Journal of Archaeological Science 37 (2010) 317–327

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

The fish of Lake Titicaca: implications for archaeology and changing ecology through stable isotope analysis

Melanie J. Miller [a], José M. Capriles [b], Christine A. Hastorf [a,*]

[a] Department of Anthropology, University of California at Berkeley, 232 Kroeber Hall, Berkeley, CA 94720-3710, USA
[b] Department of Anthropology, Washington University in St. Louis, One Brookings Drive C.B. 1114, St. Louis, MO 63130, USA

ARTICLE INFO

Article history:
Received 23 May 2009
Received in revised form
18 September 2009
Accepted 22 September 2009

Keywords:
Prehistoric fish use
Paleoecology of Lake Titicaca
South America
Carbon
Nitrogen
Stable isotopes
Archaeology
Lipid extraction

ABSTRACT

Research on past human diets in the southern Lake Titicaca Basin has directed us to investigate the carbon and nitrogen stable isotopes of an important dietary element, fish. By completing a range of analyses on modern and archaeological fish remains, we contribute to two related issues regarding the application of stable isotope analysis of archaeological fish remains and in turn their place within human diet. The first issue is the potential carbon and nitrogen isotope values of prehistoric fish (and how these would impact human dietary isotopic data), and the second is the observed changes in the fish isotopes through time. Out of this work we provide quantitative isotope relationships between fish tissues with and without lipid extraction, and a qualitative analysis of the isotopic relationships between fish tissues, allowing archaeologists to understand these relationships and how these values can be applied in future research. We test a mathematical lipid normalization equation to examine whether future researchers will need to perform lipid extraction procedures for Lake Titicaca fish. We also analyze a number of aquatic plants to better understand the range of isotopic signatures of the Lake Titicaca ecosystem. We use these data to better understand prehistoric human diet and the role that fish may have played in the past as well as potential changes in local lake ecology through time.

1. Introduction

This study grew out of the rich yet complex study of prehistoric diets, including stable isotope data from cooking pots, plants, animals and human teeth that have been collected by the Taraco Archaeological Project working in the Titicaca Basin of Bolivia. Here we present stable isotope analysis of the archaeological fish samples to understand their role in the diet of the Formative Period inhabitants of the southern Lake Titicaca Basin. Carbon and nitrogen stable isotope analysis has become very useful in a variety of dietary studies (Ambrose, 1993; Finlay et al., 2002; Schoeninger and DeNiro, 1984), and ancient human diets and their change through time (Ambrose et al., 1998; Hastorf, 1991; Katzenberg et al., 1995; Prowse et al., 2004; Richards et al., 2003; Rutgers et al., 2009; Schwarcz et al., 1985; Van Klinken et al., 2000; White et al., 1999). However, in order to place the fish in human history, it is necessary to learn about the relationships and ecology of the fish from their muscle, bone and scales, since muscle is rarely preserved in archaeological contexts, whereas bone and scales are. We investigate modern fish specimens from Lake Titicaca to compare with our archaeological analysis. Studying fish through isotopic analysis introduces an additional layer, as recent research has highlighted the important role that lipid extraction plays in retrieving correct $\delta^{13}C$ signatures from samples that contain lipids (Kojadinovic et al., 2008; Mintenbeck et al., 2008; Post et al., 2007; Sotiropoulos et al., 2004; Sweeting et al., 2006).

In this paper we contribute to the discussion of two related issues regarding the application of stable isotope analysis to archaeological fish remains and in turn their place within human diet. The first issue is the interpretation of the carbon and nitrogen isotopic values of prehistoric fish (and how these impact human dietary values), and the second is the lake–fish ecological relationship. By understanding the isotopic compositions of the fish within the larger ecology of Lake Titicaca we can better understand the human interactions in this rich and diverse region as well as the roles that fish played in human life on the Taraco Peninsula during their first 1000 years of living in settled communities. To address these two questions, we first examine modern fish and the effects of lipid extraction on carbon and nitrogen isotope values. Then we turn to the isotopic relationships between the muscle, bone, and scale tissues of modern fish to learn of their variance. The fish and aquatic plants of Lake Titicaca have not previously been analyzed

* Corresponding author.
E-mail address: hastorf@berkeley.edu (C.A. Hastorf).

0305-4403/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.jas.2009.09.043

for their isotope compositions and previous studies of aquatic environments show large variation in the isotopic ranges observed (Chisholm et al., 1982; Dufour et al., 1999; Fischer et al., 2007; France, 1995; Katzenberg and Weber, 1999; Mintenbeck et al., 2008; Sotiropoulos et al., 2004; Sweeting et al., 2006). Other studies of modern and archaeological fish species from lacustrine environments have reported a large range of carbon values. France (1995) reported pelagic consumers to range from $\delta^{13}C$ −38 to −26‰ and littoral consumers to have $\delta^{13}C$ values from −32 to −16‰. Katzenberg and Weber (1999) report fish from Lake Bikal to have $\delta^{13}C$ values ranging from −24.6 to −12.9‰. Dufour et al. (1999) report $\delta^{13}C$ values for 3 European lakes and Lake Bikal to range from −32.5 to −19.8‰. These carbon values span such a large range that it was clear to us that Lake Titicaca fish need to be investigated in their own right. Finally, we compare modern and archaeological organic carbon and nitrogen isotopes of the fish, providing new information in the foodways scholarship where fish are a potential component.

Lake Titicaca is located at an elevation of 3810 m above sea level in the south central Andes of South America. The lake covers a surface area of approximately 8400 km^2 and is divided in two unequal parts; the northern portion, known as Chucuito is larger and deeper than the southern portion, known as Wiñaymarca (Fig. 1). The Taraco Peninsula, our study area, is located in the southeastern portion of Wiñaymarca. Due to its overall shallowness, Wiñaymarca can support higher biomass densities than the northern portion, but it is also more vulnerable to climatic and environmental changes (Abbott et al., 1997; Baker et al., 2005, 2009; Binford et al., 1997; Calaway, 2005).

Faunal resources are readily available on the shoreline of the Taraco Peninsula and include over 50 species of small to large aquatic and terrestrial birds, two dozen species of fish, and a few species of frogs and toads (Dejoux and Iltis, 1992; Kent et al., 1999; Levieil and Orlove, 1990; Orlove 2002; Portugal Loayza, 2002; Steadman and Hastorf, in press;). In addition, readily available water from a number of streams and associated wetlands (known as bofedales) are ideal habitats for terrestrial vertebrates, including camelids, deer, and several rodents.

Two genera of fish (*Orestias* and *Trichomycterus*), comprising approximately 26 species, have been documented to live in Lake Titicaca (Lauzanne, 1992; Parenti, 1984; Sarmiento and Barrera, 2004). The genus *Orestias* is composed of a wide variety of species most of which are small, rarely exceeding 5 cm in length, some ranging to just above 20 cm of standard length, exhibit high genetic diversity, and are specialized to specific microhabitats within the lake (Lüssen et al., 2003; Parenti, 1984; Vaux et al., 1988). Two species of bottom-dweller filter feeder *Trichomycterus* have been described for Lake Titicaca: *T. dispar* and *T. rivulatus* and they range in size from 12 to 19 cm standard length (Fernández and Vari, 2009; Sarmiento and Barrera, 2003).

Orestias have been known to consume a broad spectrum of aquatic resources including algae, macrophytes, zooplankton, amphipods, ostracods, insects, and insect larvae (Lauzanne, 1992). Their diet is constrained by a number of factors including species, ontogeny, size, availability, and degree of specialization (Vaux et al., 1988). Lauzanne (1992) among other ichthyologists have suggested that *Orestias* specimens tend to eat more fauna as they get larger. *Trichomycterus* are filter feeders ingesting organic remains in

