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Dietary Resilience Among Hunter-Gatherers of Tierra del Fuego: Isotopic Evidence in a Diachronic Perspective

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Corresponding Author:	Mary Anne Tafuri, Ph.D. Università di Roma Roma, ITALY
Keywords:	Tierra del Fuego; stable carbon and nitrogen isotopes; Hunter-gatherers; diet; Resilience; colonialism
Abstract:	The native groups of Patagonia have relied on a hunter-gatherer economy well after the first Europeans and North Americans reached this part of the world. The large exploitation of marine mammals (i.e., seals) by such allochthonous groups has had a strong impact on the local ecology in a way that might have forced the natives to adjust their subsistence strategies. Similarly, the introduction of new foods might have changed local diet. These are the premises of our isotopic-based analysis. There is a large set of paleonutritional investigations through isotopic analysis on Fuegians groups, however a systematic exploration of food practices across time in relation to possible pre- and post-contact changes is still lacking. In this paper we investigate dietary variation in hunter-gatherer groups of Tierra del Fuego in a diachronic perspective, through measuring the isotopic ratio of carbon (δ13C) and nitrogen (δ15N) in the bone collagen of human and a selection of terrestrial and marine animal samples. The data obtained reveal an unexpected isotopic uniformity across prehistoric and recent groups, with little variation in both carbon and nitrogen mean values, which we interpret as the possible evidence of resilience among these groups and persistence of subsistence strategies, allowing inferences on the dramatic contraction (and extinction) of Fuegian populations.
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Opposed Reviewers:	
Response to Reviewers:	Reviewer #1: The paper “Dietary resilience among hunter-gatherers of Tierra del Fuego: Isotopic evidence in a diachronic perspective” presents a new isotope dataset with which to study pre and post-contact dietary changes. The authors present the first study of dietary change across this important social period, demonstrating surprising continuity given evidence in historical accounts for upheaval. Local communities appear to have retained a diet heavily focused on marine protein, which the authors describe as a form of cultural resilience. The use of isotopic data has been shown to complement historical datasets, despite the often-assumed superiority of the latter, and this is also the case here. The dataset is robust and the conclusions broadly supportable. However, to be appropriate for the broader readership of PLoS ONE I believe much more context is

required and the paper needs to be more usefully structured. Firstly, the Introduction and Archaeological/Historical Background is currently inadequate. For none specialists in the region, there is not enough information regarding the eventual hypotheses of the authors. What happened during contact? Why would the authors expect that the diet might change? Is there specific evidence for crops being introduced and locals using them? Is there evidence for local communities relying on differing extents of marine resources? This only comes in in the Discussion from Page 11-12 but needs to be in at the outset so people can understand the point of the study!

The sample of humans, 42, is also not clearly laid out and it is confusing at present. It needs to be clear where these are coming from, and which collections they are coming from, perhaps in a table. Spreading the discussion across a few pages in the text makes it hard to follow. It doesn't seem to ad up either I count 9 (pre-contact excavated subset), 2 (post-contact excavated subset), 1 (Orquera and Piana infant), 5 (Yesner et al.), 12 (Florence), 14 (Rome) = 43? Also why was only the infant from Rome left out and the others left in?

Samples are indeed 42: the comparative samples published by Yesner et al are 4. There was a typo. Also, we failed to clear out why we had to keep out the Roman infant: this was due to difficulties in the sampling process. We haave clarified this by changing the M&M section as follows "Sampling for isotopic analysis was carried out on the ribs of 14 individuals, with the exclusion of the infant, which was deliberately not sampled to avoid damage to the skeletal elements (i.e., only the skull was preserved and sampling attempts resulted very invasive)".

It is great that the authors included animals as a comparison dataset, and have a nice dataset of a number of different aquatic taxa. However, they initially state in the Introduction that these are not associated with humans, but then later in the Discussion suggest they are. Which is it? If they are not then is this because animals are missing from cemetery sites or because most did not collect them? Why were they missing from excavation? The authors then need to justify the collections they use in this situation if they are not directly associated. So more is needed here. There is also the issue that the faunal data has clearly been in part published elsewhere (Zangrando et al., and Kochi). How much has been published? What is new here? This must be clear.

This has been specified more carefully, please see new text in M&M section

There is also no stable isotope background prior to the methods section. This should be here to justify the use of the method and would also be a useful place to provide background regarding it's use in the region. How does the new study build on existing datasets of which there are clearly a few? This is clearly due to the diachronic nature of the one presented here but it is important to do this. Discussing variability in marine and aquatic ecosystems here would also make the discussion easier to follow, especially for non-specialists.

We specify that earlier isotope data have shown reliance on aquatic resource but were confined to earlier studies while no diachronic perspective was use, justifying the paper. Hence, despite we do no entirely agree with this comment we have added some background, especially in terms of earlier isotopes data. Please see the Introduction

It is also confusing why collagen was extracted using different methods? Was this linked to preservation or just laboratory differences? Why was this the case?

This was due to working in different labs, we have specified this in the M&M section

It would also be useful to report the standards used in the measurement of the data.

Please see new text in the M&M section

Why was only 1‰ added for the fossil fuel effect? General consensus would suggest 1.6‰ (so nearly 2‰) especially when modern collections are being compared to Early-Middle Holocene samples?

We preferred to apply a 1 per mil increase considering that Burton et al., work provides a direct evidence of the surface carbon reservoirs resulting from fossil fuel and biomass burning in the region. This evidence seemed more fitting to us as, specifically measured in the research area.

The stats tests also need to be set up in the methods not just added in the Results and Discussion.

Reference to statistical tests has been added to the M&M section

Care needs to be taken in the isotopic interpretation as well. For example rather than just saying the "consumption of lichens" it would be more correct to say "some consumption of lichens" (page 10, line 203). Also " a variation in diet with a decrease in the consumption of shellfish" is a big claim! It needs to be stated tentatively as one possibility. Presumably it could also be the greater consumption of higher-trophic level resources (e.g. seals and albatross). It could also be terrestrial, though the fact that both 15N and 13C go up suggests this is unlikely. Better to say would be "this therefore indicates the consumption of fewer lower marine trophic level resources or more higher trophic level marine resources". "These could be in the form of…" This would actually suggest that there was some shift. Does this go against resilience?

We adopted a more cautios approach and changed as suggested "We prompt to think that this difference between the mean 15N values could be describing a variation in the diet, indicating the consumption of fewer lower marine trophic level resources or more higher trophic level marine species".

You also actually can't really test point i) on page 14 line 309. You cannot test whether a reduction in pinnipeds was compensated through the intake of other aquatic mammals if there is no change…. All you can really say is that there was minimal change indicating relative marine resilience with the exception of point ii) whereby there is a shift in 15N and 13C which could be linked to changes which need to be phrased more conservatively and maybe elaborated on with historical data.

Indeed we cannot test this. Our assertion is a possible reconstruction we propose to explain the isotopic data obtained. We have used a more cautious language.

Line 311 here: "nitrogen populations" is a sloppy phrase, things like this need to be checked throughout. What might the greater 15N and 13C variation in pre-contact populations mean relative to post-contact populations?

There was a typo, now the phrase reads: "has driven up nitrogen values in post-contact populations;"

The appearance of "cultural resistance" at the end also seems a little over the top. This comes a little out of the blue. Perhaps if a lot of the contextual information re. hypotheses of post-colonial change were included in the Introduction and Background sections then this would be less the case.

We agree that this is a little ambitious, however we have used a language, which makes it very clear that we are being extremely cautious in presenting this point. We specifically use the contradiction between resilience/resistance to stress the profound link between ecological aspects and cultural ones. We believe this is a strong point of the paper and would prefer to keep it. We agree however, that the background needs further input, hence have added some contextual information in the Introduction section.

Page 15, line 323 isotope evidence will not show you amount of protein take! What you mean is the contribution to collagen of different types of dietary protein. Looking at health or protein amount would be better done through osteoarchaeology, which might have been an interesting aspect to think about in the current study pre and post-colonial. Is there any reason this wasn't done (i.e. characterizing diseases across collections?)?

Corrected as suggested. The osteoarchaeological investigation on both subsets is still ongoing and might be considered in future work.

	Minor comments -English needs checking throughout Page 2, Line 30: “We investigate on” should just be “We investigate dietary variation” Changed as requested Page 2, Line 34: What kind of uniformity? More specificity is required here. We have replaced with “The data obtained reveal an unexpected isotopic uniformity across prehistoric and recent groups, with little variation in both carbon and nitrogen mean values”, Page 3, Line 51: not “Fishers” Why not? I am not sure I understand this comment… Page 3, Line 61: doesn’t make sense, needs checking and re-writing. Replaced with “The ethnographic sources available describe both groups as having a well-defined marine subsistence, with no significant differences in the dietary regime of these populations”. Page 3, Line 65: for example? For example what? I struggle to understand this comment. If the reviewer refers to an example of zooarchaeological works we cite various papers… But I am not sure my intepretation of the comment is correct… Page 4, Line 70: why would they be compromised? More detail needed. We refer to this throughout the paper, Europeans and North-Americans have seriously impacted on the availability of seals in the area. We also suppor tour statement with relevant literature. Page 4, Line 76: “In the region” Corrected Page 4, Line 78: “About historical populations” not on corrected These kind of things should be checked throughout. All tables and graphs – common names of taxa as well as Latin names would be useful, whereas in the text the Latin names would be a useful addition to the common names. In the text latin names are specified, please see text here “The subset of terrestrial animals is composed by 32 samples of guanacos (Lama guanicoe) and 3 samples of andean fox (Lycalopex culpaeus). The aquatic group is more diverse: it comprises 46 samples of southern fur seals (Arctocephalus australis), 2 samples of otter (Lontra provocax), 10 samples of albatross (Thalassarche sp.) and 11 cormorants (Phalacrocorax sp.). It further includes 45 samples of pelagic fishes, which include hakes (Macronus magellanicus), snoeks (Thyrsites atun), southern hakes (Merluccius sp.) together with 2 samples of coastal fish species (Eleginops maclovinus)”. Common names have been added to the table. Beyond the Table please define the sample size for the pre and post contact groups in the methods.

	Indicated as requested, please see new Table 2 Figure 4 caption: should be box and whisker plot not whisker and box plot. Corrected Acknowledgments: why weren't permits required? Surely some were? This is a standard phrase required by PlosOne. Figures, axes headings should include units for isotopes and comparison to international standard (i.e. VPDB) check isotope publications for a guide on this. All figures corrected as requested It would also be useful to have a biplot 13C and 15N figure should the mean and s.d. for pre and post contact populations. We added the byplot as SI (SI Fig. 1) Reviewer #2: I think this is a very good paper which approach an interesting subject not frequently taken. The changes in the diet, based on isotopic studies from pre and post contact samples , indicates a continuity in the consumption o f marine resources (although probably not seals) . This is an original and un expected result, since the dietary impact during the European contact was supposed to be very strong. The data presented is new and support the conclusions. My only concern is about the use of the concept of resilience, a term which has been subject of debate in the last decade. I would like to see on the paper more discussion about the different dimensions of resilience . There is also a few questions Line 52. Why do not refer to 14C yrs BP as usual instead of uncal BP? True! we got rid of the term 'uncal' as it is implicit Line 53. Who call them canoeros? Is this a local or an anthropological denomination? It is an local denomination which was used by anthropologists throughout contact period Beyond these small details I think that this is a high quality paper and should be published. My recommendation is accepted with minor revisions.
Additional Information:	
Question	**Response**
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Competing Interests You are responsible for recognizing and disclosing on behalf of all authors any competing interest that could be perceived to bias their work, acknowledging all financial support and any other relevant financial or non-financial competing interests. Do any authors of this manuscript have competing interests (as described in the PLOS Policy on Declaration and Evaluation of Competing Interests)? **If yes**, please provide details about any and all competing interests in the box below. Your response should begin with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests:* **If no** authors have any competing interests to declare, please enter this statement in the box: "*The authors have declared that no competing interests exist.*" * typeset	The authors have declared that no competing interests exist
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be included in the Methods section of your manuscript. Please write "N/A" if your study does not require an ethics statement. **Human Subject Research (involved human participants and/or tissue)** All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or an equivalent committee, and all clinical investigation must have been conducted according to the principles expressed in the Declaration of Helsinki. Informed consent, written or oral, should also have been obtained from the participants. If no consent was given, the reason must be explained (e.g. the data were analyzed anonymously) and reported. The form of consent (written/oral), or reason for lack of consent, should be indicated in the Methods section of your manuscript. Please enter the name of the IRB or Ethics Committee that approved this study in the space below. Include the approval number and/or a statement indicating approval of this research. **Animal Research (involved vertebrate animals, embryos or tissues)** All animal work must have been conducted according to relevant national and international guidelines. If your study involved non-human primates, you must provide details regarding animal welfare and steps taken to ameliorate suffering; this is in accordance with the recommendations of the Weatherall report, "The use of non-human primates in research." The relevant guidelines followed and the committee that approved the study should be identified in the ethics statement. If anesthesia, euthanasia or any kind of animal sacrifice is part of the study, please include briefly in your statement which substances and/or methods were applied. Please enter the name of your Institutional	

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Please describe where your data may be found, writing in full sentences. **Your answers should be entered into the box below and will be published in the form you provide them, if your manuscript is accepted.** If you are copying our sample text below, please ensure you replace any instances of XXX with the appropriate details. If your data are all contained within the paper and/or Supporting Information files, please state this in your answer below. For example, "All relevant data are within the paper and its Supporting Information	All relevant data are within the paper and its Supporting Information files.

