIE 24622	14–17	15.5	I	637 ± 47	Rib	22	3.3	–19.4	10.5
SAC 10-1-1	EILAB 98122	16–18	17	M	662 ± 43	Rib	nd	3.3	–19.6	11.4
SAC 1-6-2	EILAB 196018	16–18	17	F	690 ± 40	Rib	23	3.2	–18.3	12.4
SAC 1-6-3	EILAB 196019	17–19	18	F	539 ± 46	Rib	23	3.2	–18.7	11.7
SAC 20-3-1	AIE 24621	17–21	19	M	nd	Rib	15	3.3	–18.0	11.6
SAC 10-3-1	AIE 24633	18–21	19.5	M	nd	Rib	19	3.3	–18.8	10.6
SAC 30-1-1	AIE 24617	18–24	21	F	361 ± 45	Rib	21	3.2	–18.9	11.8
SAC 10-4-1	AIE 24620	25–30	27.5	M	nd	Rib	19	3.2	–19.3	12.2
SAC 8-3-4	AIE 24630	35–45	30	M	nd	Rib	25	3.2	–18.3	11.5
SAC 10-4-3	AIE 24639	20–40	30	I	nd	Rib	16	3.3	–18.4	12.9
SAC 1-1-3	EILAB 196010	35–40	37	F	352 ± 40	Rib	23	3.1	–18.8	12.0
SAC 1-2-2	EILAB 196013	35–40	37	F	389 ± 40	Rib	6	3.1	–18.6	11.2
SAC 1-1-B	EILAB 98116	40–45	42	F	622 ± 57	Rib	nd	3.3	–19.3	10.9
SAC 4-1-1	EILAB 196025	40–45	42	F	728 ± 39	Rib	24	3.2	–18.9	11.3
SAC 1-1-6	EILAB 19119	35–50	42.5	F	nd	Rib	nd	3.2	–19.3	10.5
SAC 20-3-2	EILAB 196034	40–50	45	M	380 ± 40	Rib	23	3.2	–18.1	11.7
SAC 22-1-1	EILAB 196035	45 ± 5	45	M	704 ± 42	Rib	23	3.3	–19.7	10.1
SAC 1-3-1	AIE 24634	50–60	55	M	nd	Rib	23	3.3	–18.7	11.7
SAC 2-5-4	AIE 24615	50–60	55	F	758 ± 51	Tarsal	24	3.4	–18.2	11.8
SAC 8-1-1	AIE 24635	Adult	50	F	nd	Rib	24	3.2	–18.4	11.9
SAC 30-1-4	AIE 24636	Adult	50	I	nd	Rib	24	3.2	–18.4	11.3
SAC 12-1-1	AIE 24626	Adult	50	I	nd	Rib	22	3.4	–19.4	11.0
SAC 8-2-1	AIE 24631	Adult	50	I	nd	Pelvis	21	3.3	–19.1	10.4
SAC 20-1-1	AIE 24624	Adult	50	I	nd	Metacarpal	23	3.3	–18.0	12.1
SAC 2-8-4	AIE 24616	Adult	50	I	nd	Rib	10	3.4	–18.6	11.6

adolescents (Fig. 2) (García Guraieb, 2010; García Guraieb et al., 2015).

MATERIALS AND METHODS

Out of the 71 individuals that were recovered at the Late *Chenques*, bone samples for stable isotope analysis were taken from 52 individuals: 19 adults (older than 20 years of age) and 33 subadults, among which there are perinatal and fetal individuals, infants, children and adolescents (Table 1). Classification by sex was only attempted for older adolescents (i.e., over 17 years of age) and adults, 10 of which were female, 8 were males and 6 remained undetermined (Table 1).

Age at death was estimated using multiple bone and dental indicators. In the case of subadults, dental development and eruption (Ubelaker, 1989; Smith, 1991) and epiphyseal fusion (Scheuer and Black, 2000) were considered. In those few cases where these indicators were unavailable, long bone length was used following the standards compiled by Ubelaker (1989) and Scheuer and Black (2000). Adult age and sex estimation was carried out using the methods compiled by Buikstra and Ubelaker (1994) along with the degree of modification at the sternal end of the fourth rib (Ischan et al., 1984, 1985). Adult sex determination was based on morphological traits of skulls and coxal bones following the protocols by Buikstra and Ubelaker (1994) as well. When

TABLE 2. Descriptive statistics (mean, standard deviation, minimum, and maximum values) for carbon and nitrogen isotopes by age and sex groups of Lake Salitroso