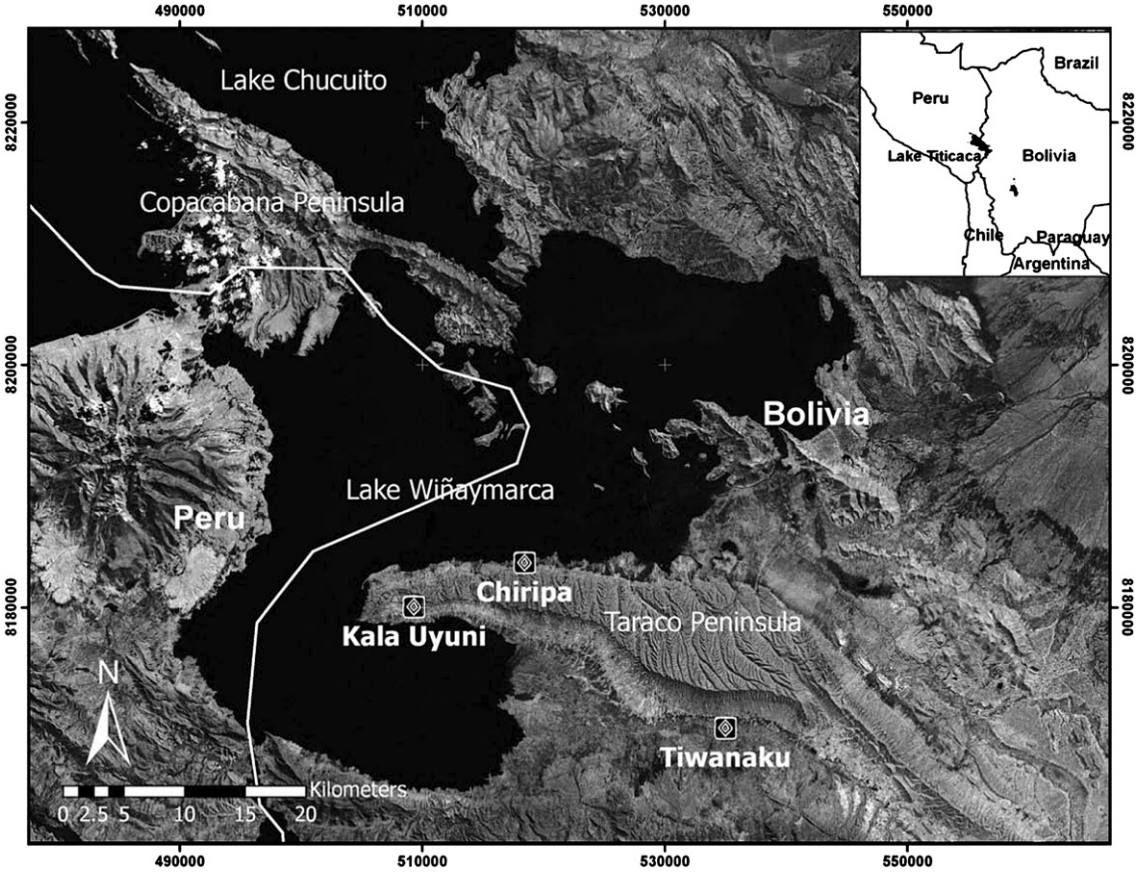

Fig. 1. Map of the region with sites mentioned in text plotted using ArcGIS 9.2. The base image is a Landsat L7 satellite image type Enhanced Thematic Mapper Plus (ETM+) panchromatic Band 8.

addition to a number of different autochthonous benthic macroinvertebrates.

In this paper we present a series of analyses that help us interpret the place of fish in past Lake Titicaca basin human diet. Our analyses demonstrate that a mathematical correction can accurately predict meaningful modern and archaeological carbon isotope values without completing lipid extraction procedures, saving researchers time and money. Comparison of fish tissues' isotope values provides archaeologists with an important relationship between hard and soft tissue remains, allowing for interpretation of fish muscle's contribution to human diets from bone or scale. We also confirm that the fish of Lake Titicaca have carbon isotope values that overlap those of maize, which is a significant factor for archaeologists studying past foodways and diet. Finally, our analyses suggest that there has been a significant shift between modern and archaeological fish carbon isotope values and we suggest a number of reasons for this finding.

2. Archaeological evidence for fish use in Lake Titicaca

Archaeologists working in the Lake Titicaca Basin agree that aquatic resources played a significant role in human diet and economy as people began settling on the landscape during the Early Formative Period (2000–800 BCE). Unfortunately, few studies have explicitly focused on building baseline research to understand the complex dynamic associated with the exploitation and consumption of aquatic resources (Capriles, 2006).

Our recent faunal research based on hundreds of weight and NISP counts from heavy fraction flotation samples recovered from the Taraco Archaeological Project excavations suggests fish remains were consistently important in the economy of the Taraco residents between the Early Formative and Tiwanaku periods with a subtle decrease over time (Bruno, 2008; Capriles et al., 2008; Moore et al., 1999). Fish consumption is also evident in the recovery of several dozen bone tools used for the manufacture of fishing nets as well as in specialized cooking features recorded as "fish pits" where thousands of fish bones were systematically processed and/or buried (Moore and Hastorf, 2000; Moore et al., 1999).

3. Materials and methods

3.1. Archaeological fish: collection procedures

The archaeological fish specimens analyzed for this study were recovered as part of the standard recovery protocol, developed by the Taraco Archaeological Project, which has been carrying out field excavations on the peninsula since 1992, focusing in early settled life, farming, herding, and ceremonial integration (Bandy and Hastorf, 2007; Hastorf, 1999; Hastorf et al., 2001). Faunal materials were collected using two recovery procedures. The entire excavated sediment for each unit of provenience was screened using 6.35-mm (0.25-inch) screens to recover large bones from macrofauna, mostly composed of domesticated camelids and to record ubiquity of birds and fish (Hastorf and Bandy, 1999; Capriles et al., 2007). At least one 10-l sample of sediment from every excavated locus was collected for fine-grained 0.5 mm water flotation recovery. Microfaunal remains, including fish bones and scales were identified from these heavy fractions of flotation samples.

The fish remains discussed in this paper come from the sites of Chiripa and Kala Uyuni (KU) and are associated with the Late Chiripa phase (800–100 BCE), within the Middle Formative period. Scales and operculum bones were selected for study from both screen and flotation heavy fractions because these bones were easier to identify to more detailed taxonomic levels. Two types of scales were identified. One includes rugosities, a distinctive character of *O. luteus*. Large and medium (larger than 5 mm) scales most probably correspond to *O. agassii* and *O. pentlandii*. The analyzed operculum bones were specifically selected due to their better preservation. These bones were measured using digital calipers and, using a transfer function, we estimated their live body size to be between 85 and 221 cm. The archaeological fish seem to have been bigger overall than the modern specimens, suggesting that the prehistoric fish caught were more diverse and grew to an older age than modern fishing allows (Capriles, 2006).

3.2. Modern fish: collection procedures

Modern *Orestias* and *Trichomycterus* fish samples were collected from Wiñaymarca Lake by local fishermen from the community of San Jose on the peninsula in 2005 and 2008. The *Orestias ispi* samples were fished from Lake Titicaca and purchased from a market in La Paz in 2005 and 2008. Bones, scales, and muscle were separated and dried in Bolivia and were then mechanically cleaned and freeze dried using a Labconco in the Center for Stable Isotope Biogeochemistry (CSIB) at the University of California-Berkeley. Samples were homogenized using a Wig-L Bug or an agate mortar and pestle, and were stored in sealed test tubes until analyzed or treated.

3.3. Modern aquatic plants: collection procedures

Modern aquatic plant samples were collected from the lake by fishermen from Santa Rosa on the Taraco Peninsula. The collected plants were air and sun-dried in Bolivia before export. Additional important Lake Titicaca aquatic plant taxa not obtained by the fisherman were sampled from collections curated in the Herbario Nacional de Bolivia (LPB) in La Paz. Plants were imported to the USA and freeze dried at the CSIB at the University of California-Berkeley. Samples were homogenized using a Wig-L Bug and were stored in sealed tubes until analyzed. Carbon and nitrogen isotopes were measured together on the same plant sample.

3.4. Stable isotope analysis and removal of lipids

The stable isotope ratio data are reported in standard delta (δ) notation as parts per thousand (per mil, ‰), with results reported relative to the Pee Dee Belemnite standard for $\delta^{13}C$, and atmospheric nitrogen for $\delta^{15}N$.

The presence of lipids in fish tissues alters their isotopic composition and causes depleted (more negative) $\delta^{13}C$ values. Lipids lack nitrogen atoms and are depleted in ^{13}C, compared to other biochemical components such as proteins (DeNiro and Epstein, 1977; Sweeting et al., 2006). The amount a $\delta^{13}C$ value alters depends on the lipid content of the tissue and has proven to be variable across different animals, with recent studies unable to show a standard shift that can be uniformly applied to all fish samples (Post et al., 2007; Sweeting et al., 2006). Lipid removal is beneficial for correcting these variations in carbon values but can adversely affect the nitrogen isotope value of the same sample (Post et al., 2007; Sotiropoulos et al., 2004; Sweeting et al., 2006). Lipid removal also presents additional problems because it requires a larger sample size, introduces extra cost into sample preparation, and requires a greater amount of time for each sample to be treated. Additionally, if a study is interested in nitrogen isotope values, samples may have to be analyzed untreated to obtain an accurate $\delta^{15}N$ with an additional lipid extraction procedure for $\delta^{13}C$, therefore doubling sample analysis time and cost. We have done this dual analysis here to try to learn

if this extraction procedure is necessary and to understand treatment effects.

3.5. Fish sample preparation: lipid removal and collagen preparation

Not all of our fish samples were treated with lipid extraction. Many samples were too small to divide into both treated and untreated portions. The samples we could process ($n = 22$, 18 modern and 4 archaeological) were placed in glass tubes for lipid extraction. Room temperature 0.5 N hydrochloric acid was added to the samples and then refrigerated. Most samples were de-calcified after 24 h. Samples were then rinsed five times with megapure water (water purified to 18 MΩ·cm specific resistivity). An extraction mixture of chloroform, methanol, and water (1.0:2.0:0.8 respectively) was used to remove the lipid fraction from bones, scale, and muscle tissue for both modern and archaeological samples (Folch et al., 1957; Post et al., 2007; Sweeting et al., 2006). These samples were then rinsed with megapure water and freeze dried in a Labconco. The bulk samples also were freeze dried, homogenized, and kept in sealed tubes until they were analyzed. All samples were analyzed for carbon and nitrogen isotopes at the Center for Stable Isotope Biogeochemistry (CSIB) Lab at the University of California at Berkeley on Europa and Delta Plus mass spectrometers. The CSIB lab reports the long-term internal precision of these machines based on bovine liver and sucrose standards at ±0.15‰ for $\delta^{13}C$ and ±0.20‰ for $\delta^{15}N$. Random samples were run in duplicate during analyses and were found to be within 2% of each other.