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Rome, 17th March 2017

Dear Editor,

We are pleased to submit a revised version of the manuscript entitled "Dietary Resilience Among Hunter-Gatherers of Tierra del Fuego: Isotopic Evidence in a Diachronic Perspective" by Mary Anne Tafuri, Atilio Francisco Javier Zangrando, Augusto Tessone' Sayuri Kochi, Jacopo Moggi-Cecchi, Fabio Di Vincenzo, Antonio Profico' and Giorgio Manzi. The manuscript presents new stable carbon and nitrogen data from humans (and associated fauna) settled in Tierra del Fuego between the first millennium AD and historical times, to explore continuity vs. discontinuity in human diet with the arrival of European and Northamerican travellers. **There was no additional external funding received for this study.**

We have tried to address all of the reviewers comments and suggestions and believe the manuscript has significantly improved. Please note that, following Reviewer # 1 suggestion, the revised manuscript has now supplementary information.

We hope you will consider the manuscript suitable for publication on PlosOne.

With best wishes,
Mary Anne Tafuri

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Full title: Dietary Resilience Among Hunter-Gatherers of Tierra del Fuego: Isotopic Evidence in a Diachronic Perspective

Short title: Isotopic Evidence of Human Dietary Resilience in Tierra del Fuego

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Abstract

The native groups of Patagonia have relied on a hunter-gatherer economy well after the first Europeans and North Americans reached this part of the world. The large exploitation of marine mammals (i.e., seals) by such allochthonous groups has had a strong impact on the local ecology in a way that might have forced the natives to adjust their subsistence strategies. Similarly, the introduction of new foods might have changed local diet. These are the premises of our isotopic-based analysis. There is a large set of paleonutritional investigations through isotopic analysis on Fuegians groups, however a systematic exploration of food practices across time in relation to possible pre- and post-contact changes is still lacking. In this paper we investigate dietary variation in hunter-gatherer groups of Tierra del Fuego in a diachronic perspective, through measuring the isotopic ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the bone collagen of human and a selection of terrestrial and marine animal samples. The data obtained reveal an unexpected isotopic uniformity across prehistoric and recent groups, with little variation in both carbon and nitrogen mean values, which we interpret as the possible evidence of resilience among these groups and persistence of subsistence strategies, allowing inferences on the dramatic contraction (and extinction) of Fuegian populations.

Introduction

The southern tip of South America is an archipelago of large and small islands that form an intricate network of channels. The landscape is characterized by mountainous terrain whose slopes are covered by a dense forest of the *Nothofagus* genus. The Beagle Channel is one of the main watercourses in this system, and runs from East to West almost in a straight line along the south coast of Isla Grande, separating it from the remaining islands of the south and southeast of the Tierra del Fuego archipelago (Fig. 1).

Fig. 1. Map of the study area with location of the sites. Key: 1: Bove's collection; 2: Aeroclub; 3: Shamakush-Mischiuen 3; 4: Paiashauaia 1; 5: Acatushun - Harberton Cementario; 6: Imiwaia.

This region was inhabited by maritime hunter-gatherers societies from 6400 BP until their nearly total transculturation in the late nineteenth century [1,2]. These groups were generically referred to as *canoeros* (i.e., people of the canoes) and included Yámanas (or Yaghans) and Alacalufs. They inhabited two distinct areas in the Fuegian Archipelago in the late 19th century: the Yámanas populated the central and eastern portion of the Beagle Channel and the group of islands up to the Cape Horn, while the Alacalufs occupied the western part of the Beagle Channel and of the Fuegian archipelago. The ethnographic sources available describe both groups as having a well-defined marine subsistence, with no significant differences in the dietary regime of these populations [3].

Earlier zooarchaeological studies on the Americas more in general and on Tierra del Fuego in particular have highlighted how the exploitation of aquatic resources, both marine and freshwater, was central to human adaptation in the area. They also pointed at the complexity of foraging strategies across the Late Pleistocene to historical times [4]. As an example, wide ranges of fish mobility were identified in the Holocene zooarchaeological record of the Beagle Channel and surrounding areas, where the exploitation of pelagic fish was also implied [5,6].

Further, faunal analyses from archaeological sites across the Beagle Channel have highlighted the subsistence of these societies by the procurement of diverse marine and coastal animal resources, including marine mammals, guanacos, birds, fish, and mussels [1]. Although recent research has observed variations in human-animal relationships during the Middle-Late Holocene [5-7], pinnipeds have remained a critical dietary staple for these marine foragers. Moreover, some scholars believe that these resources were the basis of subsistence throughout the entire occupational sequence, providing an irreplaceable food source [2,8]. In this regard, only in the nineteenth century, and with the arrival of European and North American sealers, the subsistence and behavioral patterns of these groups would have been seriously compromised [1,2,9,10]. Hence, once contact was established, we should assume that the exploitation of the Patagonian landscape and the introduction of new foods by the Europeans have had an impact on the diet of the natives.

There is a generous literature on acculturation linked to colonialism, and its discussion goes beyond the scopes of this paper. We rather focus our analysis on some of the natural and cultural processes that might have ultimately contributed to the extinction of the Fuegians or their definitive assimilation by Western cultures [11]. By contrast, we consider several accounts on the cultural continuity and the 'resistance' [e.g. 12,13] of the native groups of Patagonia to external pressure, which appears to be linked to their foraging regime and a general cultural persistence throughout time.

One way to explore continuity vs. change in dietary practices after the arrival of European populations is by conducting an isotopic investigation on human remains in the region through a diachronic perspective. Earlier isotopic studies have explored diet among ancient groups in the Beagle Channel [14-17] and in the Western Archipelago [18,19], confirming the evidence deriving from paleobotanical and faunal records. However very little is known about historical populations and variation throughout the contact period. One of the reasons for this was given by the paucity of human remains of Fuegian native populations of the 19th century.

The two skeletal collections of Fuegians recovered by Captain G. Bove in 1881 and 1883 and stored respectively in the Museo di Storia Naturale in Florence and the Museo di Antropologia G. Sergi of Sapienza of Rome, in Italy offer new contribution to this discussion. The history of the recovery and the composition of the assemblages are fully described in a paper by Marangoni et al. [20]. Both assemblages are described in Bove's reports [21], and were given to the Captain by Yaghans settled in the area of Yendegaia, near Ushuaia (Fig. 1). Considering Bove's notes, those human remains refer to family members to whom the natives related; we thus suggest a tentative attribution to the first half of the 19th century.

Pre-contact human bone remains in the research area were unearthed mainly through excavations conducted by the Proyecto Arqueológico Canal Beagle (Fig. 1), and are currently preserved in the Fin del Mundo Museum, Ushuaia. Further bone remains corresponding to four individuals were donated to the Museo Etnográfico of Buenos Aires in the early twentieth century [17]. The information regarding mortuary contexts and excavation procedures has been described in Tessone [22], Piana et al. [23] and Vila et al [24]. In most cases human remains were found in primary position in shell middens and / or rock crevices. All samples are attributed to the late Holocene, among which only two individuals have been buried in historical phases: Acatushun and Harberton Cementerio [25].

Together with human remains, faunal samples gathered from different archaeological context were added to the dataset to provide an isotopic baseline. Most species selected are usually represented in the zooarchaeological record of the Beagle Channel as common food staples (with the exception of the fox).

Materials and methods

We discuss here the isotope data of 42 humans and 166 animal specimens from different collections. The pre-contact subset excavated in the Beagle Channel counts 9 complete or almost complete skeletons (7 adults, 1 subadult and 1 infant). The post-contact subset excavated in the same region counts 2 almost complete skeletons (Acatushún and Harberton Cementerio), both corresponding to adults. Bone collagen extraction for stable isotopes analysis was carried out on ribs in good state of preservation. We included in the analysis data from comparative human material, such as the infant presented by Orquera and Piana [8], together with information reported by Yesner et al. [17] for the human remains of 4 adults from other areas of the archipelago.

The bone assemblages from Italy have a very different composition: the remains from Florence refer to a commingled set that arrived at the Museum in such a state, so that the skulls identified could not be reconnected with the post-cranial; on the basis of the crania observed we should consider a MNI of 12. Sampling for isotopic analysis was carried out on the skulls (namely, on portions of the vomer or the perpendicular plate of the ethmoid). The subset from Rome includes fifteen complete or almost complete skeletons (13 adults, 1 juvenile, 1 infant) in good state of preservation. Eleven of these were recovered by Captain Bove during his trip, while 2 further samples were later acquired by the Rome museum through a donation.

Sampling for isotopic analysis was carried out on the ribs of 14 individuals, with the exception of the infant, which was deliberately excluded to avoid damage to the skeletal elements (i.e., only the skull was preserved and sampling attempts resulted very invasive).

For all humans a general indication of sex and age at death of the individual is provided together with chronological attribution (Table 1).

The faunal bones were recovered from archaeological sites on the north coast of the Beagle Channel, spanning from the Middle to the Late Holocene (Table 2), published in detail in Zangrando et al. [26,27] and Kochi [28]. They are not directly associated with the Argentinian subset, given that most sites excavated were primary burials outside of residence areas and did not provide a faunal record. They are however comparable with the human remains in term of location and chronological attribution. In detail, the subset of terrestrial animals is composed of 32 samples of guanacos (*Lama guanicoe*) and 3 samples of andean fox (*Lycalopex culpaeus*). The aquatic group is more diverse: it comprises 46 samples of southern fur seals (*Arctocephalus australis*), 2 samples of otter (*Lontra provocax*), 10 samples of albatross (*Thalassarche* sp.) and 11 cormorants (*Phalacrocorax* sp.). It further includes 45 samples of pelagic fishes, which include hakes (*Macronus magellanicus*), snoeks (*Thyrsites atun*), southern hakes (*Merluccius* sp.) together with 2 samples of coastal fish species (*Eleginops maclovinus*). We further discuss data on modern samples of mussels (N = 15), partially presented in Zangrando et al. [26,27] and Kochi [28].

Collagen extraction and sample preparation for isotopic analysis followed two slightly different methods, given that analyses were run at respective laboratories in

Italy and Argentina. Both methods derive from Longin's [29] standard, as described below.

Italian subset

Following Brown et al. method [30], cortical bone (0.5 g) was cleaned by sand abrasion and demineralized in 0.5M solution of HCl at 4ºC for at least four days. The samples were then rinsed to neutral pH and gelatinized in pH 3 HCl at 70 ºC for 48 hours. The collagen solution was filtered off with 5–8 µm Ezee filters, frozen, and then freeze-dried. Each of the collagen extracts was weighed (ca. 1 mg) in triplicate into tin capsules, and stable carbon and nitrogen isotope ratios were measured using an automated elemental analyzer coupled in continuous-flow mode to an isotope-ratio-monitoring mass spectrometer (Costech elemental analyzer coupled to a Thermo Finnigan MAT253 mass spectrometer). Analysis was carried out at the Godwin Laboratory, University of Cambridge.

Argentinian subset

Bone fragments were cleaned with abrasive elements and ultrasonic baths. Each sample of approximately 0.3g was soaked in dilute HCl (0.5%), which was changed every 24–48 h. Then, it was rinsed with distilled water and treated with 0.125% solution of NaOH for 20 hrs. Finally, the collagen extracted was dried in an oven at 40°C for hrs [31].