Category	n	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
SAC (total sample)	51	-18.7	0.5	-19.8	-17.1	11.9	1.1	10.1	14.8
Age group									
<13 years	24	-18.7	0.6	-19.8	-17.1	12.4	1.3	10.2	14.8
Adolescent (13–19, 9 years)	8	-18.8	0.5	-19.6	-18.0	11.5	0.7	10.5	12.7
Adult (>20 years)	19	-18.8	0.4	-19.7	-18.0	11.5	0.6	10.1	12.9
Sex (≥ 17 years)									
Female	10	-18.7	0.3	-19.3	-18.2	11.5	0.5	10.5	12.4
Male	8	-18.8	0.6	-19.7	-18.0	11.3	0.6	10.1	12.2
Adult-adolescent diets									
>13 (years)	27	-18.8	0.4	-19.7	-18.0	11.5	0.6	10.1	12.9

none of these bones were available, the maximum diameter of the head of the femora and humeri was measured and compared to the range, mean and standard deviation measurements obtained for these bones from individuals whose sex had been determined using skulls and coxal bones morphological criteria (Bernal et al., 2004; García Guraieb, 2010). In the Late Chenques set presented here, this was only done in one case (SAC 1-1-6).

Ribs were selected for collagen extraction (Table 1). When they were not available or in good condition, other bone elements that did not interfere with other analyses (e.g., pathological conditions, DNA) were selected. The bone collagen extraction involved the mechanical cleaning of the sample and its rinsing in an ultrasonic bath with distilled water. To demineralize the bone, it was soaked in hydrochloric acid (HCl 2%) for 72 h, replacing the acid every 24 h. To eliminate post-depositional organic compounds, the material was placed in NaOH 0.1 M for 24 h before and after bone demineralization. Lastly, the sample was rinsed and dried in a stove at $<60^\circ\text{C}$ (Tykot, 2004).

These analyses were carried out at INGEIS (Instituto de Geocronología y Geología Isotópica, CONICET/UBA). Measurement of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in the collagen fraction was performed with a Carlo Erba EA1108 Elemental Analyzer (CHN), connected to a continuous flow Thermo Scientific Delta V Advantage mass spectrometer through a Thermo Scientific Conflo IV interface. Stable isotope results are expressed as the ratio of the heavier isotope to the lighter isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) and reported as δ values in parts “per mil” (‰) relative to internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen (Ambient Inhalable Reservoir, AIR). The quality of the isotope value of each sample was evaluated from the C/N ratio and collagen yield (van Klinken, 1999).

RESULTS AND DISCUSSION

Table 1 shows $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, collagen yields and atomic C/N together with sex and the estimated age of the 52 individuals. Out of the total sample, only individual SAC 1-2-3 was excluded from the further analysis since it presented a collagen yield $<1\%$ and a C/N ratio of 4.1, outside the acceptable range (2.9–3.6, van Klinken, 1999). The rest of the individuals ($N = 51$) showed a mean of 3.2 ± 0.08 with a range between 3.1 and 3.5 in the C/N ratio, while the collagen yields had a mean of $19\% \pm 5\%$ ($N = 43$). Considering the sample as a whole, a mean of $-18.7\% \pm 0.5\%$ for $\delta^{13}\text{C}$ was obtained, with a range between -19.8% and -17.1% . On the

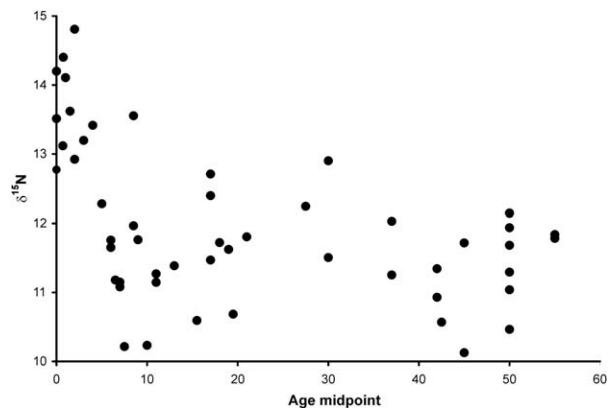


Fig. 3. $\delta^{15}\text{N}$ values for bone collagen plotted against estimated age at death for individuals from Salitroso lake.

other hand, $\delta^{15}\text{N}$ showed a mean of $11.9\% \pm 1.0\%$ with a range between 10.1% and 14.8% (Table 2).