4. Results and discussion

4.1. Modern fish tissue–isotopic relationships and lipid extraction

In order to interpret the archaeological data we need a better understanding of the isotopic fish tissue–tissue relationships. To do this, first we present the modern fish data, revealing the isotopic relationships between samples that are treated with lipid extraction and those that are not. Then we present the isotope data of these tissue–tissue relationships, patterning of bone and scale to muscle within fish tissues. Table 1 provides the modern fish carbon and nitrogen isotope data set, $n = 27$ untreated samples, $n = 18$ lipid-extracted samples, for at least 4 species.

C/N (by mass) ratios of the bulk and lipid-extracted samples are also provided in Table 1. The C/N ratios of untreated fish samples mostly fell within the accepted C/N range of 2.7–3.6 for unaltered collagen (DeNiro, 1985); there are a few untreated fish samples and one lipid-extracted sample that fall outside of this range. By comparing the samples that were analyzed as both bulk and lipid-extracted we notice that samples that had lipids removed return to the C/N range we expect. This follows the pattern that Sweeting et al. (2006) found between the lipid content in a fish tissue and the C/N values of bulk and lipid-extracted samples; as lipids are removed the C/N values are significantly decreased.

By comparing the untreated and the lipid-extracted values for the same fish we can evaluate the influence of the treatment process on carbon and nitrogen isotopes. Figs. 2, and 3 examine the relationships between these carbon and nitrogen signatures of a sample in its bulk form and the same sample after lipid extraction.

Fig. 2 displays the relationship between the C/N (bulk) and change (Δ) in carbon isotope values due to treatment effects (lipid-extracted − bulk). A linear regression determined a weak positive, yet insignificant, correlation between $\Delta\delta^{13}C$ and the C/N ratio ($\Delta\delta^{13}C = -3.156 + 1.027 \times C/N$, $N = 18$, $P = 0.059$, $R^2 = 0.205$). There is also a notable effect of tissue type, with the muscle tissues' $\delta^{13}C$ showing a greater change with treatment compared to hard tissues (with the exception of two bone samples that show a very large change in $\delta^{13}C$ between bulk and treated samples). Additionally, factors such as fish species, and fish diet (trophic level), and % lipid in the bulk sample may be driving the relationship between C/N and $\Delta\delta^{13}C$. A paired sample *t*-test showed significant differences between untreated and lipid-extracted $\delta^{13}C$ values (d.f. = 17, $t = -2.421$, $P < 0.027$).

The nitrogen isotope values for modern fish samples also show a similarly weak relationship between C/N (bulk) and $\Delta\delta^{15}N$. A linear regression also proved insignificant ($\Delta\delta^{15}N = 0.743 + - 0.091 \times C/N$, $N = 18$, $P = 0.743$, $R^2 = 0.007$). However, a greater change in $\delta^{15}N$ is observed for muscle samples than for hard tissues, indicating the differential lipid content in these tissue types and how isotope values are altered with their presence and absence. A paired sample *t*-test showed significant differences between $\delta^{15}N$ in bulk and treated samples (d.f. = 17, $t = -5.376$, $P < 0.001$).

Figs. 2 and 3 show that our lipid extraction procedure does affect tissue types and alter delta values differently. The hard tissues (bone and scale) $\delta^{13}C$ of lipid-extracted samples were, on average, 0.35‰ more positive than untreated samples (SD = 0.91‰). The bone and scales are grouped together for this calculation because both tissues have lower lipid content than other tissues, such as muscle and organs including liver (Sweeting et al., 2006). The average $\Delta\delta^{15}N$ for the same samples was 0.36‰ with a standard deviation of 0.31‰. The average change for both carbon and nitrogen isotopes due to treatment is approximately 0.35‰ in bone and scale samples. The directionality of the change for these hard tissues was not uniform; some samples became depleted and others became enriched in $\delta^{13}C$ and $\delta^{15}N$ with lipid extraction. This implies that these tissues had a relatively low lipid content overall in their untreated form and that its presence in these tissues has a small, but noted effect on their carbon and nitrogen isotope values.

Our modern *Orestias* and *Trichomycterus* muscle samples have an average $\Delta\delta^{13}C$ of 0.64‰ (SD = 0.33‰) and an average $\Delta\delta^{15}N$ of 0.74‰ (SD = 0.20‰). Sweeting et al. (2006) report similar average changes between lipid-extracted and bulk muscle samples for bass fish, with means of $\Delta\delta^{13}C = 0.67‰$, SD = 0.30‰ and $\Delta\delta^{15}N = 0.71‰$, SD = 0.26‰ (Sweeting et al., 2006:597, Table 1). This larger degree of change in isotope values of the muscle tissue validates the need for accounting for lipid presence in any fish study using muscle tissue. Additionally, these changes in muscle isotope values were directional (with the exception of one sample's $\delta^{13}C$). Treated samples were more enriched than bulk samples for both carbon and nitrogen isotopes.

Previous research examining lipid extraction effects have developed a number of mathematical equations to correct for lipid effects on $\delta^{13}C$ in aquatic tissues. These equations have had mixed results in their application to other data sets (Kojadinovic et al., 2008; Mintenbeck et al., 2008). Post et al. (2007) provide a corrective equation for predicting lipid-extracted $\delta^{13}C$ in aquatic organisms. We decided to verify its application to samples from our locality. The equation is as follows (Post et al., 2007:186):

$$\delta^{13}C(\text{normalized}) = \delta^{13}C(\text{untreated}) - 3.32 + 0.99 \times C:N.$$

The results from applying this equation to our Lake Titicaca material are listed in Table 1 as predicted $\delta^{13}C$ lipid-extracted values. We found that the Post et al. (2007) formula was relatively accurate in predicting the lipid-extracted value from the bulk fish samples. Using Post's formula on the Lake Titicaca fish samples, the overall average difference between the predicted carbon value and our measured value was 0.29‰ (with the measured values being slightly more depleted than the predicted). When we compared the

Table 1
Modern fish carbon and nitrogen stable isotope data (acceptable C/N ratios are in bold type).

Identifier	Taxa	Tissue type	$\delta^{13}C$ untreated	$\delta^{15}N$ untreated	C/N untreated	$\delta^{13}C$ lipid-extracted	$\delta^{15}N$ lipid-extracted	C/N lipid-extracted	Predicted $\delta^{13}C$ lipid-extracted (from Post et al., 2007)	$\Delta\delta^{13}C$ (lipid-extracted–untreated)	$\Delta\delta^{15}N$ (lipid-extracted–untreated)
Fish 1 B	*Orestias luteus*	Bone	−12.99	4.12	**3.1**	−13.14	4.66	3.09	−13.24	−0.15	0.54
Fish 3 B	*Trichomycterus* sp.	Bone	−15.32	6.54	**3.24**	−13.42	6.39	3.13	−15.43	1.9	−0.15
Fish 5 B	*Orestias agassii*	Bone	−11.88	4.96	3.71	−11.95	4.77	2.96	−11.53	−0.07	−0.19
Fish 5 S	*Orestias agassii*	Scales	−11.77	4.09	**3.34**	−11.97	4.81	3.01	−11.78	−0.2	0.72
Fish 7 B	*Orestias agassii*	Bone	−13.46	4.53	**3.18**	−13.94	5.07	2.91	−13.63	−0.48	0.54
Fish 7 S	*Orestias agassii*	Scales	−14.12	4.49	**3.41**	−14.52	5.05	3.14	−14.06	−0.4	0.56
Fish 8 B	*Orestias luteus*	Bone	−12.37	4.81	**3.34**	−12.18	5.13	2.99	−12.38	0.19	0.32
Fish 9 B	*Orestias agassii*	Bone	−11.98	4.3	**3.41**	−11.35	4.25	3.04	−11.92	0.63	−0.05
Fish 9 S	*Orestias agassii*	Scales	−10.98	4.48	**3.34**	−11.2	4.48	3.04	−10.99	−0.22	0
Fish 10 B	*Orestias* sp.	Bone	−13.62	5.64	3.92	−13.07	5.88	2.97	−13.06	0.55	0.24
Fish 10 S	*Orestias* sp.	Scales	−12.79	6.12	**3.6**				−12.55		
Fish 11 B	*Trichomycterus* sp.	Bone	−12.05	4.44	3.85	−12.19	5.18	**3.01**	−11.56	−0.14	0.74
Fish 13 B	*Orestias ispi*	Bone	−16.11	7.3	4.45	−13.95	7.75	**3.17**	−15.02	2.16	0.45
Fish 14 M	*Orestias agassii*	Muscle	−14.44	5.5	**3.37**	−13.64	6.05	**3.45**	−14.42	0.8	0.55
Fish 15 M	*Orestias luteus*	Muscle	−16.14	6.7	**3.31**	−15.66	7.69	**3.49**	−16.18	0.48	0.99
Fish 16 M	*Orestias* sp.	Muscle	−14.52	6.29	**3.25**	−14.02	7.2	**3.42**	−14.62	0.5	0.91
Fish 17 M	*Trichomycterus* sp.	Muscle	−14.68	5.5	**3.41**	−14.48	6.28	**3.54**	−14.62	0.2	0.78
Fish 18 M	*Orestias ispi*	Muscle	−16.69	9.47	**3.63**	−15.53	10.23	**3.56**	−16.42	1.16	0.76
Fish 19 M	*Orestias ispi*	Muscle	−15.92	8.4	**3.45**	−15.23	8.87	3.78	−15.82	0.69	0.47
Fish 2005 (1)	*Trichomycterus* sp.	Muscle/bone/scale	−11.53	6.78	**3.45**				−11.44		
Fish 2005 (2)	*Trichomycterus* sp.	Muscle/bone/scale	−12.67	8.55	5.58				−10.47		
Fish 2005 (3)	*Trichomycterus* sp.	Muscle/bone/scale	−14.27	7.28	4.48				−13.16		
Fish 2005 (4)	*Trichomycterus* sp.	Muscle/bone/scale	−14.20	5.54	6.85				−10.74		
Fish 2005 (5)	*Orestias agassii*	Muscle/bone/scale	−11.73	5.06	**3.51**				−11.58		
Fish 2005 (6)	*Orestias agassii*	Muscle/bone/scale	−12.16	6.34	**3.48**				−12.03		
Fish 2005 (7)	*Orestias luteus*	Muscle/bone/scale	−15.25	4.06	**3.60**				−15.01		
Fish 2005 (8)	*Orestias luteus*	Muscle/bone/scale	−11.81	5.41	**3.38**				−11.78		