Measurement of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the collagen fraction was performed with a Carlo Erba EA1108 Elemental Analyzer (CHN), connected to a continuous flow Thermo Scientific Delta V Advantage mass spectrometer through a Thermo Scientific ConFlo IV interface.

For both subsets stable isotope results are expressed as the ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and reported as δ values in parts per thousand (‰), relative to internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen (Ambient Inhalable Reservoir, AIR). Based on replicate analyses of international and laboratory (i.e., alanine, nylon, caffeine, USGS40, EMC) standards, measurement errors are less than ± 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$. The collagen yield, the percentage of carbon and nitrogen, and the atomic C/N ratio of each sample were also recorded to check collagen quality [32-34]. Finally, the modern fauna samples were corrected for carbon by adding 1‰ due to the Suess effect [35]. The historic human samples of Bove's collections were not corrected for this effect [36], considering their estimated chronology.

Results obtained where analyzed through descriptive statistics and non-parametric tests using IBM SPSS© ver. 23.

Results

Table 1 provides data on collagen yields, C/N ratios and collagen isotopic values of the sampled humans, with mean carbon and nitrogen isotope values and C/N ratios from collagen of animals presented in Table 2. For all the samples studied collagen yield and C/N ratios confirm that isotopic signals are to be considered reliable.

Table 1. The human assemblage. Stable carbon and nitrogen isotope ratios for human collagen with data quality indicator (% of collagen %C, %N, C/N). Key: f=female, m=male; n.d.=not determined.

ID	Provenience	sample info	sex	age at death	cal. age (AD/BC)	contact	% coll	$\delta^{13}C_{VPDB}$	%C	$\delta^{15}N_{AIR}$	%N	C/N
TF1	Rome	Fuegian1	f	40+	ca. 1800	post	28.2	-11.6	43.9	18.9	16.3	3.1
TF2	Rome	Fuegian2	f	20-30	ca. 1800	post	28.8	-11.9	45.6	18.9	17.1	3.1
TF3	Rome	Fuegian3	f	40+	ca. 1800	post	28.8	-11.2	46.0	19.3	17.2	3.1
TF4	Rome	Fuegian4	f	40+	ca. 1800	post	24.9	-11.9	42.8	18.4	15.9	3.1
TF5	Rome	Fuegian5	m	40+	ca. 1800	post	29.5	-12.0	44.8	18.3	16.5	3.2
TF6	Rome	Fuegian6	m	40+	ca. 1800	post	26.2	-11.9	46.6	18.0	17.2	3.1
TF7	Rome	Fuegiano7	f	40+	ca. 1800	post	26.6	-12.1	44.0	17.5	16.4	3.1
TF8	Rome	Fuegian8	m	30-40	ca. 1800	post	18.7	-12.1	44.3	17.6	16.6	3.1
TF9	Rome	Fuegian9	f	40+	ca. 1800	post	26.0	-12.5	43.8	17.5	16.4	3.1
TF10	Rome	Fuegian10	f	20-30	ca. 1800	post	29.3	-11.2	45.4	18.0	16.9	3.1
TF11	Rome	Fuegian11	f	40+	ca. 1800	post	39.8	-11.3	41.4	18.6	15.4	3.1
TF12	Rome	Fuegian12	m	15	ca. 1800	post	29.4	-11.6	46.6	17.4	17.4	3.1
TF13	Rome	Fuegian13	f	16	ca. 1800	post	26.2	-11.6	43.6	17.6	16.2	3.1
TF14	Rome	Fuegian13A	m	40+	ca. 1800	post	24.4	-13.0	43.2	17.5	15.8	3.2
TF15	Florence	3116	f	adult	ca. 1800	post	25.1	-12.5	43.4	19.4	15.4	3.3
TF16	Florence	3119	f	adult	ca. 1800	post	23.5	-12.3	43.4	18.6	15.8	3.2
TF17	Florence	3120	f	adult	ca. 1800	post	27.4	-12.1	40.1	19.0	14.7	3.2
TF18	Florence	3122	m	adult	ca. 1800	post	23.1	-12.3	38.9	19.7	14.3	3.2
TF19	Florence	3124	m	adult	ca. 1800	post	20.9	-12.0	42.4	19.3	15.5	3.2
TF20	Florence	3126	m	adult	ca. 1800	post	24.1	-11.4	45.6	19.6	16.2	3.3
TF21	Florence	3127	m	adult	ca. 1800	post	24.5	-12.5	42.0	19.6	15.1	3.2
TF22	Florence	3129	m	adult	ca. 1800	post	22.4	-12.1	45.3	20.4	16.5	3.2
TF23	Florence	3131	m	adult	ca. 1800	post	24.9	-12.0	43.3	19.7	15.9	3.2
TF24	Florence	3133	m	adult	ca. 1800	post	17.5	-12.1	45.8	19.5	16.8	3.2
TF25	Florence	3132	f?	adult	ca. 1800	post	25.6	-12.5	43.2	18.7	15.7	3.2
TF26	Florence	3134	m	adult	ca. 1800	post	18.5	-15.0	45.1	12.3	16.3	3.2
TF 27	MFM	Mischiuen III	f	13-17	1300-1400	pre	n.d.	-11.1	46.5	18.1	16.6	3.3
TF 28	MFM	2668	n.d.	adult	n.d.[d]	pre	n.d.	-11.5	43.1	18.2	15.4	3.3
TF 29	MFM	2669	n.d.	adult	n.d.[d]	pre	n.d.	-13.3	41.5	15.8	15.1	3.2
TF 30	MFM	795	n.d.	adult	n.d.[d]	pre	n.d.	-11.9	45.0	18.0	16.0	3.3
TF 31	MFM	1607	n.d.	juvenile	n.d.[d]	pre	n.d.	-11.9	40.3	18.4	14.4	3.3
TF 32	MFM	SHE	m	35-45	520-650	pre	n.d.	-12.4	41.4	18.3	14.7	3.3
TF 33	MFM	Acatushun	f	30-40	1600-1800	post	n.d.	-13.9	46.4	16.7	16.5	3.3
TF 34	MFM	Paiashauaia	f	35-45	530-670	pre	n.d.	-11.9	43.9	18.3	15.7	3.3
TF 35	MFM	Aeroclub	n.d.	adult	pre-contact[e]	pre	n.d.	-11.2	40.6	19.2	14.8	3.2
TF 36	MFM	BI1	f	25-49	1300-1415	pre	n.d.	-11.2	42.3	19.2	15.4	3.2
TF 37	MFM	Harberton	m	25-35	1600-1800	post	n.d.	-11.6	42.6	18.6	15.4	3.2
TF 38[b]	M. etngráfico	Isla Hoste	n.d.	adult	pre-contact[f]	pre	n.d.	-13.3	n.d.	17.2	n.d.	n.d.
TF 39[b]	M. etngráfico	Ushuaia	n.d.	adult	pre-contact[f]	pre	n.d.	-12.6	n.d.	18.8	n.d.	n.d.
TF 40[b]	M. etngráfico	Isla Hoste	m	adult	pre-contact[f]	pre	n.d.	-16.8	n.d.	13.2	n.d.	n.d.
TF 41[b]	M. etngráfico	Isla Navarino	m	adult	pre-contact[f]	pre	n.d.	-18.5	n.d.	10.6	n.d.	n.d.
TF 42[c]	---	Shamakush I	n.d.	0-6 months	950-1300	pre	n.d.	-12.8	n.d.		n.d.	n.d.

[a] Radiocarbon data was calibrated using SHCal04 curve [37] from Calib Rev 6.0.1 program
[b] published in Yesner et al. [17]
[c] published in Orquera and Piana [8]
[d] Undated sample from unknown depositional context
[e] prehistoric midden
[f] date within the last 1500 years before contact

Table 2. The faunal assemblage. Mean carbon and nitrogen ratios and C/N (with standard deviations) for animal taxa examined.

Taxa	**common name**	**N**	**$\delta^{13}C_{VPDB}$**		**$\delta^{15}N_{AIR}$**		**C/N**	
Terrestrial			**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
Lama guanicoe	guanacos	32	-20.6	2.2	0.5	2.5	3.2	0.03
Lycalopex culpaeus	andean fox	3	-18.3	2.3	9	3.3	3.3	0.06
Riverine-marine								
Lontra provocax	otter	2	-10.7	1.8	21.1	4.2	3.2	0
Eleginops maclovinus	Patagonian blenny	2	-12.1	0.5	16.4	0.1	3.6	0.2
Marine								
Arctocephalus australis	seal	46	-11.8	0.5	17.3	0.6	3.2	0.04
Thalassarche sp.	albatross	10	-13.1	0.5	18.6	0.9	3.1	0.02
Phalacrocorax sp.	cormorants	11	-12.7	0.3	15.4	0.6	3.2	0.05
Macruronus magellanicus	hakes	14	-11.6	0.3	15.8	0.9	3.2	0.02
Thyrsites atun	snoeks	14	-12.6	0.7	15.3	0.9	3.2	0.04
Mytilidae	mussels	15	-16.2	0.6	11.1	0.5	3.2	0.05
Merluccius sp.	hake	17	-12.8	0.7	17	0.9	3.3	0.05

Carbon isotope values of collagen from terrestrial animals are typical of mammals feeding on C_3 plants (mean $\delta^{13}C$ between -18.3‰ and -20.6‰). Variation in nitrogen isotope values ($\delta^{15}N$) is highest, and it ranges from 9‰ (fox) to 0.5‰ (guanaco); the very low values for the guanacos can be explained by some consumption of lichens (*Usnea* sp.) with very low $\delta^{15}N$ values (-10.8 to -17.5 ‰) [28].

As expected, the marine vertebrate resources have higher $\delta^{13}C$ and $\delta^{15}N$ values than the terrestrial herbivores, with means of -12.2‰±0.8‰ and 17.1‰±1.9‰ respectively. Little variation in isotope means is observed for seals, seabirds and fish. Three species of pelagic fish (*Macruronus magellanicus, Thyrsites atun, Merluccius* sp.) have $\delta^{13}C$ values with means ranging from -11.6‰ to -12.8‰, and mean $\delta^{15}N$ values range from 17.0‰ to 15.3‰. Carbon isotope values from collagen of seabirds and southern fur seal are similar to those for pelagic fish. For $\delta^{15}N$ values, it is worth noticing that albatrosses have higher mean values than other marine resources. In the other extreme of the marine food web, mussels are presented with the lower mean values for $\delta^{13}C$ (-16.2‰±0.6) and $\delta^{15}N$ (11.1‰±0.5), which are significantly different from those of the marine vertebrates.

Among the estuary resources, *Lontra provocax* has much heavier isotope mean values than marine resources (-10.7‰ for $\delta^{13}C$ and 21.1‰ for $\delta^{15}N$), and *Eleginops maclovinus* has analogous values to those of pelagic fish.

The $\delta^{13}C$ and $\delta^{15}N$ values of human remains range from almost completely marine-derived diets (-11.1‰ and 18.1‰) to values that could reflect entirely terrestrial food composition (-18.5‰ and 10.6‰). However, more than 90% of values are grouped in the ranges -11.1 and -13.3 ‰ for $\delta^{13}C$ and 18.1 and 17.2 ‰ for $\delta^{15}N$, which indicates mainly marine-based diets (Fig. 2).

Fig. 2. **Distribution of the human data.** Frequency of stable carbon and nitrogen isotope values.

The mean $\delta^{13}C$ and $\delta^{15}N$ values of the humans are -12.4±1.4‰ and 18.0±1.9‰, and the medians respectively -12.1‰ and 18.4‰. If we exclude the few cases (N=3) that could reflect up to 50% terrestrial food composition, the means and medians do not significantly change: mean $\delta^{13}C$ and $\delta^{15}N$ values are -12.0±0.6‰ and 18.5±0.9‰, and the medians respectively are -12.0‰ and 18.5‰. The correlation between $\delta^{13}C$ and $\delta^{15}N$ in human values is significant ($r^2 = 73.9$ $p = 0.0001$) (Fig. 3).

In general, the humans cluster in a rather homogeneous group with no significant differences between males and females measured in either carbon or nitrogen values for the three subsets (S1 Table).

Fig. 3. **Stable isotope biplot.** $\delta^{13}C$ and $\delta^{15}N$ (‰) of human samples with associated animal mean values and standard deviations. The three human subsets are kept separate.