Adult and adolescent diets

To study the main characteristics of the weaning process in these populations, the first step was to evaluate the age and sex differences in diets among individuals over 13 years of age. No differences were found in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between adults and adolescents (Table 2). Likewise, minor differences are observed when compared by sex, with differences of 0.1% and 0.2% for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Table 2). Thus, there is no variation in the protein portion of the diet for all individuals over 13 years of age (Table 2). $\delta^{13}\text{C}$ showed a mean of $-18.8\% \pm 0.4\%$ with a range from -19.7% to -18.0% and $\delta^{15}\text{N}$ had a mean of $11.5\% \pm 0.6\%$ with a range between 10.1% and 12.9% (Table 2).

Nitrogen isotope ratios

$\delta^{15}\text{N}$ values show age-related variation, with subadults under 4 years of age showing higher values relative to those of older individuals (Fig. 3).³ Individuals

³To plot adults of undetermined age (“adult” in the age column of Table 1), they were arbitrarily assigned to the 50 years age category. This creates an artificial concentration of values at this age. However, as the focus of this work lies on the age related trends of the subadult portion of the plot and adults are considered mostly as a group, this methodological decision does not affect the interpretation of results.

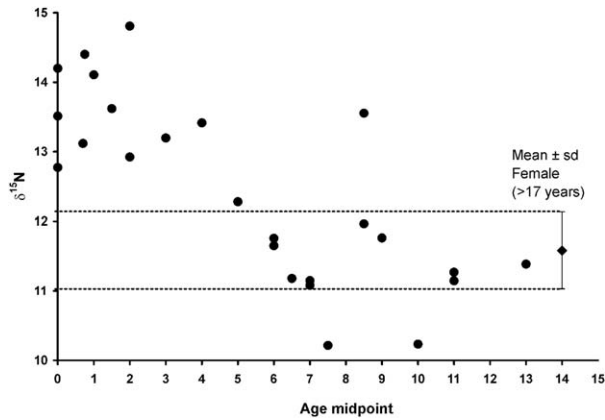


Fig. 4. $\delta^{15}\text{N}$ values for subadults (<13 years) bone collagen plotted against estimated age at death. Also included are female mean \pm SD isotope values.

between 0 and 4 years old had values between 12.7‰ and 14.8‰ (Table 1 and Fig. 3), whereas values from individuals >4 years old are distributed between 10.1‰ and 12.9‰ (Table 1 and Fig. 3). Thus, only the extremes from these two distributions overlap with the sole exception of one outlier from this last age group who recorded a $\delta^{15}\text{N}$ value of 13.5‰ at 8.5 years of age.

Isotopic variation among individuals under 13 years of age is depicted in Figure 4. The first aspect to consider is the group of unborn/perinatal individuals, which includes three individuals. Nitrogen isotope values for this age group range from 12.7‰ to 14.2‰. The lowest value comes from individual SAC 1-1-2, who was ~36-weeks old and shows the closest value to the female mean. In fact, this value shows a difference of 1.2‰ with respect to the female mean. This individual was found in the abdominal cavity of SAC 1-1-3 (Goñi and Barrientos, 2000), a female adult of an estimated age of 35–40 years (Table 1), suggesting it might be a fetus or a stillbirth. The female adult recorded a $\delta^{15}\text{N}$ value of 12.0‰, which implies a difference of 0.7‰ with respect to the fetus/perinate mentioned above (see Burt, 2013). The remaining two perinates present values that are further away from the female diet. The age estimation for these individuals is presented within a precision range of up to ± 0.5 years, because dental age was not available for them (Table 1). Particularly for this age group, such a range can imply a large difference in paleodiets as individuals can be either exclusively nursing, weaning or completely weaned (Schurr, 1997). Thus, it is possible that different situations are represented in these three individuals, two of them being breastfed and the third one, with values closer to adult female diets, representing an unborn individual (see Reynard and Turros, 2015).