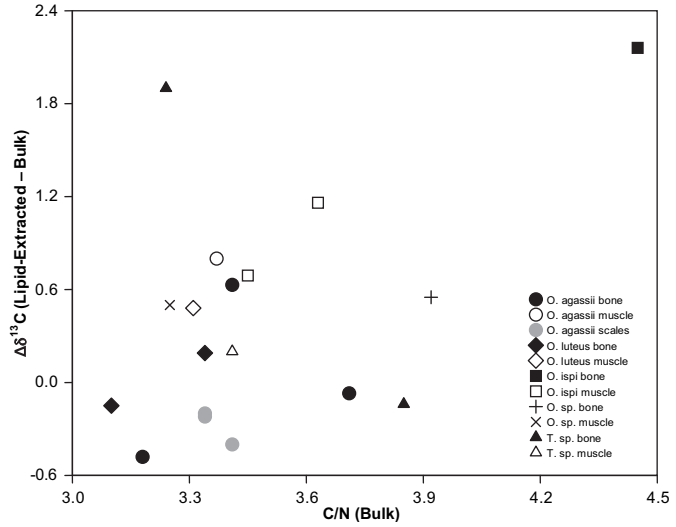

Fig. 2. Graph displaying the relationship between C/N (bulk) and change in carbon isotope values related to treatment (lipid-extracted–bulk) for modern fish samples.

Post et al. (2007) formula results based on tissue type, the formula was more effective in predicting the carbon values for bone and scale samples than for muscle samples. This is notably important for archaeologists as they usually only retrieve bone and scale. The average predicted carbon value for bone and scale was only 0.15‰ more enriched than the measured value, while the muscle samples predicted carbon values were 0.59‰ more depleted than the measured value. Therefore the equation that Post et al. (2007) presented proves to be a reliable mathematical correction for our modern Lake Titicaca fish samples.

Since our focus is to understand the fish component of past human diet, local lake isotopic variability, and the relationships that humans have had with the lake fish as a food source through time, isotopic changes of less than 1–2‰ are not likely to dramatically alter our data or our interpretations of that data. Working with archaeological samples inherently requires developing methods that are the least destructive and require a minimal amount of material. Fish scales may be plentiful but since fish scales are quite small, they are not always identifiable to genus (let alone species), and cannot be grouped with other scales to reach a minimum sample size without assuming an unwarranted interdependence.

Through our examination of the effects of chemical extraction we are now able to quantify how lipid presence or absence alters isotope values. Future research that relies on these two fish genera can use these data to guide decisions about whether it is required to extract the lipids or mathematically correct for lipid effects on isotope signatures. Since we analyzed 18 sample pairs to compare treatment effects on the isotope values, we are able to present an ideal graph of modern fish values in Fig. 4. This figure illustrates the lipid-extracted $\delta^{13}C$ and untreated $\delta^{15}N$ data from the same samples in one graph.

4.2. Modern fish isotopic tissue–tissue relationships

Due to the small sample sizes we have of our modern fish and the numerous analyses we undertook, we were only able to run three sample pairs from the same fish (directly displaying the tissue–tissue offsets between bone and scale, but unfortunately not the relationship to muscle). The three fish we sampled for bone and scale show that C and N isotopic values in these tissue types are very similar, but that the small amount of variation found (average variation = 0.31‰) is not constant (i.e., directional) by tissue type.

The overall relationship between *Orestias* species' muscle compared to *Orestias* bone and scale is patterned for carbon and nitrogen isotopes. We can see from these results that bone and scale samples are more enriched in $\delta^{13}C$ than in the muscle samples. Muscle tissue also appears to be more enriched in $\delta^{15}N$ than bone and scale samples.

Unfortunately the *Trichomycterus* sample size was too small to observe the same pattern between muscle and bone. However, we can see from these three *Trichomycterus* samples that the bone and scale samples are more enriched in $\delta^{13}C$ than muscle (Fig. 4) and muscle tissue is enriched in $\delta^{15}N$ relative to the harder tissues. The average difference in our small set of samples is about 2‰ enrichment in $\delta^{15}N$ for muscle (compared to hard tissues) and $\delta^{13}C$ is approximately 2‰ more depleted in muscle than in bone or scale. By establishing a value of tissue–tissue offset for carbon and

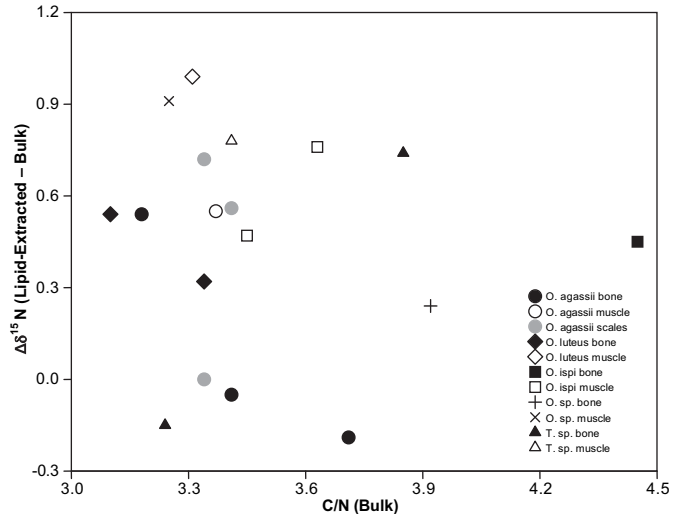

Fig. 3. Graph displaying the relationship between C/N (bulk) and change in nitrogen isotope values related to treatment (lipid-extracted–bulk) for modern fish samples.

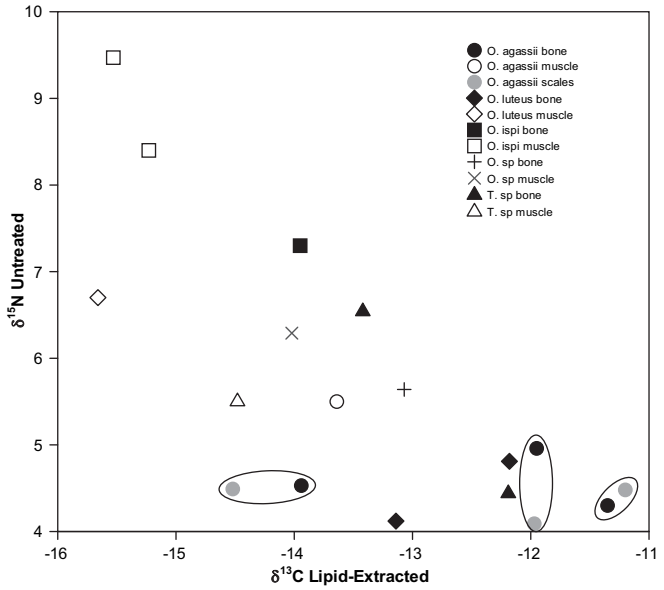

Fig. 4. Ideal graph displaying the lipid-extracted carbon values and untreated nitrogen values for modern fish by tissue type. Samples within ellipses are from the same fish.

nitrogen between tissues, archaeologists will be able to extrapolate fish muscle tissue values from bone and scale samples with greater accuracy. It is this predicted fish muscle value that can begin to inform us about past human fish consumption, since it is the muscle that humans would have most often consumed, metabolized, and incorporated into their own bodies and chemical signatures. Modern Bolivians who live near Lake Wiñaymarca are known to eat fish (especially the smaller species) in their entirety, including the bones. This may have also been the way prehistoric inhabitants consumed fish. The human digestive tract is not equipped to digest bones very well (or hair or scale) so it is fish muscle that was digested and metabolized and therefore recorded in human tissue chemistry.

4.3. Modern fish and lake plants

In order to better understand the fish isotopic values, it is helpful to investigate the relationship between the fish and their lake environment. The fish isotopes reflect their dietary compositions, and through analysis of lake plants we can begin to understand a part of their food web. Additionally, studying the fish–lake relationship will aid us in understanding the human dietary relationships with this significant aquatic food source. This broader approach allows us to see that the fish isotopes are unique.

Modern fish ($n = 18$, 15 *Orestias* sp., 3 *Trichomycterus* sp.) and modern lake plants ($n = 21$, with approximately 14 genera represented) were analyzed for their carbon and nitrogen stable isotope signatures. Table 2 presents the data for Lake Titicaca aquatic plants. Fig. 5 shows the carbon and nitrogen signatures of modern lake plants and modern fish.