Discussion

The transculturation from nomadic/mobile hunting-gathering to sedentary/residential lifestyle is a key process in the history of the Fuegian populations. The overexploitation of pinnipeds produced by European and American sealers, the demographic concentrations in missions and ranches, and the introduction of illnesses by Europeans during the 19th century have produced deep structural changes in the subsistence patterns and demography of native groups. Our data and statistical analysis suggest no differences in dietary practices across time detectable at an isotopic level, which is in counter-tendency with our expectations, and offer some interesting convolution.

The Kolmogorov-Smirnov test shows that there is no statistical difference in $\delta^{15}N$ between pre- and post-contact populations ($p= 0.185$; $n=14$ and $n=28$ respectively), with slight differences in the distribution of data (S2 Fig.). For nitrogen values, isotope data show that post-contact populations kept diets focused on marine resources of high trophic levels. This is particularly interesting: if seals – one of the preferred food sources among the Yamana – were becoming less available because of

Europeans and Americans' exploitation we should assume post-contact consumption of low trophic level species and higher values among pre-contact groups, as a consequence of consumption of high trophic level species. Surprisingly, the post-contact nitrogen data cluster around higher values, against this expectation (Fig. 4). However, the reported nitrogen values for some species of seabirds and pelagic fish are similar, and even higher, to those of fur seals in the southern South Atlantic, indicating analogous trophic levels. Therefore, a change in subsistence redirected towards the exploitation of such minor resources should not necessarily have implied a change in the trophic levels.

Fig. 4. **Pre- and post-contact data distribution.** Box and whisker plot of nitrogen and carbon values for pre- and post-contact human populations. Nitrogen median values show little differences between pre-contact and post-contact phases.

The descriptive statistics presented in Table 3 is also noteworthy: it shows a difference of 1.2‰ between the mean $\delta^{15}N$ values, and a lesser distance between the medians (0.5‰), which do not support a significant change in trophic terms.

Table 3. **Summary of pre- and post-contact data.** Descriptive statistics for the human sample only, according to chronological phase.

	$\delta^{13}C_{VPDB}$		$\delta^{15}N_{AIR}$	
	Pre-contact	Post-contact	Pre-contact	Post-contact
N	14	28	14	28
Min.	-18.5	-15.0	10.6	12.3
Max.	-11.1	-11.2	19.2	20.4
Mean	-12.8	-12.2	17.2	18.4
Stand. Dev.	2.1	0.8	2.5	1.5

Median	-12.3	-12.1	18.2	18.7

We prompt to think that this difference in the mean $\delta^{15}N$ values could be describing a variation in the diet, indicating the consumption of fewer lower marine trophic level resources or more high-trophic level marine species. A decrease in the consumption of shellfish by post-contact populations might be supported by historical documents: the setting-up of the Anglican Mission in Ushuaia by the end of 1860s led to the demographic concentration and semi-sedentism of the native population in few years. There were almost permanently residing between 10 and 18 Yamana families, and every year the Mission received hundreds of Yamana people where they stayed from four to six months [38-40]. Such concentration of persons and such reduction in mobility certainly should have resulted in the overexploitation of local resources, especially shellfish [1]. Given the $\delta^{15}N$ lighter values on shellfish in comparison with other marine resources, the high nitrogen values in post-contact human remains could be describing accordingly less accessibility to those resources.

The Fuegian landscape shows little seasonal variation in the environment, with oxygen isotopic analyses indicating a shift in mean annual temperature that ranges between 1 and 2°C across several centuries (i.e., between the Medieval Climatic Anomaly and the Little Ice Age) [41], with paleobotanical data [42,43] suggesting little changes in the local vegetation over the last six millennia. Similarly, archaeological data [44] show that human occupation in the area is uninterrupted, with a general uniformity in cultural practices. Across time human groups settled in Tierra del Fuego display a general continuity, either in cultural practices or in technological complexity, with rare innovations. This appears to be true also for what concerns diet, as types of food consumed and levels of protein intake appear to remain unchanged across several centuries. According to Orquera and colleagues [44], “in spite of its simplicity, the system was successful. Its collapse happened by the end of the 19th century, and was caused by the catastrophic pinniped depredation carried out by Euro-American, Chilean and Argentine groups, as well as by the introduction of sickness against which the indigenous populations had no immunity”. Under such circumstances, it is possible that the subsistence patterns of the marine hunter-gatherers of the Beagle Channel based on the exploitation of a high diversity of resources have given to these populations a resilience to cope with the hunting of fur seals by industrial populations. The overall consistency in isotope data might hence be interpreted in cultural terms. We propose here that: i) post-contact societies might have experienced a significant reduction in the consumption of pinnipeds, which was compensated through the intake of other aquatic mammals (i.e., seabirds and fish with similar nitrogen levels), with effects of such shift in the diet not detectable at the isotopic level; at the same time, ii) a significant decrease in the consumption of molluscs by post-contact populations might have driven up their nitrogen values;

The human ecological resilience of the Fuegian groups expressed in the search for nutrional alternatives can be perceived, as anticipated, as the evidence of a cultural resistance, which goes along with a general stability of these people. Here, the idea of resistance is not linked to noncompliance [45], as in the lack of negotiation of traditions. Rather, it is perceived as the tendency to persist in the expression of specific traditions (the hunting/fishing of aquatic species), despite external stimuli and/or the knowledge of alternatives. For the Fuegians, such resistance seems to translate in a striking uniformity of dietary practices across several centuries, as mirrored in the isotopic ratios of the skeletal tissues. Furthermore, considering that the contribution to collagen of different types of dietary proteins appears to remain constant throughout time, we might need to reconsider the influence of diseases as the main factor in the dramatic contraction (and eventually extinction) of Fuegian populations.

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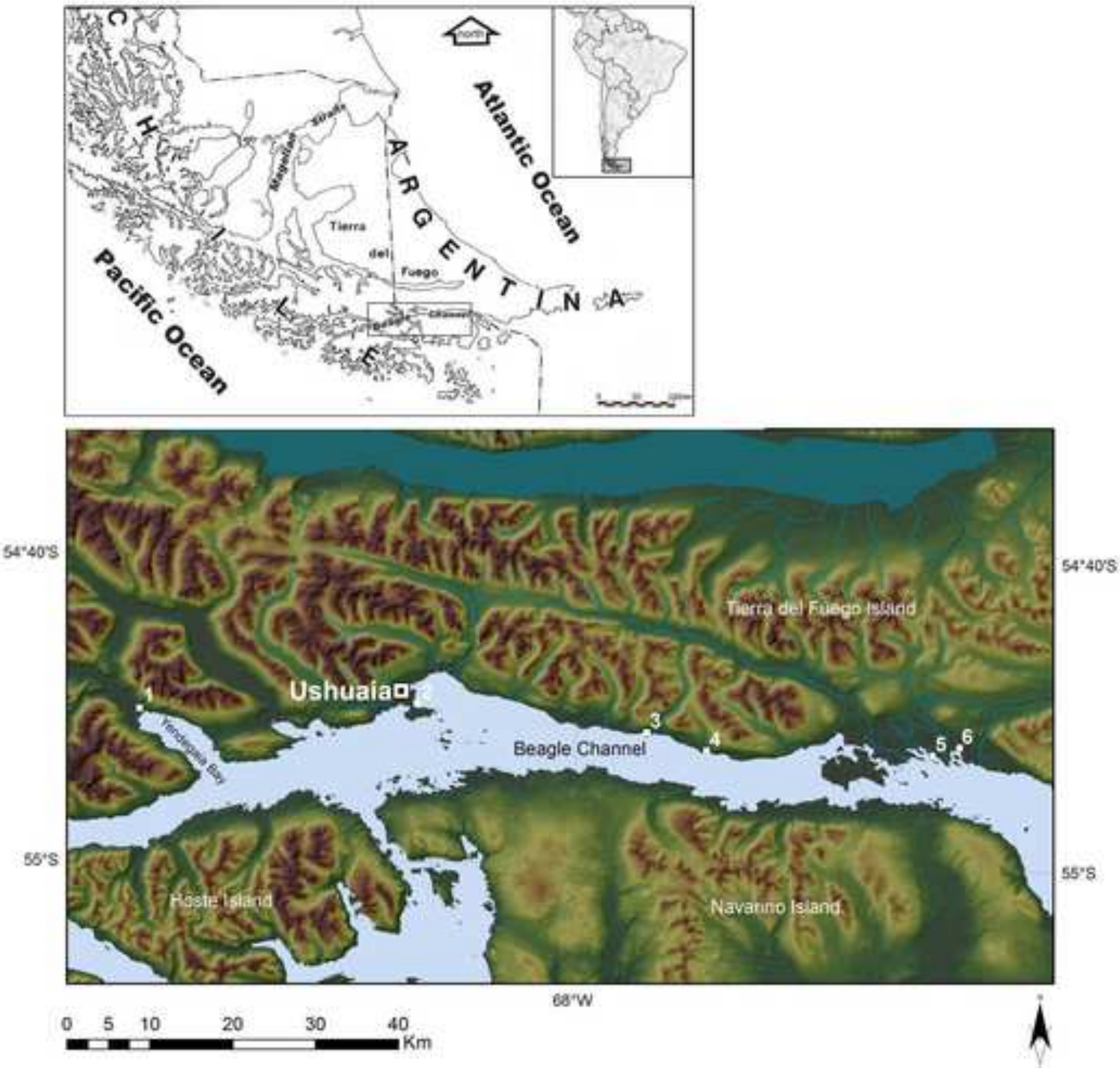
Supporting information

S1 Table. Statistics report. Summary of the Mann-Whitney U test for human carbon and nitrogen data according to sex. The three subsets are kept separate. For the Ushuaia subset 7 individuals were excluded, as no sex estimate was available.

S2 Figure. Biplot of stable carbon and nitrogen data. Mean humans $\delta^{13}C$ and $\delta^{15}N$ values (with sd) for pre-contact (n=14) and post-contact (n=28) subsets.



CHILE
ARGENTINA
Atlantic Ocean
Pacific Ocean
Magellan Strait
Tierra del Fuego
54°40'S
55°S
68°W
Tierra del Fuego Island
Ushuaia
1
2
3
4
5
6
Beagle Channel
Hoste Island
Navarino Island
0 5 10 20 30 40 Km

Figure 2

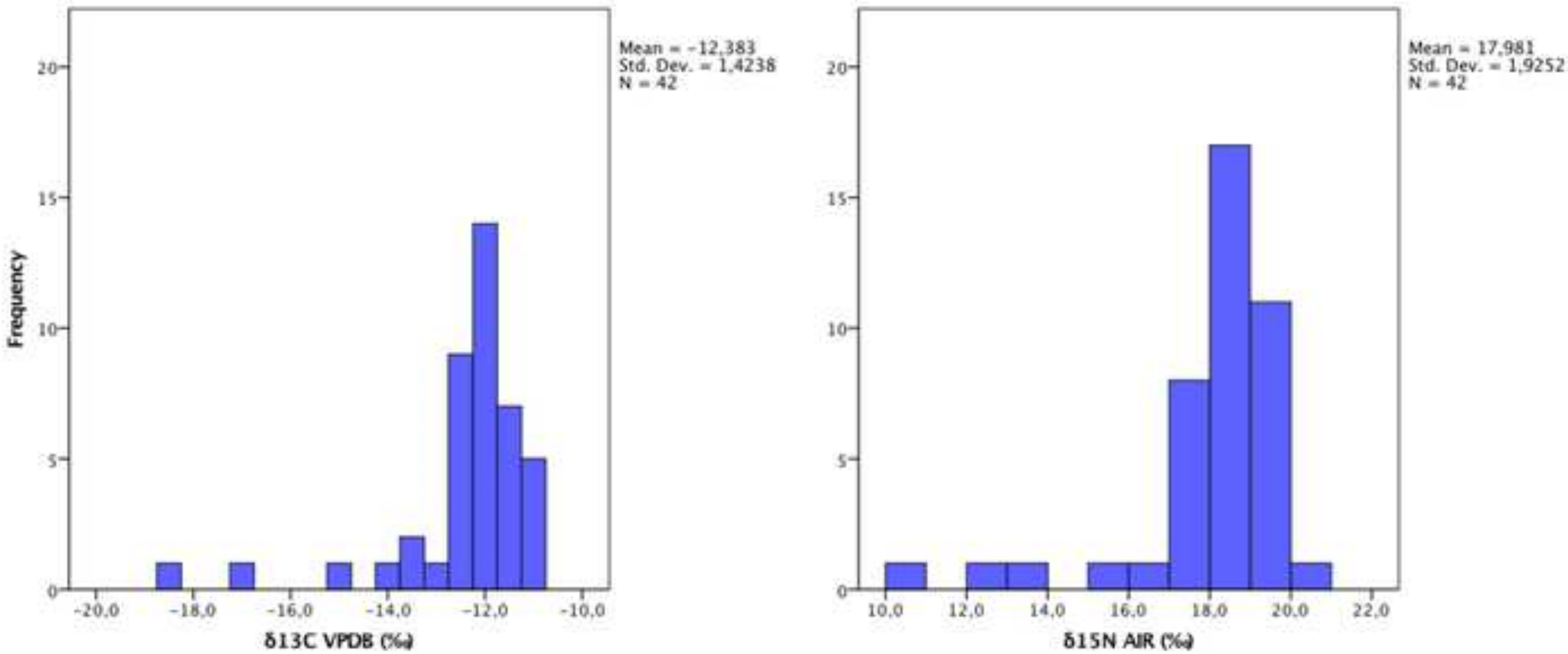

Mean = -12,383
Std. Dev. = 1,4238
N = 42
Frequency
20
15
10
5
0
-20,0
-18,0
-16,0
-14,0
-12,0
-10,0
δ13C VPDB (‰)
Mean = 17,981
Std. Dev. = 1,9252
N = 42
20
15
10
5
0
10,0
12,0
14,0
16,0
18,0
20,0
22,0
δ15N AIR (‰)