Between 0.1 and 4 years of age, $\delta^{15}\text{N}$ values are distributed between 12.9‰ and 14.8‰ and are always higher than the values from all individuals older than 13 years of age and from the unborn/perinatal individual mentioned above. It is interesting that both values—maximum and minimum—were obtained at age 2 (Table 1). Thus, the age when the end of exclusive breastfeeding would be recorded—considering the maximum value as a benchmark—at the same time shows the lowest $\delta^{15}\text{N}$ value among the individuals between 0.1 and 4 years of age. This characteristic discloses one of the difficulties of these studies, that is, establishing the end of exclusive

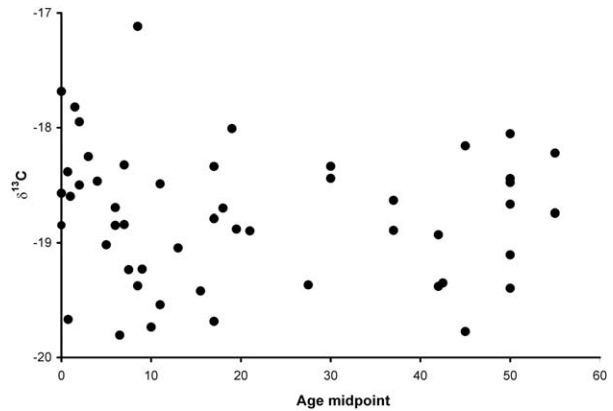


Fig. 5. $\delta^{13}\text{C}$ values for bone collagen plotted against estimated age at death for individuals from Salitroso lake.

breastfeeding and the beginning of weaning with the incorporation of alternative foods. Schurr and Powell (2005) point out that the maximum value is prone to analytical and age estimation errors and to the presence of outliers in the sample. They suggest that using the second highest value is more reliable than using the first. In SAC, the second highest value, $\delta^{15}\text{N}$ 14.4‰, was obtained at age 0.75 ± 0.25 years.

Regarding the onset of the weaning process, some remarks must be made. First, when discussing the highest and lowest value at age 2, $\delta^{15}\text{N}$ variability in female individuals must be considered as a measure of expected variability for the infants (Waters-Rist et al., 2011). Dispersion measures are similar for both groups: female and infants. If we consider the standard deviation, the female value is 0.5‰ whereas for individuals between 0.1 and 4 years old the standard deviation is 0.6‰; the situation is the same if the range is considered, which in both cases is 1.9‰ (Table 2). Second, it is important to point out that, at 0.75 and 2 years old, values are at 3.3 and 2.9‰, respectively, above the female mean. These differences are close to what is expected for one trophic level and to what has been recorded both in other skeletal samples of similar characteristics (Schurr and Powell, 2005; Turner et al., 2007; Jay et al., 2008; Nitsch et al., 2011; Waters-Rist et al., 2011), and in mother-child actualistic studies (Fogel et al., 1989; Fuller et al., 2006). And finally, we must consider the high intra-population variability ethnographically observed for the main milestones of the weaning process such as the first incorporation of liquids and solid food (e.g., Fouts et al., 2001, 2005; Konner, 2005; Sellen, 2007). If we consider these issues, the end of exclusive breastfeeding can be established between 0.75 and 2 years of age for Lake Salitroso populations.

After reaching the maximum value, there is a tendency towards lower $\delta^{15}\text{N}$ values and between 5 and 6 years of age the isotopic values begin to overlap the range for females (Fig. 4). On the one hand, we find individual 1-6-1, who is ~5 years old, with a value of 12.2‰, placed between the maximum and minimum values of female individuals and 0.2‰ higher than the standard deviation over the female mean (Table 2). On the other hand, the two 6-year-old individuals (SAC 1-6-4: 11.6‰ and SAC 8-3-1: 11.7‰, Table 1) show values that are close to the female mean (Table 2). This circumstance

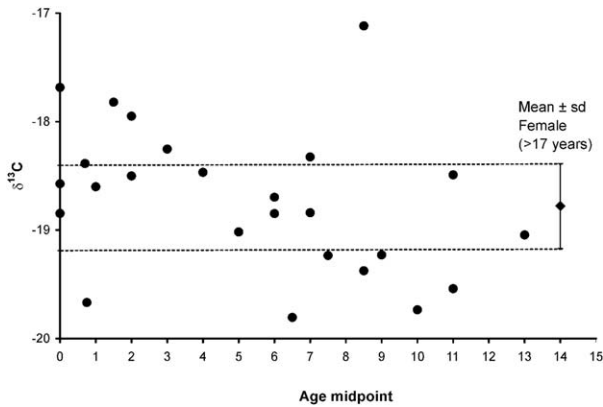


Fig. 6. $\delta^{13}\text{C}$ values for subadults (<13 years) bone collagen plotted against estimated age at death. Also included are female mean \pm SD isotope values.

marks the end of the weaning process, as the range values of both subadult and adult diets begin to overlap.