The aquatic plants' isotope values reflect their adaptations to this lacustrine environment. The modern plant carbon isotope values cover a very large range, 25.23‰. France (1995) also reported a wide range of carbon isotope values for littoral environments in Ontario, covering a range of about 16‰. Littoral producers are relatively enriched in $\delta^{13}C$ compared to pelagic producers and this

Table 2
Modern lake plants carbon and nitrogen stable isotope data.

Identifier	Taxa or local name	Tissue type	$\delta^{13}C$	$\delta^{15}N$
LG 1	*Lemna gibba*	Leaf + stem	−26.5	1.54
RT 2	*Ranunculus trichophyllus*	Leaf + stem	−18.64	12.24
EPC 3	*Elodea potamegeton* (from Copacabana)	Leaf + stem	−9.71	2.3
PP 4	*Potamogeton pectinatus*	Leaf + stem	−7.21	3.69
EPH 5	*Elodea potamegeton* (from Huatayata)	Leaf + stem	−11.45	4.03
STM 6	*Schoenoplectus tatora*	Leaf + stem	−26.85	5.9
MC 7	*Myriophyllum coacollu*	Leaf + stem	−7.77	4.02
STR 8	*Schoenoplectus tatora* (below water, near rhizome)	Stem	−26.73	2.07
LPu2	*Schoenoplectus tatora* wrapped in unidentified algae	Algae on plant	−18.60	1.07
LPu4a	*Myriophyllum coacollu*	Leaf	−8.53	3.11
LPu4b	*Myriophyllum coacollu*	Stem	−14.60	2.07
LPu4c	*Myriophyllum coacollu*	Roots	−9.14	0.42
LPu5	*Chara*	Leaf + stem	−11.55	−2.58
LP6a	Si-Yu	Stem	−3.00	−0.58
LP6b	Si-Yu	Leaf	−5.78	1.41
LP6c	Si-Yu	Leaf + stem	−4.07	0.75
LPu1	unidentified lake grass	Leaf	−10.92	−5.27
LPu2007	Unidentified lake plant	Leaf + Stem	−28.23	9.87
Algae (lima, from lake)	Algae from lake (lima)	Algae	−21.64	7.02
Unknown lake plant (3)	Unidentified lake plant	Leaf + stem	−27.68	−2.70
Unknown lake plant (5)	Unidentified lake plant	Leaf + stem	−26.81	−4.95

All taxa are from lake Wiñaymarca except those identified from other localities.

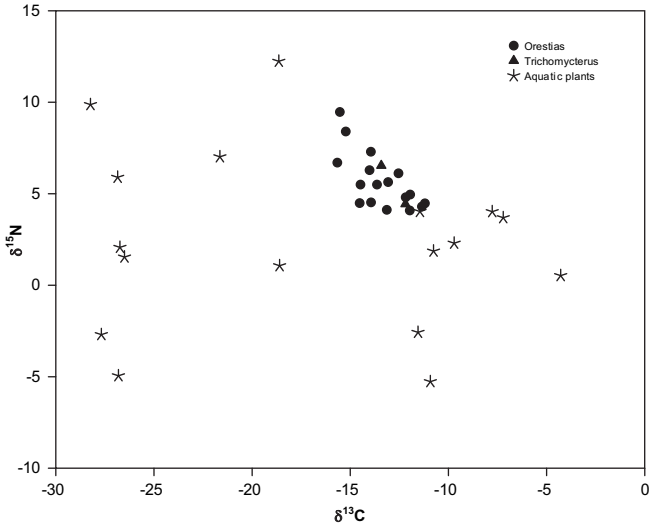

Fig. 5. Carbon and nitrogen stable isotopes for Lake Wiñaymarca aquatic plants and modern fish. Modern fish samples (bone, scale, and muscle samples) are represented by their lipid-extracted carbon values and their untreated nitrogen values. Modern aquatic plants that were sampled in separate parts are represented by the averaged value of these tissues.

is believed to be caused by a number of factors including limited CO_2 and fractionation of DIC (France, 1995; Post, 2002). Many of the plants we analyzed from Lake Wiñaymarca have very enriched $\delta^{13}C$ values. Sternberg et al. (1984) explain that the photosynthetic adaptations (and therefore the carbon products that give a plant its characteristic isotopic signature) of aquatic plants are particularly unique. While the carbon isotopic signatures of terrestrial plants allow researchers to be able to identify the type of photosynthetic process the plant is using (such as C3, C4, or CAM), aquatic plants' carbon isotopes do not allow for this type of interpretation. Enriched carbon values in aquatic plants may still result from a C3 pathway existing within certain systems, the slow diffusion of CO_2 in water, and other factors (Andrews and Abel, 1979; Benedict et al., 1980; O'Leary, 1981; Sternberg et al., 1984).

The nitrogen range for these plants is unique, since the range of nitrogen values for these samples is exceptionally broad, spanning 17.51‰. A large range of nitrogen values has been documented in other ecosystems and is related to the $\delta^{15}N$ of nitrogen sources within a lake system and fractionation of nitrogen during absorption and incorporation by these plants (Evans, 2001; Handley and Raven, 1992).

The isotope values we observe for the aquatic plants may also be related to other unique factors particular to this lake. Seasonal variation in primary producers has been documented and it is possible that the plants we sampled have isotope values that fluctuate with changing seasonal conditions (McCutchan and Lewis, 2001; Post, 2002). Additionally, the spatial distribution of the plants within the lake can play a role in the isotopic signatures of these aquatic flora (France, 1995).

The fish samples' isotope values range from −11.2 to −15.53‰ $\delta^{13}C$ (lipid-extracted) and 4.09 to 9.47‰ $\delta^{15}N$ (from the bulk samples). These carbon values are quite enriched, and relate to the unique, local ecosystem of the lake and the diets of these species. These values correspond with those reported by France (1995) for littoral consumers. Carbon is conserved between an animal and its diet, with the fractionation between trophic levels reported to be around 1‰ enrichment (DeNiro and Epstein, 1978; Peterson and Fry, 1987). Therefore these fish are consuming a diet that is within approximately ±1‰ of their observed $\delta^{13}C$ values.

Nitrogen isotopes are useful in interpreting the relationships between consumers in food webs because a large enrichment of $\delta^{15}N$ is observed as an organism ascends the food chain. The average fractionation of nitrogen in soft tissue as one rises through the food chain is $+3.4 \pm 1.1‰$ (Minagawa and Wada, 1984). Our nitrogen values also are disbursed, which may indicate the variation in trophic levels between these fish, or may also relate to the size or age of the fish we sampled. The values we report suggest that some fish were consuming more plant matter while others were probably consuming a fair amount of protein, possibly from snails or other invertebrates and maybe even some piscivory was occurring. The *Orestias ispi* muscle tissue has the highest $\delta^{15}N$ values. The small *ispi* fish probably consumes a greater proportion of protein or possibly vegetation that is also highly enriched in $\delta^{15}N$.

The isotopic composition of fish tissues serve as long-term representations of their diets, since turnover rates of tissues range from months to years (Hesslein et al., 1993). Due to this, we can infer that seasonal consumption variation will not alter the isotopic values of the tissues analyzed, and that the isotope values we observe are an average dietary signal of the overall diet. Comparing our plant values to the tight fish isotope values demonstrates that a number of the plants that we sampled are probably not components of the modern fish diet. Other sources of food for the fish, such as invertebrates, were not analyzed in this study but their presence and influence on the diet is visible in the $\delta^{15}N$ values. Since the lake plants $\delta^{15}N$ span a very broad range, including negative values, the range of positive values we see in the fish indicate that a significant portion of their diet was derived from other sources, the topic of future research.

Identifying the isotopic signatures of possible local foods provides archaeologists with another tool to better investigate production and consumption. Most importantly for the local archaeology is the evidence we have that the isotope values for the fish and some of the aquatic plants of Lake Titicaca directly overlap those of terrestrial C4 plants, most importantly maize (*Zea mays* L.), for both carbon and nitrogen isotopes (Hastorf and DeNiro, 1985; Miller and Hastorf, unpublished data). Berryman et al. (2007) reported human bone collagen values from the southern Lake Titicaca basin site of Khonkho Wankane to have carbon values ranging from −20 to −8‰. Human collagen from the Late Formative individuals at that site averaged −18.1‰ $\delta^{13}C$ and from the Tiwanaku time period the average was more enriched, −12.8‰ $\delta^{13}C$. This introduces a number of issues when one is attempting to tease out the introduction and emphasis of maize in local diets through time in the region, since carbon cannot be relied on as the sole distinguishing marker since both corn and fish foods were present in the area at some point during the Formative phase, albeit at different ratios in different communities (Logan, 2006).

4.4. Modern and archaeological fish

There is a significant amount of archaeological evidence on the Taraco Peninsula suggesting that lake resources, especially fish, played an important role in the lives of prehistoric peoples. By analyzing modern fish we are able to show that for $\delta^{13}C$, bone and scale are more enriched than muscle tissue and that muscle tissue is more enriched than bone or scale in $\delta^{15}N$. These $\delta^{13}C$ and $\delta^{15}N$ calibrations allow us to interpret the archaeological bone and scale values, informing about the fish muscle that prehistoric peoples would have consumed.