Figure 3

Click here to download Figure Figure 3.tif

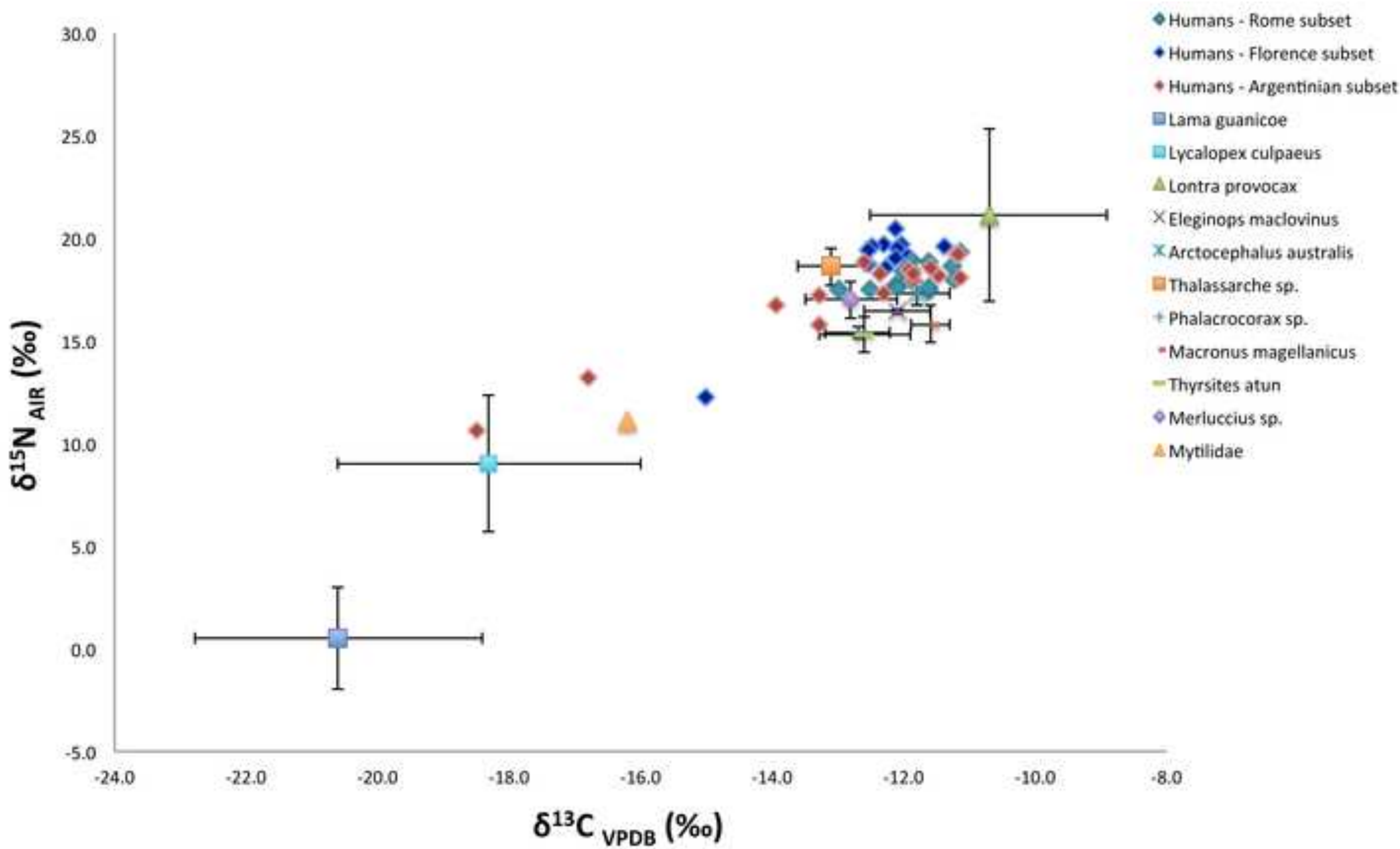


Figure 4

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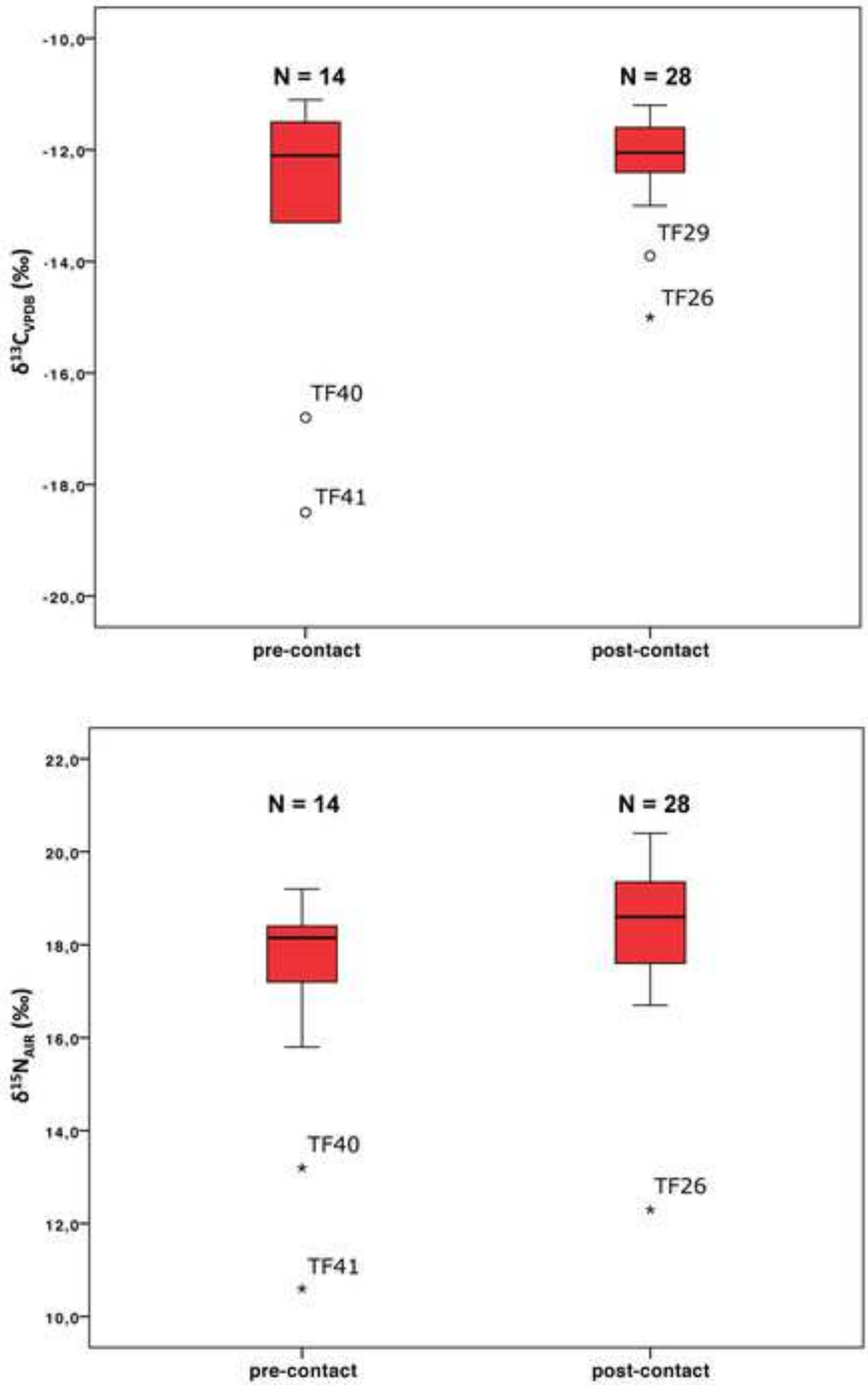

S1 Table

Click here to access/download
Supporting Information
S1 Table.docx

S2 Fig.

Click here to access/download
Supporting Information
S2 Figure.docx

Full title: Dietary Resilience Among Hunter-Gatherers of Tierra del Fuego: Isotopic Evidence in a Diachronic Perspective

Short title: Isotopic Evidence of Human Dietary Resilience in Tierra del Fuego

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Abstract

The native groups of Patagonia have relied on a hunter-gatherer economy well after the first Europeans and North Americans reached this part of the world. The large exploitation of marine mammals (i.e., seals) by such allochthonous groups has had a strong impact on the local ecology in a way that might have forced the natives to adjust their subsistence strategies. Similarly, the introduction of new foods might have changed local diet. These are the premises of our isotopic-based analysis. There is a large set of paleonutritional investigations through isotopic analysis on Fuegians groups, however a systematic exploration of food practices across time in relation to possible pre- and post-contact changes is still lacking. In this paper we investigate dietary variation in hunter-gatherer groups of Tierra del Fuego in a diachronic perspective, through measuring the isotopic ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the bone collagen of human and a selection of terrestrial and marine animal samples. The data obtained reveal an unexpected isotopic uniformity across prehistoric and recent groups, with little variation in both carbon and nitrogen mean values, which we interpret as the possible evidence of resilience among these groups and persistence of subsistence strategies, allowing inferences on the dramatic contraction (and extinction) of Fuegian populations.

Introduction

The southern tip of South America is an archipelago of large and small islands that form an intricate network of channels. The landscape is characterized by mountainous terrain whose slopes are covered by a dense forest of the *Nothofagus* genus. The Beagle Channel is one of the main watercourses in this system, and runs from East to West almost in a straight line along the south coast of Isla Grande, separating it from the remaining islands of the south and southeast of the Tierra del Fuego archipelago (Fig. 1).

Fig. 1. Map of the study area with location of the sites. Key: 1: Bove's collection; 2: Aeroclub; 3: Shamakush-Mischiuen 3; 4: Paiashauaia 1; 5: Acatushun - Harberton Cementario; 6: Imiwaia.

This region was inhabited by maritime hunter-gatherers societies from 6400 BP until their nearly total transculturation in the late nineteenth century [1,2]. These groups were generically referred to as *canoeros* (i.e., people of the canoes) and included Yámanas (or Yaghans) and Alacalufs. They inhabited two distinct areas in the Fuegian Archipelago in the late 19th century: the Yámanas populated the central and eastern portion of the Beagle Channel and the group of islands up to the Cape Horn, while the Alacalufs occupied the western part of the Beagle Channel and of the Fuegian archipelago. The ethnographic sources available describe both groups as having a well-defined marine subsistence, with no significant differences in the dietary regime of these populations [3].

Earlier zooarchaeological studies on the Americas more in general and on Tierra del Fuego in particular have highlighted how the exploitation of aquatic resources, both marine and freshwater, was central to human adaptation in the area. They also pointed at the complexity of foraging strategies across the Late Pleistocene to historical times [4]. As an example, wide ranges of fish mobility were identified in the Holocene zooarchaeological record of the Beagle Channel and surrounding areas, where the exploitation of pelagic fish was also implied [5,6].

