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

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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 ¹³C 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 relation. 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.
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 $^{13}\text{C}/^{12}\text{C}$, to $^{13}\text{C}/^{12}\text{C}$, and littoral consumers to have $^{13}\text{C}$ values from $^{13}\text{C}/^{12}\text{C}$ to $^{13}\text{C}/^{12}\text{C}$.

Katzenberg and Weber (1999) report fish from Lake Bikal to have $^{13}\text{C}$ values ranging from $^{13}\text{C}/^{12}\text{C}$ to $^{13}\text{C}/^{12}\text{C}$. Dufour et al. (1999) report $^{13}\text{C}$ values for 3 European lakes and Lake Bikal to range from $^{13}\text{C}/^{12}\text{C}$ to $^{13}\text{C}/^{12}\text{C}$. 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). In addition, readily available water from a number of streams and associated wetlands (known as bofedales) are ideal habitats for terrestrial vertebrates, including camels, 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 (Lüssem 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.](image-url)
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 for fine-grained 0.5 mm water flotation recovery. Microfaunal 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. Modern fish: collection procedures

Modern Orestias and Trichomycterus fish samples were collected from Wilkaymarca 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 and were stored in sealed 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 δ13C, and atmospheric nitrogen for δ15N.

The presence of lipids in fish tissues alters their isotopic composition and causes depleted (more negative) δ13C values. Lipids lack nitrogen atoms and are depleted in 15N, compared to other biochemical components such as proteins (DeNiro and Epstein, 1977; Sweeting et al., 2006). The amount a δ13C 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 δ15N with an additional lipid extraction procedure for δ13C, 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 (1:2:0.08 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 (%CV for δ13C and δ15N vary between 0.15 and 0.20 respectively). A linear regression determined a weak positive, yet insignificant, correlation between δ13C and C/N ratio (ΔDδ13C = −3.156 + 1.027 × C/N, N = 18, P = 0.059, R² = 0.205). There is also a notable effect of tissue type, with the muscle tissues’ δ13C showing a greater change with treatment compared to hard tissues (with the exception of two bone samples that show a very large change in δ13C 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 ΔDδ13C. A paired sample t-test showed significant differences between untreated and lipid-extracted δ13C 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 ΔDδ15N. A linear regression also proved insignificant (ΔDδ15N = −0.743 + −0.091 × C/N, N = 18, P = 0.743, R² = 0.007). However, a greater change in ΔDδ15N 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 δ315N 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) δ13C 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 ΔDδ15N 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 δ13C and δ15N 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 ΔDδ13C of 0.64% (SD = 0.33%) and an average ΔDδ15N 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 ΔDδ13C = 0.67% (SD = 0.30%) and ΔDδ15N = 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 δ13C). 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 δ13C 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 correction equation for predicting lipid-extracted δ13C 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 δ13C 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>
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<th>δ¹³C untreated</th>
<th>δ¹⁵N untreated</th>
<th>δ¹³C lipid-extracted</th>
<th>δ¹⁵N lipid-extracted</th>
<th>C/N lipid-extracted</th>
<th>Predicted δ¹³C lipid-extracted (from Post et al., 2007)</th>
<th>M.J. Miller et al. / Journal of Archaeological Science 37 (2010) 317–327</th>
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<td>3.48</td>
<td>-12.03</td>
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that are the least destructive and require a minimal amount of archaeological samples inherently requires developing methods to alter our data or our interpretations of that data. Working with isotopic changes of less than 1–2‰, humans have had with the lake fish as a food source through time, modern Lake Titicaca fish samples. The presented proves to be a reliable mathematical correction for our measured value. Therefore the equation that 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 δ13C and untreated δ15N 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 δ13C than in the muscle samples. Muscle tissue also appears to be more enriched in δ15N 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 δ13C than muscle (Fig. 4) and muscle tissue is enriched in δ15N relative to the harder tissues. The average difference in our small set of samples is about 2‰ enrichment in δ15N for muscle (compared to hard tissues) and δ13C is approximately 2‰, more depleted in muscle than in bone or scale. By establishing a value of tissue–tissue offset for carbon and...
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 relationships 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 δ13C compared to pelagic producers and this is believed to be caused by a number of factors including limited CO2 and fractionation of DIC (France, 1995; Post, 2002). Many of the plants we analyzed from Lake Wiñaymarca have very enriched δ13C 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 CO2 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 δ15N 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‰ δ13C (lipid-extracted) and 4.09 to 9.47‰ δ15N (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 δ13C values.

![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 values of these tissues.](image-url)
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 $+3.4 \pm 1.3_{\text{w}}$ (Minagawa and Wada, 1984). Our nitrogen values also are disbrushed, 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 tissue ratio 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 isolate values we observe are an average dietary signal of the overall diet. Comparing our plant values to the light fish isolate 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 isolate 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 Khonko Wankane to have carbon values ranging from $-20$ to $-8_{\text{w}}$, human collagen from the Late Formative individuals at that site averaged $-18.1_{\text{w}}$ $\delta^{13}$C and from the Tiwanaku time period the average was more enriched, $-12.8_{\text{w}}$ $\delta^{13}$C. This introduces a number of issues when one is attempting to tease out the introduction and emphasis of maize in local diets throughout 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 consumed and influences on the diet is visible in the $\delta^{15}$N.

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 isotope values 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 as well it predicted the $\delta^{13}$C lipido-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 $0.63_{\text{w}}$ (SD = 1.23$_{\text{w}}$) 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 $0.99_{\text{w}}$ (SD = 0.52$_{\text{w}}$) more enriched. The $\Delta^{13}$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 $1.03_{\text{C}}$ (SD = 0.22$_{\text{C}}$) more enriched than their bulk value; one had the exact opposite outcome with the lipid-extracted sample 1.1$_{\text{C}}$ 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, ranging from $-8.27$ to $-7.61_{\text{w}}$, 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 $-8.8_{\text{w}}$ 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 $-11.09_{\text{w}}$ with an average of $-8.40_{\text{w}}$ (SD = 2.3$_{\text{w}}$). 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$_{\text{w}}$.

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$_{\text{w}}$, with one unusually enriched fish $\delta^{15}$N of 12.82$_{\text{w}}$. The average fish $\delta^{15}$N value is 6.91$_{\text{w}}$ (SD = 2.19$_{\text{w}}$) including the outlier, SD = 1.03$_{\text{w}}$ 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
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‰ to 4‰) might explain the observed differences. The modern carbon isotope values are consistent with those found in ancient samples (Lauzanne, 1992). 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 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 ($F = 0.081, d.f. = 1, P = 0.6$). 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 ($F = 0.081, d.f. = 1, P = 0.6$). 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 ($F = 0.081, d.f. = 1, P = 0.6$).
there were no significant differences in δ¹¹⁴N 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 Titicaca and that these species are consuming similar levels of plants and proteins over the past 3000 years. This is further supported by the observed change of fish isotopes, which have 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 δ¹³C and more enriched in δ¹⁵N. The average δ³⁴S 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 δ¹⁵N 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 δ¹³C and 2‰ more enriched in δ¹⁵N compared to hard tissues. If this holds true for archaeological samples then the fish muscle would average –10.2‰ δ¹³C and 8.3‰ δ¹⁵N. 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.

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