Table 3 presents the archaeological fish carbon and nitrogen data ($n = 11$, 10 *Orestias* sp., 1 *Trichomycterus* sp., Table 3). In Fig. 6 modern and archaeological fish isotopes are compared. This graph presents the $\delta^{13}C$ lipid-extracted and $\delta^{15}N$ untreated data for both modern and archaeological fish. The C/N values of bulk archaeological samples fell outside of the range of accepted (unaltered) values (DeNiro, 1985), but samples that were chemically treated with lipid extraction had their C/N values corrected and therefore we believe that these treated archaeological carbon and nitrogen signatures are an accurate reflection of the fish chemistry, the diagenesis of these samples is minimal.

Due to small sample sizes, only 4 of the archaeological samples were large enough to produce a lipid-extracted sample. We tested the Post et al. (2007) equation to see how well it predicted the $\delta^{13}C$ lipid-extracted values for all archaeological samples. For the 4 samples that were lipid-extracted we found that the Post equation worked relatively well. The Post equation produced $\delta^{13}C$ values that were more enriched than our measured values for 3 of the 4 samples, with the predicted values on average 0.63‰ (SD = 1.23‰) more enriched than the measured lipid-extracted values. When we compare the Post predicted $\delta^{13}C$ to the measured untreated $\delta^{13}C$ we see that Post predicted values are all more enriched than the measured untreated samples, on average the predicted values are 0.99‰ (SD = 0.52‰) more enriched. The $\Delta\delta^{13}C$ that we observed in our own 4 samples between lipid-extracted and bulk samples is very similar; 3 of the lipid-extracted values were on average 1.03‰ (SD = 0.22‰) more enriched than their bulk value; one had the exact opposite outcome with the lipid-extracted sample 1.1‰ depleted than the untreated sample.

All but one of the archaeological samples have enriched carbon signatures, with 3 samples having very similar $\delta^{13}C$ values, rangingfrom −8.27 to −7.61‰, and one outlier value of −16.23 $\delta^{13}C$. These values are more enriched than most other values we have encountered in our literature reviews, with occasional exceptions of enriched values such as those reported by Fischer et al. (2007) with $\delta^{13}C$ values ranging from −13.1 to −8.8‰ for fish from Stone Age sites in Denmark. When we include the values that the Post et al. (2007) equation predicts for lipid-extracted $\delta^{13}C$ we see that the samples are all quite enriched and that the one outlier is still the most depleted sample observed. The Post predicted $\delta^{13}C$ samples range from −4.86 to −11.09‰, with an average of −8.40‰ (SD = 2.3‰). The outlier sample with a relatively depleted $\delta^{13}C$ is an *Orestias* sample; this value could be real or this could be the result of diagenesis (although the C/N value of the lipid-extracted sample is 3.03 and therefore falls into the "good" range). Since this sample does not follow the pattern of the other archaeological samples we have decided to exclude it from our statistical analyses. Excluding this depleted sample, the range of observed carbon isotope values is still significantly broad, spanning about 6‰.

These carbon signatures suggest that the diet, age and/or habitat of the archaeological fish were different from those of the modern fish. Possible explanations for these very high archaeological carbon values include diet (consumption of lake plants and vertebrates with enriched carbon values), local ecosystem carbon cycling, size and age of fish caught in the past versus today, and possible climatic changes. The archaeological fish may have had a diet composed of food sources with a larger range of carbon values in the past, although these too would be quite enriched, and some fish may have preferentially consumed certain foods that others did not. The large range of carbon values could also be indicating temporal diet changes (Finlay et al., 2002).

The majority of the $\delta^{15}N$ values for these archaeological samples range between 5.05 and 7.66‰, with one unusually enriched fish $\delta^{15}N$ of 12.82‰. The average fish $\delta^{15}N$ value is 6.91‰ (SD = 2.19‰ including the outlier, SD = 1.03‰ if the outlier is excluded).

Unfortunately, we do not have samples of lake plants from prehistoric times so we are unable to assess the range of isotopic values that those plants may have had. However, based on our knowledge of the modern lake plants, it is reasonable to conclude that prehistoric aquatic plants may have had isotopic values within

Table 3
Archaeological fish carbon and nitrogen stable isotope data.

Identifier	Site and sector	Taxa	Tissue type	$\delta^{13}C$ untreated	$\delta^{15}N$ untreated	C/N untreated	$\delta^{13}C$ lipid-extracted	$\delta^{15}N$ lipid-extracted	C/N lipid-extracted	Predicted $\delta^{13}C$ lipid-extracted (from Post et al., 2007)	$\Delta\delta^{13}C$ (lipid-extracted –untreated)	$\Delta\delta^{15}N$ (lipid-extracted–untreated)
Archaeological fish L 5080 (e)	KU-AQ	*Trichomycterus* sp.	Bone	−6.72	7.05	4.32	−7.82	7.17	**3.51**	−5.76	−1.1	0.12
Archaeological fish L 5080 (g)	KU-AQ	*Orestias* sp.	Bone	−8.49	7.29	5.11	−7.61	7.93	**2.93**	−6.75	0.88	0.64
Archaeological fish L 5080 (h)	KU-AQ	*Orestias* sp.	Bone	−6.51	5.39	5.02				−4.86		
Archaeological Fish L 5178/1 (a)	KU-AC	*Orestias agassii*	Scales	−9.55	5.88	3.7	−8.27	6.22	**3.15**	−9.21	1.28	0.34
Archaeological fish L 5178/1 (b)	KU-AC	*Orestias luteus*	Scales	−11.25	7.43	4.6				−10.02		
Archaeological fish L 5178/1 (i)	KU-AC	*Orestias* sp.	Bone	−17.17	5.81	4.84	−16.23	6.2	**3.03**	−15.70	0.94	0.39
Archaeological Fish L 1405/3	Chiripa-Santiago	*Orestias* sp.	Scales	−10.08	7.66	4.73				−8.72		
Archaeological fish L 1431-1	Chiripa-Monticulo 1	*Orestias* sp.	Scales	−6.1	12.82	3.84				−5.62		
Archaeological Fish L 60/1	Chiripa-Llusco	*Orestias* sp.	Scales	−10.2	4.95	4.13				−9.43		
Archaeological fish L 1429	Chiripa-Monticulo 1	*Orestias* sp.	Scales	−9.64	6.72	3.92				−9.08		
Archaeological fish L 679/1	Chiripa-Santiago	*Orestias* sp.	Scales	−11.44	5.05	3.71				−11.09		

KU represents the site of Kala Uyuni.

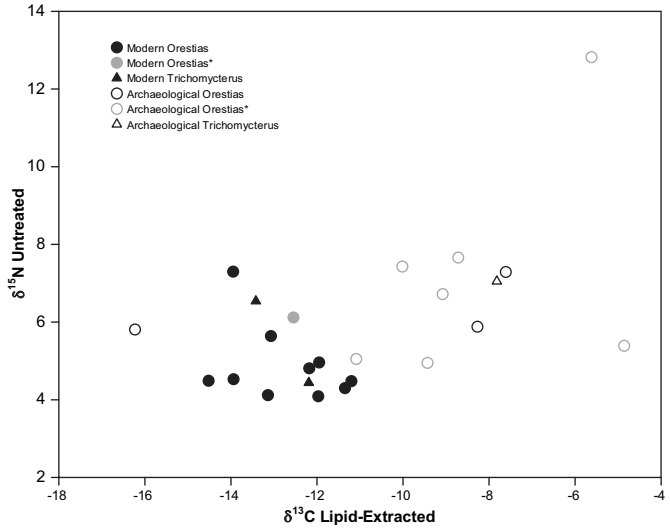

Fig. 6. Carbon and nitrogen stable isotope values for modern and archaeological fish. Starred samples are derived from the equation in Post et al. (2007) for predicting lipid-extracted stable carbon isotope values.

a similar range. There appears to be more variation in the fish carbon sources than in the nitrogen sources (the carbon range is about three times greater than the nitrogen range). The tight nitrogen range suggests that the majority of the fish were all occupying a similar trophic level. We have not accounted for the size or age of these archaeological samples and it has been noted that feeding strategies/preferences change as fish age (Lauzanne, 1992; Sweeting et al., 2007; Vaux et al., 1988). It is worth remembering that small fish are underrepresented in archaeological studies due to the difficulty of recovering and analyzing smaller sizes.

There is no overlap between the archaeological and modern sample carbon isotopes, with the exception of the one outlier archaeological sample, $\delta^{13}C = -16.23$. This was an unexpected result that we cannot offer a definitive explanation for as yet. This disparity could be related to changes in the local lake environment of the past. The global change in carbon isotopes that has been documented in recent samples (around 2‰) does not account for our large divide between modern and archaeological samples (Friedli et al., 1986).

The $\delta^{13}C$ values are different in many ways. We performed a two-way ANOVA (excluding the one outlier with the depleted carbon value) to test the differences between chronology and taxonomy found in the $\delta^{13}C$ results. The results showed significant differences between modern and archaeological fish ($F = 47.486$, d.f. $= 1$, $P < 0.001$), but not between taxonomic genera, *Orestias* and *Trichomycterus* ($F = 0.012$, d.f. $= 1$, $P = 0.9$) nor the interaction of chronology and taxonomy ($F = 0.081$, d.f. $= 1$, $P = 0.6$). Post-hoc Tukey comparisons of means determined significant differences only between modern and archaeological samples ($P < 0.001$) and specifically between modern *Orestias* and archaeological *Orestias* ($P < 0.001$), modern *Trichomycterus* and archaeological *Orestias* ($P = 0.007$), and modern *Orestias* and archaeological *Trichomycterus* ($P = 0.03$). This shows that the temporal differences are more important than the taxonomic differences and suggests that a significant change in the carbon cycling or sources occurred through time, or that the age or size of fish caught has shifted over time.