Further, faunal analyses from archaeological sites across the Beagle Channel have highlighted the subsistence of these societies by the procurement of diverse marine and coastal animal resources, including marine mammals, guanacos, birds, fish, and mussels [1]. Although recent research has observed variations in human-animal relationships during the Middle-Late Holocene [5-7], pinnipeds have remained a critical dietary staple for these marine foragers. Moreover, some scholars believe that these resources were the basis of subsistence throughout the entire occupational sequence, providing an irreplaceable food source [2,8]. In this regard, only in the nineteenth century, and with the arrival of European and North American sealers, the subsistence and behavioral patterns of these groups would have been seriously compromised [1,2,9,10]. Hence, once contact was established, we should assume that the exploitation of the Patagonian landscape and the introduction of new foods by the Europeans have had an impact on the diet of the natives.

There is a generous literature on acculturation linked to colonialism, and its discussion goes beyond the scopes of this paper. We rather focus our analysis on some of the natural and cultural processes that might have ultimately contributed to the extinction of the Fuegians or their definitive assimilation by Western cultures [11]. By contrast, we consider several accounts on the cultural continuity and the 'resistance' [e.g. 12,13] of the native groups of Patagonia to external pressure, which appears to be linked to their foraging regime and a general cultural persistence throughout time.

One way to explore continuity vs. change in dietary practices after the arrival of European populations is by conducting an isotopic investigation on human remains in the region through a diachronic perspective. Earlier isotopic studies have explored diet among ancient groups in the Beagle Channel [14-17] and in the Western Archipelago [18,19], confirming the evidence deriving from paleobotanical and faunal records. However very little is known about historical populations and variation throughout the contact period. One of the reasons for this was given by the paucity of human remains of Fuegian native populations of the 19th century.

The two skeletal collections of Fuegians recovered by Captain G. Bove in 1881 and 1883 and stored respectively in the Museo di Storia Naturale in Florence and the Museo di Antropologia G. Sergi of Sapienza of Rome, in Italy offer new contribution to this discussion. The history of the recovery and the composition of the assemblages are fully described in a paper by Marangoni et al. [20]. Both assemblages are described in Bove's reports [21], and were given to the Captain by Yaghans settled in the area of Yendegaia, near Ushuaia (Fig. 1). Considering Bove's notes, those human remains refer to family members to whom the natives related; we thus suggest a tentative attribution to the first half of the 19th century.

Pre-contact human bone remains in the research area were unearthed mainly through excavations conducted by the Proyecto Arqueológico Canal Beagle (Fig. 1), and are currently preserved in the Fin del Mundo Museum, Ushuaia. Further bone remains corresponding to four individuals were donated to the Museo Etnográfico of Buenos Aires in the early twentieth century [17]. The information regarding mortuary contexts and excavation procedures has been described in Tessone [22], Piana et al. [23] and Vila et al [24]. In most cases human remains were found in primary position in shell middens and / or rock crevices. All samples are attributed to the late Holocene, among which only two individuals have been buried in historical phases: Acatushun and Harberton Cementerio [25].

Together with human remains, faunal samples gathered from different archaeological context were added to the dataset to provide an isotopic baseline. Most species selected are usually represented in the zooarchaeological record of the Beagle Channel as common food staples (with the exception of the fox).

Materials and methods

We discuss here the isotope data of 42 humans and 166 animal specimens from different collections. The pre-contact subset excavated in the Beagle Channel counts 9 complete or almost complete skeletons (7 adults, 1 subadult and 1 infant). The post-contact subset excavated in the same region counts 2 almost complete skeletons (Acatushún and Harberton Cementerio), both corresponding to adults. Bone collagen extraction for stable isotopes analysis was carried out on ribs in good state of preservation. We included in the analysis data from comparative human material, such as the infant presented by Orquera and Piana [8], together with information reported by Yesner et al. [17] for the human remains of 4 adults from other areas of the archipelago.

The bone assemblages from Italy have a very different composition: the remains from Florence refer to a commingled set that arrived at the Museum in such a state, so that the skulls identified could not be reconnected with the post-cranial; on the basis of the crania observed we should consider a MNI of 12. Sampling for isotopic analysis was carried out on the skulls (namely, on portions of the vomer or the perpendicular plate of the ethmoid). The subset from Rome includes fifteen complete or almost complete skeletons (13 adults, 1 juvenile, 1 infant) in good state of preservation. Eleven of these were recovered by Captain Bove during his trip, while 2 further samples were later acquired by the Rome museum through a donation.

Sampling for isotopic analysis was carried out on the ribs of 14 individuals, with the exception of the infant, which was deliberately excluded to avoid damage to the skeletal elements (i.e., only the skull was preserved and sampling attempts resulted very invasive).

For all humans a general indication of sex and age at death of the individual is provided together with chronological attribution (Table 1).

The faunal bones were recovered from archaeological sites on the north coast of the Beagle Channel, spanning from the Middle to the Late Holocene (Table 2), published in detail in Zangrando et al. [26,27] and Kochi [28]. They are not directly associated with the Argentinian subset, given that most sites excavated were primary burials outside of residence areas and did not provide a faunal record. They are however comparable with the human remains in term of location and chronological attribution. In detail, the subset of terrestrial animals is composed of 32 samples of guanacos (*Lama guanicoe*) and 3 samples of andean fox (*Lycalopex culpaeus*). The aquatic group is more diverse: it comprises 46 samples of southern fur seals (*Arctocephalus australis*), 2 samples of otter (*Lontra provocax*), 10 samples of albatross (*Thalassarche* sp.) and 11 cormorants (*Phalacrocorax* sp.). It further includes 45 samples of pelagic fishes, which include hakes (*Macronus magellanicus*), snoeks (*Thyrsites atun*), southern hakes (*Merluccius* sp.) together with 2 samples of coastal fish species (*Eleginops maclovinus*). We further discuss data on modern samples of mussels (N = 15), partially presented in Zangrando et al. [26,27] and Kochi [28].

Collagen extraction and sample preparation for isotopic analysis followed two slightly different methods, given that analyses were run at respective laboratories in

Italy and Argentina. Both methods derive from Longin's [29] standard, as described below.

Italian subset

Following Brown et al. method [30], cortical bone (0.5 g) was cleaned by sand abrasion and demineralized in 0.5M solution of HCl at 4ºC for at least four days. The samples were then rinsed to neutral pH and gelatinized in pH 3 HCl at 70 ºC for 48 hours. The collagen solution was filtered off with 5–8 µm Ezee filters, frozen, and then freeze-dried. Each of the collagen extracts was weighed (ca. 1 mg) in triplicate into tin capsules, and stable carbon and nitrogen isotope ratios were measured using an automated elemental analyzer coupled in continuous-flow mode to an isotope-ratio-monitoring mass spectrometer (Costech elemental analyzer coupled to a Thermo Finnigan MAT253 mass spectrometer). Analysis was carried out at the Godwin Laboratory, University of Cambridge.

Argentinian subset

Bone fragments were cleaned with abrasive elements and ultrasonic baths. Each sample of approximately 0.3g was soaked in dilute HCl (0.5%), which was changed every 24–48 h. Then, it was rinsed with distilled water and treated with 0.125% solution of NaOH for 20 hrs. Finally, the collagen extracted was dried in an oven at 40°C for hrs [31].

Measurement of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the collagen fraction was performed with a Carlo Erba EA1108 Elemental Analyzer (CHN), connected to a continuous flow Thermo Scientific Delta V Advantage mass spectrometer through a Thermo Scientific ConFlo IV interface.

For both subsets stable isotope results are expressed as the ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and reported as δ values in parts per thousand (‰), relative to internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen (Ambient Inhalable Reservoir, AIR). Based on replicate analyses of international and laboratory (i.e., alanine, nylon, caffeine, USGS40, EMC) standards, measurement errors are less than ± 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$. The collagen yield, the percentage of carbon and nitrogen, and the atomic C/N ratio of each sample were also recorded to check collagen quality [32-34]. Finally, the modern fauna samples were corrected for carbon by adding 1‰ due to the Suess effect [35]. The historic human samples of Bove's collections were not corrected for this effect [36], considering their estimated chronology.

Results obtained where analyzed through descriptive statistics and non-parametric tests using IBM SPSS© ver. 23.

Results

Table 1 provides data on collagen yields, C/N ratios and collagen isotopic values of the sampled humans, with mean carbon and nitrogen isotope values and C/N ratios from collagen of animals presented in Table 2. For all the samples studied collagen yield and C/N ratios confirm that isotopic signals are to be considered reliable.

Table 1. The human assemblage. Stable carbon and nitrogen isotope ratios for human collagen with data quality indicator (% of collagen %C, %N, C/N). Key: f=female, m=male; n.d.=not determined.

ID	Provenience	sample info	sex	age at death	cal. age (AD/BC)	contact	% coll	δ13C VPDB	%C	δ15N AIR	%N	C/N
TF1	Rome	Fuegian1	f	40+	ca. 1800	post	28.2	-11.6	43.9	18.9	16.3	3.1
TF2	Rome	Fuegian2	f	20-30	ca. 1800	post	28.8	-11.9	45.6	18.9	17.1	3.1
TF3	Rome	Fuegian3	f	40+	ca. 1800	post	28.8	-11.2	46.0	19.3	17.2	3.1
TF4	Rome	Fuegian4	f	40+	ca. 1800	post	24.9	-11.9	42.8	18.4	15.9	3.1
TF5	Rome	Fuegian5	m	40+	ca. 1800	post	29.5	-12.0	44.8	18.3	16.5	3.2
TF6	Rome	Fuegian6	m	40+	ca. 1800	post	26.2	-11.9	46.6	18.0	17.2	3.1
TF7	Rome	Fuegiano7	f	40+	ca. 1800	post	26.6	-12.1	44.0	17.5	16.4	3.1
TF8	Rome	Fuegian8	m	30-40	ca. 1800	post	18.7	-12.1	44.3	17.6	16.6	3.1
TF9	Rome	Fuegian9	f	40+	ca. 1800	post	26.0	-12.5	43.8	17.5	16.4	3.1
TF10	Rome	Fuegian10	f	20-30	ca. 1800	post	29.3	-11.2	45.4	18.0	16.9	3.1
TF11	Rome	Fuegian11	f	40+	ca. 1800	post	39.8	-11.3	41.4	18.6	15.4	3.1
TF12	Rome	Fuegian12	m	15	ca. 1800	post	29.4	-11.6	46.6	17.4	17.4	3.1
TF13	Rome	Fuegian13	f	16	ca. 1800	post	26.2	-11.6	43.6	17.6	16.2	3.1
TF14	Rome	Fuegian13A	m	40+	ca. 1800	post	24.4	-13.0	43.2	17.5	15.8	3.2
TF15	Florence	3116	f	adult	ca. 1800	post	25.1	-12.5	43.4	19.4	15.4	3.3
TF16	Florence	3119	f	adult	ca. 1800	post	23.5	-12.3	43.4	18.6	15.8	3.2
TF17	Florence	3120	f	adult	ca. 1800	post	27.4	-12.1	40.1	19.0	14.7	3.2
TF18	Florence	3122	m	adult	ca. 1800	post	23.1	-12.3	38.9	19.7	14.3	3.2
TF19	Florence	3124	m	adult	ca. 1800	post	20.9	-12.0	42.4	19.3	15.5	3.2
TF20	Florence	3126	m	adult	ca. 1800	post	24.1	-11.4	45.6	19.6	16.2	3.3
TF21	Florence	3127	m	adult	ca. 1800	post	24.5	-12.5	42.0	19.6	15.1	3.2
TF22	Florence	3129	m	adult	ca. 1800	post	22.4	-12.1	45.3	20.4	16.5	3.2
TF23	Florence	3131	m	adult	ca. 1800	post	24.9	-12.0	43.3	19.7	15.9	3.2
TF24	Florence	3133	m	adult	ca. 1800	post	17.5	-12.1	45.8	19.5	16.8	3.2
TF25	Florence	3132	f?	adult	ca. 1800	post	25.6	-12.5	43.2	18.7	15.7	3.2
TF26	Florence	3134	m	adult	ca. 1800	post	18.5	-15.0	45.1	12.3	16.3	3.2
TF 27	MFM	Mischiuen III	f	13-17	1300-1400	pre	n.d.	-11.1	46.5	18.1	16.6	3.3
TF 28	MFM	2668	n.d.	adult	n.d.[d]	pre	n.d.	-11.5	43.1	18.2	15.4	3.3
TF 29	MFM	2669	n.d.	adult	n.d.[d]	pre	n.d.	-13.3	41.5	15.8	15.1	3.2
TF 30	MFM	795	n.d.	adult	n.d.[d]	pre	n.d.	-11.9	45.0	18.0	16.0	3.3
TF 31	MFM	1607	n.d.	juvenile	n.d.[d]	pre	n.d.	-11.9	40.3	18.4	14.4	3.3
TF 32	MFM	SHE	m	35-45	520-650	pre	n.d.	-12.4	41.4	18.3	14.7	3.3
TF 33	MFM	Acatushun	f	30-40	1600-1800	post	n.d.	-13.9	46.4	16.7	16.5	3.3
TF 34	MFM	Paiashauaia	f	35-45	530-670	pre	n.d.	-11.9	43.9	18.3	15.7	3.3
TF 35	MFM	Aeroclub	n.d.	adult	pre-contact[e]	pre	n.d.	-11.2	40.6	19.2	14.8	3.2
TF 36	MFM	BI1	f	25-49	1300-1415	pre	n.d.	-11.2	42.3	19.2	15.4	3.2
TF 37	MFM	Harberton	m	25-35	1600-1800	post	n.d.	-11.6	42.6	18.6	15.4	3.2
TF 38[b]	M. etngráfico	Isla Hoste	n.d.	adult	pre-contact[f]	pre	n.d.	-13.3	n.d.	17.2	n.d.	n.d.
TF 39[b]	M. etngráfico	Ushuaia	n.d.	adult	pre-contact[f]	pre	n.d.	-12.6	n.d.	18.8	n.d.	n.d.
TF 40[b]	M. etngráfico	Isla Hoste	m	adult	pre-contact[f]	pre	n.d.	-16.8	n.d.	13.2	n.d.	n.d.
TF 41[b]	M. etngráfico	Isla Navarino	m	adult	pre-contact[f]	pre	n.d.	-18.5	n.d.	10.6	n.d.	n.d.
TF 42[c]	---	Shamakush I	n.d.	0-6 months	950-1300	pre	n.d.	-12.8	n.d.		n.d.	n.d.