Finally, the $\delta^{15}\text{N}$ values continue to decrease between 7 and 11 years of age. This group presents the lowest mean of all, $\delta^{15}\text{N} = 11.1\text{‰}$ (without considering the outlier of this group; SAC 1-4-1: 13.5‰), 0.4‰ lower relative to the female mean. Previous researches have found that the post-weaning group usually has lower $\delta^{15}\text{N}$ values than the female mean in different populations (Schurr, 1997, 1998; Richards et al., 2002; Ogrinc and Budja, 2005; Eerkens et al., 2011; Nitsch et al., 2011). This has been explained by two alternative hypotheses: different diets of this age groups (Richards et al., 2002; Nitsch et al., 2011) or physiological/metabolic aspects related to the high velocity of bone turnover (Schurr, 1997, 1998; Millard, 2000; however, see Waters-Rist and Katzenberg, 2010). It is beyond the aim and scope of this article to settle this question and varied situations are probably included in the aforementioned cases.

Carbon isotope ratios

As opposed to $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ shows little variation according to age. Most of the individuals (47/51) have $\delta^{13}\text{C}$ values between -20‰ and -18‰ (Table 1 and Fig. 5). Isotopic variation of individuals under 13 years of age is depicted in Figure 6. It is worth noting that three of the four individuals with less negative values than -18‰ are between 0 and 2 years old.

The mean for individuals between 0 and 4 years old is $-18.4\text{‰} \pm 0.5\text{‰}$, i.e., 0.3‰ less negative than female individuals. Differences are not statistically significant (Mann–Whitney $P > 0.05$). Thus, even if the distribution of stable carbon values for individuals between 0 and 13 years of age is similar to the one observed for $\delta^{15}\text{N}$, when it comes to $\delta^{13}\text{C}$, values are generally found within the standard deviation for female individuals and, except for those four cases already mentioned, within the range for adult individuals.

Regarding $\delta^{13}\text{C}$, Fuller et al. (2006) carried out a longitudinal study on nails and hair from infants and mothers and suggested that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ could complement each other, the first one being a good indicator of the end of breastfeeding and the latter, of the introduction of solid supplementary food (Fuller et al., 2006). According to these authors, the repetition of a pattern in several archaeological investigations where there are high

values of $\delta^{15}\text{N}$ together with little variation of $\delta^{13}\text{C}$, as in the case of SAC, suggests that the death of the individuals took place while they were still breastfeeding but they had already been introduced to supplementary solid food (Fuller et al., 2006). If that were the case, in SAC, we would be witnessing an early incorporation of supplementary solid food and a late cessation of breastfeeding.

Comparison with other hunter–gatherer populations

On the basis of the obtained results for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, we suggest that in this Patagonian group the first intake of supplementary food would have taken place at an early age, possibly within the first year of life. This derives from the little variation of $\delta^{13}\text{C}$ values in relation to the estimated age of the individuals. On the other hand, cessation of breastfeeding would have occurred several years later, at around age five, a time when $\delta^{15}\text{N}$ values begin to overlap those of diets from individuals older than 13 years of age.

This characterization of the age of breastfeeding and weaning shows similarities with other analyzed archaeological hunter–gatherers (Schurr and Powell, 2005; Clayton et al., 2006; Waters-Rist et al., 2011; Eerkens and Bartelink, 2013). Particularly, SAC samples show the most similarities with those from southwestern Siberia (Waters-Rist et al., 2011) and central California (Eerkens and Bartelink, 2013). Both cases share the characteristic of being societies with subsistence strategies based only on foraging activities without any food production. Also, the case of southwestern Siberia corresponds to groups inhabiting a high latitude environment like the case of Patagonia. These studies used an intra-individual sampling methodology. Waters-Rist et al. (2011) obtained $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data from three different growth areas of long bones in 49 subadults whereas Eerkens and Bartelink (2013) analyzed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in an average of 7 or 8 serial sections of the first molars of 17 adult individuals. Despite the different methodological approaches, these studies recorded similar trajectories of breastfeeding and weaning. According to $\delta^{15}\text{N}$, both in the Siberian and Californian populations, there is a period of exclusive breastfeeding between 0 and 2 years of age and complete weaning takes place at about 5- to 6-years old (Waters-Rist et al., 2011; Eerkens and Bartelink, 2013). In the specific case of SAC, we identified the end of exclusive breastfeeding to be between 0.75 and 2 years of age. Weaning would be complete after 4 years of age, while by about 6 years of age, the diet would be similar to that of an adult, which implies a long and gradual weaning process.