We performed a two-way ANOVA (excluding the one outlier with the enriched nitrogen value) to clarify the differences between chronology and taxonomy found in the $\delta^{15}N$ results. Interestingly

there were no significant differences in $\delta^{15}N$ values for any interactions we have studied, including between modern and archaeological fish values, between *Orestias* and *Trichomycterus*, and between any chronological or genera pairs. This suggests that fish have remained at similar trophic positions through time in Lake Wiñaymarca and that these species are consuming similar levels of plants and proteins over the past 3000 years. This also further supports the theory that the underlying change of fish isotopes has not necessarily been a direct shift in dietary practices but may have more to do with the local carbon changing over time, or a change in fishing practices (reflecting differences in size and age of fish caught).

Examining these archaeological bone and scale samples in light of the patterns we observed in the modern analogues, we would expect the muscle tissue of these fish to be more depleted in $\delta^{13}C$ and more enriched in $\delta^{15}N$. The average $\delta^{13}C$ value for archaeological fish is $-8.2‰$ (excluding the outlier of $-16.23‰$). Archaeological fish muscle would have been more depleted in carbon than this. The average $\delta^{15}N$ value for the archaeological fish is $6.3‰$ (excluding the outlier of $12.82‰$), the fish muscle should be more enriched in nitrogen than this value. In our analysis of modern fish we noted (with caution) that modern fish muscle is approximately $2‰$ depleted in $\delta^{13}C$ and $2‰$ more enriched in $\delta^{15}N$ compared to hard tissues. If this holds true for archaeological samples then the fish muscle would average $-10.2‰$ $\delta^{13}C$ and $8.3‰$ $\delta^{15}N$. Further analysis of a larger sample of modern fish would help clarify the fractionation values between tissue types and may alter this current interpretation. Additionally, studying more archaeological samples from different sites and time periods would strengthen our understanding of the prehistoric fish and their chemistry.

5. Conclusions

In this paper we have examined modern and archaeological *Orestias* and *Trichomycterus* fish samples for their carbon and nitrogen isotopes in order to better understand the diets of prehistoric inhabitants of the Lake Titicaca region and the role of fish in those diets. We found that lipid extraction of fish samples was helpful in identifying the true carbon isotope values but detrimentally altered the nitrogen values. The Post et al. (2007) equation was useful in predicting lipid-extracted carbon values and may allow researchers to save time, money, and sample resources in the future by applying this mathematical equation to tissue analysis from modern and archaeological faunal remains. Our analysis was unable to reliably quantify the tissue–tissue offset values between modern fish tissue types but we noted significant carbon depletion and nitrogen enrichment in muscle tissue relative to bone and scale. This is of particular importance to archaeologists studying human diet.

Fish bone and scale samples from archaeological contexts were analyzed and when compared to modern analogous fish, we found a significant difference in the carbon values between these two groups. This points to changing carbon sources over time (changing fish diets), that the carbon cycling within the local lake ecosystem has changed, fish of different ages or sizes were eaten, or perhaps climatic changes are influencing this observed shift. Further investigation of this phenomenon would provide useful information to modern biologists, geologists, conservationists, and archaeologists.

Our analyses of prehistoric fish remains from archaeological sites on the Taraco Peninsula provide us with important clues about the interpretation of the isotopic values of these prehistoric food sources. For millennia people have relied on Lake Titicaca to provide food, and stable isotope analysis of these fish assist us in better understanding diet and foodways, especially when we examine human bone collagen carbon and nitrogen isotopes and tooth enamel carbon isotopes. Unfortunately, our analysis of modern and archaeological fish confirmed that the carbon and nitrogen signatures of these fish species overlap the carbon and nitrogen isotope values of maize. Isotopic and archaeological evidence points to the significance of fish and maize through time. Stable isotopes will not be the only analytical tool we will need to tease apart these food contributions to human diets.

Acknowledgments

We would like to thank the members of the Taraco Archaeological Project, the Directorate of Archaeology, Ministry of Culture in La Paz, and the local residents of the Taraco Peninsula who introduced us to the importance of fish in their lives. We would also like to thank Dr Bob Feranec and Dr Alan Shabel for guidance with lipid extraction protocols. Many thanks to Paul Brooks, Stefania Mambelli, and Dr Todd Dawson, of CSIB, for their assistance in stable isotope analysis at UC Berkeley. We would also like to thank Alejandra Domic and Dr Stephan Beck for helping with the identification of Lake Titicaca plants and suggestions for statistical analysis. We thank the communities of San José, Santa Rosa and Chiripa, and especially Petrona Aruquipa. Melanie J. Miller initially was supported by the National Science Foundation with a Research Experience for Undergraduates, under Hastorf and Bandy's National Science Foundation grant BCS Archaeology 0234011 C for the initial isotopic analysis. Further research has been supported by a Committee on Research Faculty Research Grant, University of California-Berkeley and Washington University in St. Louis. We are also grateful to our anonymous reviewers for their helpful critiques and comments.

References

Abbott, M.B., Binford, M., Brenner, M., Kelts, K., 1997. A 3500 ^{14}C yr high-resolution record of water-level changes in Lake Titicaca, Bolivia/Peru. Quaternary Research 47, 169–180.

Ambrose, S., 1993. Isotopic analysis of paleodiets: methodological and interpretive considerations. In: Sandford, M.K. (Ed.), Investigations of Ancient Tissue. Gordon and Breach Science Publishers, Langhorne, PA, pp. 59–130. 60-71, 82–96.

Ambrose, S., Butler, B.M., Hanson, D.B., Hunter-Anderson, R.L., Krueger, H.W., 1998. Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific. American Journal of Physical Anthropology 104, 343–361.

Andrews, T.J., Abel, K.M., 1979. Photosynthetic carbon metabolism in seagrasses. ^{14}C-Labeling evidence for the C_3 pathway. Plant Physiology 63, 650–656.

Baker, P.A., Fritz, S.C., Garland, J., Ekdahl, E., 2005. Holocene hydrologic variation at Lake Titicaca, Bolivia/Peru, and its relationship to North Atlantic climate variation. Journal of Quantitative Science 20, 655–662.

Baker, P.A., Fritz, S.C., Burns, S.J., Ekdahl, E., Rigsby, C.A., 2009. The nature and origin of decadal to millennial scale climate variability in the Southern Tropics of South America: The Holocene Record of Lago Umayo, Peru

Bandy, M., Hastorf, C.A., 2007. Kala Uyuni, an early political center in the Southern Lake Titicaca Basin, No. 64. Contributions of the Archaeological Research Facility Monograph Publications. University of California, Berkeley, CA.

Binford, M., Kolata, A., Brenner, M., Janusek, J., Seddon, M., Abbott, M., Curtis, J., 1997. Climate variation and the rise and fall of an Andean civilization. Quaternary Research 47, 235–248.

Benedict, C.R., Wong, W.W.L., Wong, J.H.H., 1980. Fractionation of the stable isotopes of inorganic carbon by seagrasses. Plant Physiology 65, 512–517.

Berryman, C.A., Blom, D.E., Tykot, R.H., 2007. Paleodietary insight into the rise of the state in the Southern Lake Titicaca basin: the view from Khonkho Wankane. In: Symposium Khonkho Wankane and Beyond: Recent Research in the Machaca Region of Bolivia. Co-organized by Ohnstad, A., Smith, S. C., at the 72nd Annual Society for American Archaeology Meeting, Austin, Texas, April 27.

Bruno, M.C., 2008. Waranq Waranqa: Ethnobotanical Perspectives on Agricultural Intensification in the Lake Titicaca Basin (Taraco Peninsula, Bolivia). Department of Anthropology, Washington University, St. Louis.

Calaway, M.J., 2005. Ice-cores, sediments and civilisation collapse: a cautionary tale from Lake Titicaca. Antiquity 79, 778–790.

Capriles, J.M., 2006. A Zooarchaeological Analysis of Fish Remains from the Lake Titicaca Formative Period (Ca. 1000 B.C.–A.D. 500) Site of Kala Uyuni, Bolivia. MA thesis, Department of Anthropology, Washington University, St. Louis.