[a] Radiocarbon data was calibrated using SHCal04 curve [37] from Calib Rev 6.0.1 program

[b] published in Yesner et al. [17]

[c] published in Orquera and Piana [8]

[d] Undated sample from unknown depositional context

[e] prehistoric midden

[f] date within the last 1500 years before contact

Table 2. The faunal assemblage. Mean carbon and nitrogen ratios and C/N (with standard deviations) for animal taxa examined.

Taxa	common name	N	$\delta^{13}C_{VPDB}$		$\delta^{15}N_{AIR}$		C/N	
Terrestrial			**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
Lama guanicoe	guanacos	32	-20.6	2.2	0.5	2.5	3.2	0.03
Lycalopex culpaeus	andean fox	3	-18.3	2.3	9	3.3	3.3	0.06
Riverine-marine								
Lontra provocax	otter	2	-10.7	1.8	21.1	4.2	3.2	0
Eleginops maclovinus	Patagonian blenny	2	-12.1	0.5	16.4	0.1	3.6	0.2
Marine								
Arctocephalus australis	seal	46	-11.8	0.5	17.3	0.6	3.2	0.04
Thalassarche sp.	albatross	10	-13.1	0.5	18.6	0.9	3.1	0.02
Phalacrocorax sp.	cormorants	11	-12.7	0.3	15.4	0.6	3.2	0.05
Macruronus magellanicus	hakes	14	-11.6	0.3	15.8	0.9	3.2	0.02
Thyrsites atun	snoeks	14	-12.6	0.7	15.3	0.9	3.2	0.04
Mytilidae	mussels	15	-16.2	0.6	11.1	0.5	3.2	0.05
Merluccius sp.	hake	17	-12.8	0.7	17	0.9	3.3	0.05

Carbon isotope values of collagen from terrestrial animals are typical of mammals feeding on C_3 plants (mean $\delta^{13}C$ between -18.3‰ and -20.6‰). Variation in nitrogen isotope values ($\delta^{15}N$) is highest, and it ranges from 9‰ (fox) to 0.5‰ (guanaco); the very low values for the guanacos can be explained by some consumption of lichens (*Usnea* sp.) with very low $\delta^{15}N$ values (-10.8 to -17.5 ‰) [28].

As expected, the marine vertebrate resources have higher $\delta^{13}C$ and $\delta^{15}N$ values than the terrestrial herbivores, with means of -12.2‰±0.8‰ and 17.1‰±1.9‰ respectively. Little variation in isotope means is observed for seals, seabirds and fish. Three species of pelagic fish (*Macruronus magellanicus, Thyrsites atun, Merluccius* sp.) have $\delta^{13}C$ values with means ranging from -11.6‰ to -12.8‰, and mean $\delta^{15}N$ values range from 17.0‰ to 15.3‰. Carbon isotope values from collagen of seabirds and southern fur seal are similar to those for pelagic fish. For $\delta^{15}N$ values, it is worth noticing that albatrosses have higher mean values than other marine resources. In the other extreme of the marine food web, mussels are presented with the lower mean values for $\delta^{13}C$ (-16.2‰±0.6) and $\delta^{15}N$ (11.1‰±0.5), which are significantly different from those of the marine vertebrates.

Among the estuary resources, *Lontra provocax* has much heavier isotope mean values than marine resources (-10.7‰ for $\delta^{13}C$ and 21.1‰ for $\delta^{15}N$), and *Eleginops maclovinus* has analogous values to those of pelagic fish.

The $\delta^{13}C$ and $\delta^{15}N$ values of human remains range from almost completely marine-derived diets (-11.1‰ and 18.1‰) to values that could reflect entirely terrestrial food composition (-18.5‰ and 10.6‰). However, more than 90% of values are grouped in the ranges -11.1 and -13.3 ‰ for $\delta^{13}C$ and 18.1 and 17.2 ‰ for $\delta^{15}N$, which indicates mainly marine-based diets (Fig. 2).

Fig. 2. **Distribution of the human data.** Frequency of stable carbon and nitrogen isotope values.

The mean $\delta^{13}C$ and $\delta^{15}N$ values of the humans are -12.4±1.4‰ and 18.0±1.9‰, and the medians respectively -12.1‰ and 18.4‰. If we exclude the few cases (N=3) that could reflect up to 50% terrestrial food composition, the means and medians do not significantly change: mean $\delta^{13}C$ and $\delta^{15}N$ values are -12.0±0.6‰ and 18.5±0.9‰, and the medians respectively are -12.0‰ and 18.5‰. The correlation between $\delta^{13}C$ and $\delta^{15}N$ in human values is significant (r^2 = 73.9 p = 0.0001) (Fig. 3).

In general, the humans cluster in a rather homogeneous group with no significant differences between males and females measured in either carbon or nitrogen values for the three subsets (S1 Table).

Fig. 3. **Stable isotope biplot.** $\delta^{13}C$ and $\delta^{15}N$ (‰) of human samples with associated animal mean values and standard deviations. The three human subsets are kept separate.

Discussion

The transculturation from nomadic/mobile hunting-gathering to sedentary/residential lifestyle is a key process in the history of the Fuegian populations. The overexploitation of pinnipeds produced by European and American sealers, the demographic concentrations in missions and ranches, and the introduction of illnesses by Europeans during the 19th century have produced deep structural changes in the subsistence patterns and demography of native groups. Our data and statistical analysis suggest no differences in dietary practices across time detectable at an isotopic level, which is in counter-tendency with our expectations, and offer some interesting convolution.

The Kolmogorov-Smirnov test shows that there is no statistical difference in $\delta^{15}N$ between pre- and post-contact populations ($p= 0.185$; $n=14$ and $n=28$ respectively), with slight differences in the distribution of data (S2 Fig.). For nitrogen values, isotope data show that post-contact populations kept diets focused on marine resources of high trophic levels. This is particularly interesting: if seals – one of the preferred food sources among the Yamana – were becoming less available because of

Europeans and Americans' exploitation we should assume post-contact consumption of low trophic level species and higher values among pre-contact groups, as a consequence of consumption of high trophic level species. Surprisingly, the post-contact nitrogen data cluster around higher values, against this expectation (Fig. 4). However, the reported nitrogen values for some species of seabirds and pelagic fish are similar, and even higher, to those of fur seals in the southern South Atlantic, indicating analogous trophic levels. Therefore, a change in subsistence redirected towards the exploitation of such minor resources should not necessarily have implied a change in the trophic levels.

Fig. 4. **Pre- and post-contact data distribution.** Box and whisker plot of nitrogen and carbon values for pre- and post-contact human populations. Nitrogen median values show little differences between pre-contact and post-contact phases.

The descriptive statistics presented in Table 3 is also noteworthy: it shows a difference of 1.2‰ between the mean $\delta^{15}N$ values, and a lesser distance between the medians (0.5‰), which do not support a significant change in trophic terms.

Table 3. **Summary of pre- and post-contact data.** Descriptive statistics for the human sample only, according to chronological phase.

	$\delta^{13}C_{VPDB}$		**$\delta^{15}N_{AIR}$**	
	Pre-contact	Post-contact	Pre-contact	Post-contact
N	14	28	14	28
Min.	-18.5	-15.0	10.6	12.3
Max.	-11.1	-11.2	19.2	20.4
Mean	-12.8	-12.2	17.2	18.4
Stand. Dev.	2.1	0.8	2.5	1.5

Median	-12.3	-12.1	18.2	18.7

We prompt to think that this difference in the mean δ^{15}N values could be describing a variation in the diet, indicating the consumption of fewer lower marine trophic level resources or more high-trophic level marine species. A decrease in the consumption of shellfish by post-contact populations might be supported by historical documents: the setting-up of the Anglican Mission in Ushuaia by the end of 1860s led to the demographic concentration and semi-sedentism of the native population in few years. There were almost permanently residing between 10 and 18 Yamana families, and every year the Mission received hundreds of Yamana people where they stayed from four to six months [38-40]. Such concentration of persons and such reduction in mobility certainly should have resulted in the overexploitation of local resources, especially shellfish [1]. Given the δ^{15}N lighter values on shellfish in comparison with other marine resources, the high nitrogen values in post-contact human remains could be describing accordingly less accessibility to those resources.

The Fuegian landscape shows little seasonal variation in the environment, with oxygen isotopic analyses indicating a shift in mean annual temperature that ranges between 1 and 2°C across several centuries (i.e., between the Medieval Climatic Anomaly and the Little Ice Age) [41], with paleobotanical data [42,43] suggesting little changes in the local vegetation over the last six millennia. Similarly, archaeological data [44] show that human occupation in the area is uninterrupted, with a general uniformity in cultural practices. Across time human groups settled in Tierra del Fuego display a general continuity, either in cultural practices or in technological complexity, with rare innovations. This appears to be true also for what concerns diet, as types of food consumed and levels of protein intake appear to remain unchanged across several centuries. According to Orquera and colleagues [44], "in spite of its simplicity, the system was successful. Its collapse happened by the end of the 19th century, and was caused by the catastrophic pinniped depredation carried out by Euro-American, Chilean and Argentine groups, as well as by the introduction of sickness against which the indigenous populations had no immunity". Under such circumstances, it is possible that the subsistence patterns of the marine hunter-gatherers of the Beagle Channel based on the exploitation of a high diversity of resources have given to these populations a resilience to cope with the hunting of fur seals by industrial populations. The overall consistency in isotope data might hence be interpreted in cultural terms. We propose here that: i) post-contact societies might have experienced a significant reduction in the consumption of pinnipeds, which was compensated through the intake of other aquatic mammals (i.e., seabirds and fish with similar nitrogen levels), with effects of such shift in the diet not detectable at the isotopic level; at the same time, ii) a significant decrease in the consumption of molluscs by post-contact populations might have driven up their nitrogen values;

The human ecological resilience of the Fuegian groups expressed in the search for nutritional alternatives can be perceived, as anticipated, as the evidence of a cultural resistance, which goes along with a general stability of these people. Here, the idea of resistance is not linked to noncompliance [45], as in the lack of negotiation of traditions. Rather, it is perceived as the tendency to persist in the expression of specific traditions (the hunting/fishing of aquatic species), despite external stimuli and/or the knowledge of alternatives. For the Fuegians, such resistance seems to translate in a striking uniformity of dietary practices across several centuries, as mirrored in the isotopic ratios of the skeletal tissues. Furthermore, considering that the contribution to collagen of different types of dietary proteins appears to remain constant throughout time, we might need to reconsider the influence of diseases as the main factor in the dramatic contraction (and eventually extinction) of Fuegian populations.