Thus, this characterization of weaning as a gradual and lengthy process in this Patagonian foragers is coincident with Sellen and Smay's results in their cross-cultural study. As mentioned above, the authors found, that compared to agricultural and pastoral societies, hunter-gatherers had the oldest ages for complete cessation of breastfeeding (i.e., complete weaning) but they tended to start the weaning process earlier, despite the apparent lack of adequate weaning foods such as cereals and animal milk in foragers (Sellen and Smay, 2001).

This triggers questions about which foods would be used during the weaning process in hunter–gatherer societies, especially in high latitude environments, such as southern Patagonia, where the plant resources are scarce and with marked seasonal availability. In this

regard, some references found in the ethnohistorical and ethnographic records for these Patagonian populations are illustrative. In his writings about the Selknam from Tierra del Fuego Island, Martín Gusinde ([1936]1982) made some notes on breastfeeding and weaning practices. In spite of their insular environment, the Selknam were terrestrial hunter-gatherers who share many cultural characteristics with other populations from continental Patagonia. This ethnographer noticed that exclusive breastfeeding took place during the first months of an individual's life and, that, the first supplementary food was guanaco fat and meat that were given to children as pacifiers or chewing toys (Gusinde, [1936] 1982). Other sources mention that choique meat and fat were particularly reserved to women and small children (Palermo, 1983). These reports from Patagonia are also coincident with Sellen and Smay's cross-cultural study that identified in many hunter-gatherers groups the frequent use of meats, fat and other animal products as adequate weaning foods even for infants (sometimes with the aid of pre-mastication by an adult) (Sellen and Smay, 2001).

In addition, as in most populations analyzed by Sellen and Smay (2001) the use of fruits and starchy vegetables as weaning foods was also recorded in the ethnohistorical record from Patagonia. One of the references is the use of a plant—yocón (*Diposis patagonica*)—to feed babies and infants during the weaning process (Ladio and Lozada, 2000). Other Patagonian edible resources are fruits (*Berberis buxifolia*, *Berberis empetrifolia*, *Empetrum rubrum*), roots (*Azorella trifurcata*, *Arjona Patagonica*), tubers (*Tropaeolum patagonicum*), and mushrooms (*Cyttaria darwinii*, *Cyttaria hockeri*) as well as bird eggs (e.g., *Pterocnemia pennata* and *Chloephaga pita*) (Musters, [1871] 1991; Onelli, [1904] 1998; Martinic, 1995; Ladio and Lozada, 2000; Ochoa and Ladio, 2011), some of which had the potential to be used as alternative weaning and children foods.

CONCLUSIONS

This study constitutes the first presentation of archaeological data for stable nitrogen and carbon isotopes available to discuss the processes of breastfeeding and weaning among hunter-gatherers from southern Patagonia. The analyzed sample is characterized by a high number of individuals with a narrow temporal and spatial distribution, together with a high proportion of subadults. This combination of characteristics is rather unusual for archaeological studies of hunter-gatherer groups worldwide. The aims of the study were to characterize weaning and breastfeeding among this Patagonian forager group and to test Sellen and Smay's observation that foragers tend to have an early onset of weaning, around the first year of life, but a late complete cessation of breastfeeding.

The results obtained and discussed here show that $\delta^{15}\text{N}$ data have a clear relationship with age, with individuals under 4-years old having the highest values. These results have been interpreted as the end of exclusive breastfeeding to be between 0.75 and 2 years of age and the cessation of breastfeeding about 5–6 years of age. In addition, $\delta^{13}\text{C}$ shows little variation with age. These combined results suggest that among this Patagonian hunter-gatherer population the weaning process was gradual and lengthy, with an early incorporation of supplementary solid food and a late cessation of

breastfeeding. This pattern is consistent both with Sellen and Smay's findings on foragers' weaning practices and results obtained by other researchers in other isotopic studies of archaeological hunter-gatherer populations. Finally, it is worth noting that this case study corresponds to human groups inhabiting high latitude environments during the Late Holocene, which gives them a distinctive feature because of the scarcity and low seasonal availability of plant foods for the weaning process to take place. Future research will be directed to investigate the relation between these inferred weaning and breastfeeding practices with mobility strategies and demographic characteristics of these populations.

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