Capriles, J.M., Moore, K.M., Domic, A.I., 2007. Recuperación diferencial de conjuntos arqueofaunísticos: implicancias para el análisis de restos de peces en la cuenca del Lago Titicaca. Archaeobios 1, 15–21.
Capriles, J.M., Domic, A.I., Moore, K.M., 2008. Fish remains from the Formative period (1000 BC–AD 400) of Lake Titicaca, Bolivia: zooarchaeology and taphonomy. Quaternary International 180, 115–126.
Chisholm, B.S., Nelson, D.E., Schwarcz, H.P., 1982. Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216, 1131–1132.
Dejoux, C., Iltis, A., 1992. Lake Titicaca: a Synthesis of Limnological Knowledge. Kluwer Academic, Dordrecht.
DeNiro, M.J., 1985. Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to paleodietary reconstruction. Nature 317, 806–809.
DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197, 261–263.
DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42, 495–506.
Dufour, E., Bocherens, H., Mariotti, A., 1999. Paleodietary implications of isotopic variability in Eurasian lacustrine fish. Journal of Archaeological Science 26, 617–627.
Evans, R.D., 2001. Physiological mechanisms influencing plant nitrogen isotope composition. Trends in Plant Science 6, 121–126.
Fernández, L., Vari, R., 2009. New species of *Trichomycterus* from the Andean Cordillera of Argentina (Siluriformes: Trichomycteridae). Copeia, 195–202.
Finlay, J.C., Khandawala, S., Power, M.E., 2002. Spatial scales of carbon flow in a river food web. Ecology 83, 1845–1859.
Fischer, A., Olsen, J., Richards, M., Heinemeier, J., Sveinbjornsdottir, A.E., Bennike, P., 2007. Coast-inland mobility and diet in the Danish Mesolithic and Neolithic: evidence from stable isotope values of humans and dogs. Journal of Archaeological Science 34, 2125–2150.
Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497–509.
France, R.L., 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40, 1310–1313.
Friedli, H., Lotscher, H., Oeschger, H., Siegenthaler, U., Stauffer, B., 1986. Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries. Nature 324, 237–238.
Handley, L.L., Raven, J.A., 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell and Environment 15, 965–985.
Hastorf, C.A.,1991. Gender, space and food in prehistory. In: Engendering Archaeology: Women and Prehistory, ed. by J. Gero and M. Conkey. Blackwell Press, Oxford, pp 132–159.
Hastorf, C. A., Bandy, M., 1999. Excavation methodology and field procedures. In: Early Settlement in Chiripa, Bolivia: Research of the Taraco Archaeological Project, No. 57 Contributions Archaeological Research Facility Monograph Publications, University of California, Berkeley, pp. 29–30.
Hastorf, C.A., DeNiro, M.J., 1985. Reconstruction of prehistoric plant production and cooking practices by a new isotopic method. Nature 315, 489–491.
Hastorf, C.A., Bandy, M., Steadman, L., Whitehead, W., Dean, E., de France, S., Steadman, D., Goddard, R., Kojan, D., Paz, J.L., Moore, K., 2001. El Periodo Formativo en Chiripa, Bolivia. Textos Antropologicos 13 (1–2), 17–91.
Hesslein, R.H., Hallard, K.A., Ramlal, P., 1993. Replacement of sulfur, carbon, and nitrogen, in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Canadian Journal of Fisheries and Aquatic Sciences 50, 2071–2076.
Katzenberg, M.A., Weber, A., 1999. Stable isotope ecology and paleodiet in the Lake Bikal region of Siberia. Journal of Archaeological Science 26, 651–659.
Katzenberg, M.A., Schwarcz, H.P., Knyf, M., Melbye, F.J., 1995. Stable isotope evidence for maize horticulture and paleodiet in southern Ontario, Canada. American Antiquity 60, 335–350.
Kent, A.M., Webber, T., Steadman, D.W., 1999. Distribution, relative abundance, and prehistory of birds on the Taraco Peninsula, Bolivian altiplano. Ornitología Neotropical 10, 151–178.
Kojadinovic, J., Richard, P., Le Corre, M., Cosson, R.P., Bustamante, P., 2008. Effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values in seabird muscle, liver, and feathers. Waterbirds 31, 169–178.
Levieil, D.P., Orlove, B., 1990. Local control of aquatic resources: community and ecology in Lake Titicaca, Peru. American Anthropologist 92, 362–382.
Lauzanne, L., 1992. Native species: the *Orestias*. In: Dejoux, C., Iltis, A. (Eds.), Lake Titicaca. Kluwer Academic, New York, pp. 405–419.
Logan, A.L., 2006. The application of phytolith and starch grain analysis to understanding formative period subsistence, ritual, and trade on the Taraco Peninsula, highland Bolivia. MS thesis, Department of Anthropology, University of Missouri.
Lüssen, A., Falk, T.M., Villwock, W., 2003. Phylogenetic patterns in populations of Chilean species of the genus *Orestias* (Teleostei: Cyprinodontidae): results of mitochondrial DNA analysis. Molecular Phylogenetics and Evolution 29, 151–160.
McCutchan Jr., J.H., Lewis Jr., W.M., 2001. Seasonal variation in stable isotope ratios of stream algae. Verhandlungen Internationale Vereinigung für Limnologie 27, 3304–3307.
Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48, 1135–1140.
Mintenbeck, K., Brey, T., Jacob, U., Knust, R., Struck, U., 2008. How to account for the lipid effect on carbon stable-isotope ratio ($\delta^{13}C$): sample treatment effects and model bias. Journal of Fish Biology 72, 815–830.
Moore, K., Hastorf, C.A., 2000. The re-interpretation of cultural context in excavation and analysis of archaeobiological samples from Chiripa, Bolivia, in: Symposium Integrating Plants, Animals and People in Archaeological Interpretation. Co-organized by Miller, N., Moore, K., at the 65th Annual Meetings of the Society for American Archaeology, Philadelphia, PA, April 7.
Moore, K.M., Steadman, D., deFrance, S., 1999. Herds, fish, and fowl in the domestic and ritual economy of formative Chiripa. In: Hastorf, C.A. (Ed.), Early Settlement in Chiripa, Bolivia: Research of the Taraco Archaeological Project, No. 57. Contributions Archaeological Research Facility Monograph Publications. University of California, Berkeley, pp. 105–116.
O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553–567.
Orlove, B.S., 2002. Lines in the Water: Nature and Culture at Lake Titicaca. University of California Press, Berkeley, CA.
Parenti, L.R., 1984. A taxonomic revision of the Andean killifish genus *Orestias* (Cyprinodontiformes, Cyprinodontidae). Bulletin of the American Museum of Natural History 178, 107–214.
Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18, 293–320.
Portugal Loayza, J., 2002. Los Urus: Aprovechamiento y Manejo de Recursos Acuáticos, Lidema. Konrad Adenauer Stiffung, La Paz.
Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.
Post, D.M., Layman, C.A., Albrey Arrington, D., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189.
Prowse, T., Schwarcz, H.P., Saunders, S., Macchiarelli, R., Bondioli, L., 2004. Isotopica paleodiet studies of skeletons from the Imperial Roman-age cemetery of Isola Sacra, Rome, Italy. Journal of Archaeological Science 31, 259–272.
Richards, M.P., Schulting, R.J., Hedges, R.E.M., 2003. Archaeology: sharp shift in diet at onset of Neolithic. Nature 425, 366.
Rutgers, L.V., van Strydonck, M., Boudin, M., van der Linde, C., 2009. Stable isotope data from the early Christian catacombs of ancient Rome: new insights into the dietary habits of Rome's early Christians. Journal of Archaeological Science 36, 1127–1134.
Sarmiento, J., Barrera, S., 2003. Peces. In: Flores Bedregal, E., Miranda, C. (Eds.), Fauna Amenazada de Bolivia, Animales sin Futuro? Ministerio de Desarrollo Sostenible, La Paz, pp. 77–102.
Sarmiento, J., Barrera, S., 2004. Fish. In: Ibish, P.L., Merida, G. (Eds.), Biodiversity: The Richness of Bolivia. Knowledge and Conservation Status, Ministerio de Desarrollo Sostenible. Fundacion Amigos de la Naturaleza, Santa Cruz, pp. 126–133.
Schoeninger, M.J., DeNiro, M.J., 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48, 625–639.
Schwarcz, H.P., Melbye, J., Katzenberg, M.A., 1985. Stable isotopes in human skeletons of southern Ontario: reconstructing paleodiet. Journal of Archaeological Science 12, 187–206.
Sotiropoulos, M.A., Tonn, W.M., Wassenaar, L.I., 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. Ecology of Freshwater Fish 13, 155–160.
Steadman, D., Hastorf, C.A., in press. Prehistoric birds from the Lake Titicaca region, Bolivia: long-term change in an Andean bird community. Journal of Nature Conservation (in press).
Sternberg, L., DeNiro, M.J., Keeley, J.E., 1984. Hydrogen, oxygen, and carbon isotope ratios of cellulose from submerged aquatic Crassulacean acid metabolism plants. Plant Physiology 76, 68–70.
Sweeting, C.J., Polunin, N.V.C., Jennings, S., 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Communications in Mass Spectrometry 20, 595–601.
Sweeting, C.J., Barry, J., Barnes, C., Polunin, N.V.C., Jennings, S., 2007. Effects of body size and environment on diet-tissue $\delta^{15}N$ fractionation in fishes. Journal of Experimental Marine Biology and Ecology 340, 1–10.
Van Klinken, G.J., Richards, M.P., Hedges, R.E.M., 2000. An overview of causes for stable isotopic variations in part European human populations: environmental, ecophysiological, and cultural effects. In: Ambrose, S.H., Katzenberg, M.A. (Eds.), Biogeochemical Approaches to Paleodietary Analysis. Kluwer Academic/Plenum Publishers, New York, pp. 39–63.
Vaux, P., Wurtsbaugh, W., Trevino, H., Marino, L., Bustamante, E., Torres, J., Richerson, P., Alfaro, R., 1988. Ecology of the pelagic fishes of Lake Titicaca, Peru-Bolivia. Biotropica 20, 220–229.
White, C.D., Longstaffe, F.J., Law, K., 1999. Seasonal stability and variation in diet as reflected in human mummy tissues from the Kharga Oasis and the Nile Valley. Palaeogeography, Palaeoclimatology. Palaeoecology 147, 209–222.