Acknowledgements

We thank Mike Hall and James Rolfe at the Godwin Lab, Department of Earth Sciences, University of Cambridge for help with isotopic analyses on the Italian material, and Catherine Kneale, Louise Butterworth and Hazel Reade, McDonald Institute for Archaeological Research, for help in sample preparation and analysis. Access to the collection and permit to sample the specimens at the Museum in Florence was granted by the Curator, Dr. Monica Zavattaro, whose assistance is gratefully acknowledged. We thank Maria Luana Belli and Aurelio Marangoni for assistance with sampling of the skeletons preserved in Rome. No further permits were required for the described study, which complied with all relevant regulations. We thank the reviewers for precious comments on the paper.

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Supporting information

S1 Table. Statistics report. Summary of the Mann-Whitney U test for human carbon and nitrogen data according to sex. The three subsets are kept separate. For the Ushuaia subset 7 individuals were excluded, as no sex estimate was available.

S2 Figure. Biplot of stable carbon and nitrogen data. Mean humans $\delta^{13}C$ and $\delta^{15}N$ values (with sd) for pre-contact (n=14) and post-contact (n=28) subsets.

Reviewer's Responses to Questions

Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: Partly

Reviewer #2: Yes

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2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: Yes

Reviewer #2: Yes

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3. Have the authors made all data underlying the findings in their manuscript fully available?

The PLOS Data policy requires authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception (please refer to the Data Availability Statement in the manuscript PDF file). The data should be provided as part of the manuscript or its supporting information, or deposited to a public repository. For example, in addition to summary statistics, the data points behind means, medians and variance measures should be available. If there are restrictions on publicly sharing data—e.g. participant privacy or use of data from a third party—those must be specified.

Reviewer #1: Yes

Reviewer #2: Yes

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4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors here.

Reviewer #1: Yes

Reviewer #2: Yes

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5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1: The paper “Dietary resilience among hunter-gatherers of Tierra del Fuego: Isotopic evidence in a diachronic perspective” presents a new isotope dataset with which to study pre and post-contact dietary changes. The authors present the first study of dietary change across this important social period, demonstrating surprising continuity given evidence in historical accounts for upheaval. Local communities appear to have retained a diet heavily focused on marine protein, which the authors describe as a form of cultural resilience. The use of isotopic data has been shown to complement historical datasets, despite the often-assumed superiority of the latter, and this is also the case here.

The dataset is robust and the conclusions broadly supportable. However, to be appropriate for the broader readership of PLoS ONE I believe much more context is required and the paper needs to be more usefully structured. Firstly, the Introduction and Archaeological/Historical Background is currently inadequate. For none specialists in the region, there is not enough information regarding the eventual hypotheses of the authors. What happened during contact? Why would the authors expect that the diet might change? Is there specific evidence for crops being introduced and locals using them? Is there evidence for local communities relying on differing extents of marine resources? This only comes in in the Discussion from Page 11-12 but needs to be in at the outset so people can understand the point of the study!

The sample of humans, 42, is also not clearly laid out and it is confusing at present. It needs to be clear where these are coming from, and which collections they are coming from, perhaps in a table. Spreading the discussion across a few pages in the text makes it hard to follow. It doesn't seem to ad up either I count 9 (pre-contact excavated subset), 2 (post-contact excavated subset), 1 (Orquera and Piana infant), 5 (Yesner et al.), 12 (Florence), 14 (Rome) = 43? Also why was only the infant from Rome left out and the others left in?

Samples are indeed 42: the comparative samples published by Yesner et al are 4. There was a typo. Also, we failed to clear out why we had to keep out the Roman infant: this was due to difficulties in the sampling process. We haave clarified this by changing the M&M section as follows "Sampling for isotopic analysis was carried out on the ribs of 14 individuals, with the exclusion of the infant, which was deliberately not sampled to avoid damage to the skeletal elements (i.e., only the skull was preserved and sampling attempts resulted very invasive)".

It is great that the authors included animals as a comparison dataset, and have a nice dataset of a number of different aquatic taxa. However, they initially state in the Introduction that these are not associated with humans, but then later in the Discussion suggest they are. Which is it? If they are not then is this because animals are missing from cemetery sites or because most did not collect them? Why were they missing from excavation? The authors then need to justify the collections they use in this situation if they are not directly associated. So more is needed here. There is also the issue that the faunal data has clearly been in part published elsewhere (Zangrando et al., and Kochi). How much has been published? What is new here? This must be clear.

This has been specified more carefully, please see new text in M&M section

There is also no stable isotope background prior to the methods section. This should be here to justify the use of the method and would also be a useful place to provide background regarding it's use in the region. How does the new study build on existing datasets of which there are clearly a few? This is clearly due to the diachronic nature of the one presented here but it is important to do this. Discussing variability in marine and aquatic ecosystems here would also make the discussion easier to follow, especially for non-specialists.

We specify that earlier isotope data have shown reliance on aquatic resource but were confined to earlier studies while no diachronic perspective was use, justifying the paper. Hence, despite we do no entirely agree with this comment we have added some background, especially in terms of earlier isotopes data. Please see the Introduction

It is also confusing why collagen was extracted using different methods? Was this linked to preservation or just laboratory differences? Why was this the case?

This was due to working in different labs, we have specified this in the M&M section

It would also be useful to report the standards used in the measurement of the data.

Please see new text in the M&M section

Why was only 1‰ added for the fossil fuel effect? General consensus would suggest 1.6‰ (so nearly 2‰) especially when modern collections are being compared to Early-Middle Holocene samples?

We preferred to apply a 1 per mil increase considering that Burton et al., work provides a <u>direct</u> evidence of the surface carbon reservoirs resulting from fossil fuel and biomass burning in the region. This evidence seemed more fitting to us as, specifically measured in the research area.

The stats tests also need to be set up in the methods not just added in the Results and Discussion.

Reference to statistical tests has been added to the M&M section

Care needs to be taken in the isotopic interpretation as well. For example rather than just saying the “consumption of lichens” it would be more correct to say “some consumption of lichens” (page 10, line 203). Also “ a variation in diet with a decrease in the consumption of shellfish” is a big claim! It needs to be stated tentatively as one possibility. Presumably it could also be the greater consumption of higher-trophic level resources (e.g. seals and albatross). It could also be terrestrial, though the fact that both 15N and 13C go up suggests this is unlikely. Better to say would be “this therefore indicates the consumption of fewer lower marine trophic level resources or more higher trophic level marine resources”. “These could be in the form of…” This would actually suggest that there was some shift. Does this go against resilience?

We adopted a more cautios approach and changed as suggested “We prompt to think that this difference between the mean δ^{15}N values could be describing a variation in the diet, indicating the consumption of fewer lower marine trophic level resources or more higher trophic level marine species”.

You also actually can’t really test point i) on page 14 line 309. You cannot test whether a reduction in pinnipeds was compensated through the intake of other aquatic mammals if there is no change…. All you can really say is that there was minimal change indicating relative marine resilience with the exception of point ii) whereby there is a shift in 15N and 13C which could be linked to changes which need to be phrased more conservatively and maybe elaborated on with historical data.

Indeed we cannot test this. Our assertion is a possible reconstruction we propose to explain the isotopic data obtained. We have used a more cautious language.

Line 311 here: “nitrogen populations” is a sloppy phrase, things like this need to be checked throughout. What might the greater 15N and 13C variation in pre-contact populations mean relative to post-contact populations?

There was a typo, now the phrase reads: “has driven up nitrogen values in post-contact populations;”

The appearance of “cultural resistance” at the end also seems a little over the top. This comes a little out of the blue. Perhaps if a lot of the contextual information re. hypotheses of post-colonial change were included in the Introduction and Background sections then this would be less the case.

We agree that this is a little ambitious, however we have used a language, which makes it very clear that we are being extremely cautious in presenting this point. We specifically use the contradiction between resilience/resistance to stress the profound link between ecological aspects and cultural ones. We believe this is a strong point of the paper and would prefer to keep it. We agree however, that the background needs further input, hence have added some contextual information in the Introduction section.

Page 15, line 323 isotope evidence will not show you amount of protein take! What you mean is the contribution to collagen of different types of dietary protein. Looking at health or protein amount would be better done through osteoarchaeology, which might have been an interesting aspect to think about in the current study pre and post-colonial. Is there any reason this wasn’t done (i.e. characterizing diseases across collections?)?

Corrected as suggested. The osteoarchaeological investigation on both subsets is still ongoing and might be considered in future work.

Minor comments

-English needs checking throughout

Page 2, Line 30: “We investigate on” should just be “We investigate dietary variation”

Changed as requested

Page 2, Line 34: What kind of uniformity? More specificity is required here.

We have replaced with “The data obtained reveal an unexpected isotopic uniformity across prehistoric and recent groups, with little variation in both carbon and nitrogen mean values”,

Page 3, Line 51: not “Fishers”

Why not? I am not sure I understand this comment…

Page 3, Line 61: doesn't make sense, needs checking and re-writing.

Replaced with "The ethnographic sources available describe both groups as having a well-defined marine subsistence, with no significant differences in the dietary regime of these populations".

Page 3, Line 65: for example?

For example what? I struggle to understand this comment. If the reviewer refers to an example of zooarchaeological works we cite various papers… But I am not sure my intepretation of the comment is correct…

Page 4, Line 70: why would they be compromised? More detail needed.

We refer to this throughout the paper, Europeans and North-Americans have seriously impacted on the availability of seals in the area. We also suppor tour statement with relevant literature.

Page 4, Line 76: "In the region"

Corrected

Page 4, Line 78: "About historical populations" not on

corrected

These kind of things should be checked throughout.

All tables and graphs – common names of taxa as well as Latin names would be useful, whereas in the text the Latin names would be a useful addition to the common names.

In the text latin names are specified, please see text here "The subset of terrestrial animals is composed by 32 samples of guanacos (Lama guanicoe) and 3 samples of andean fox (Lycalopex culpaeus). The aquatic group is more diverse: it comprises 46 samples of southern fur seals (Arctocephalus australis), 2 samples of otter (Lontra provocax), 10 samples of albatross (Thalassarche sp.) and 11 cormorants (Phalacrocorax sp.). It further includes 45 samples of pelagic fishes, which include hakes (Macronus magellanicus), snoeks (Thyrsites atun), southern hakes (Merluccius sp.) together with 2 samples of coastal fish species (Eleginops maclovinus)". Common names have been added to the table.

Beyond the Table please define the sample size for the pre and post contact groups in the methods.

Indicated as requested, please see new Table 2

Figure 4 caption: should be box and whisker plot not whisker and box plot.

Corrected

Acknowledgments: why weren't permits required? Surely some were?

This is a standard phrase required by PlosOne.

Figures, axes headings should include units for isotopes and comparison to international standard (i.e. VPDB) check isotope publications for a guide on this.

All figures corrected as requested

It would also be useful to have a biplot 13C and 15N figure should the mean and s.d. for pre and post contact populations.

We added the byplot as SI (SI Fig. 1)

Reviewer #2: I think this is a very good paper which approach an interesting subject not frequently taken. The changes in the diet, based on isotopic studies from pre and post contact samples , indicates a continuity in the consumption o f marine resources (although probably not seals) . This is an original and un expected result, since the dietary impact during the European contact was supposed to be very strong. The data presented is new and support the conclusions. My only concern is about the use of the concept of resilience, a term which has been subject of debate in the last decade. I would like to see on the paper more discussion about the different dimensions of resilience . There is also a few questions

Line 52. Why do not refer to 14C yrs BP as usual instead of uncal BP?

True! we got rid of the term ‘uncal’ as it is implicit

Line 53. Who call them canoeros? Is this a local or an anthropological denomination?

It is an local denomination which was used by anthropologists throughout contact period

Beyond these small details I think that this is a high quality paper and should be published. My recommendation is accepted with minor revisions.

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6. If you would like your identity to be revealed to the authors, please include your name here (optional).

Your name and review will not be published with the manuscript.

Reviewer #1: (No Response)

Reviewer #2: Gustavo Politis