Human-induced ecological change in the open oceans appears to be accelerating. Fisheries, climate change and elevated nutrient inputs are variously blamed, at least in part, for altering oceanic ecosystems. Yet it is challenging to assess the extent of anthropogenic change in the open oceans, where historical records of ecological conditions are sparse, and the geographical scale is immense. We developed millennial-scale amino acid nitrogen isotope records preserved in ancient animal remains to understand changes in food web structure and nutrient regimes in the oceanic realm of the North Pacific Ocean (NPO). Our millennial-scale isotope records of amino acids in bone collagen in a wide-ranging oceanic seabird, the Hawaiian petrel (Pterodroma sandwichensis), showed that trophic level declined over time. The amino acid records do not support a broad-scale increase in nitrogen fixation in the North Pacific subtropical gyre, rejecting an earlier interpretation based on bulk and amino acid specific $\delta^{15}N$ chronologies for Hawaiian deep-sea corals and bulk $\delta^{15}N$ chronologies for the Hawaiian petrel. Rather, our work suggests that the food web structure in the NPO has shifted at a broad geographical scale, a phenomenon potentially related to industrial fishing.

1. Introduction

Anthropogenic impacts are an increasing threat to marine ecosystems. Warming of marine water alters ocean circulation, contributes to sea-level rise, and has unmistakable impacts on coastal coral reef communities [1]. Rising inorganic nitrogen from rivers, nitrogen fixation, and nitrogen deposition impart eutrophication, promote hypoxia, and alter ecological community structure [2]. The collapse of coastal fisheries is also well known [3]. While anthropogenic disturbance in coastal regions is obvious, human influences on the open oceans are more insidious. Rising temperatures and nutrient-driven increases in primary production are recognized in the open oceans [2,4,5]. Higher in the oceanic food web, fisheries contribute to depletion of top predators and cetaceans, and in the Central Pacific alone, annual catch is ca 1.4 million tonnes [6–8]. While there may be a temptation to consider oceanic food webs, especially in the deep ocean, as relatively insulated from human impacts, this is probably a misperception considering that loss of coastal marine resources and technological advances compel exploitation far beyond continental shelves [9]. Even so, evaluating the state of ecosystems is difficult without historical records of change, a formidable challenge for the vast oceanic realm.
The legacy of climate and certain human impacts is recorded in the ratio of \(^{15}\text{N}/^{14}\text{N}\) in bulk tissues (\(\delta^{15}\text{N}\)) and amino acids (\(\delta^{15}\text{N}_{\text{aa}}\)) of marine organisms [10,11]. Nitrogen added to the base of the food web by nitrogen fixing diazotrophs is distinguished by a uniquely low \(\delta^{15}\text{N}\) of about 0‰, compared with 5–6‰ for marine nitrate [4,12,13]. Such isotopic differences in source nitrogen are faithfully passed on to organisms higher in the food web, but with approximately a 3‰ increase in \(\delta^{15}\text{N}\) per trophic level [14]. While \(\delta^{15}\text{N}_{\text{aa}}\) is commonly used to study nitrogen cycling and food web structure, \(\delta^{15}\text{N}_{\text{aa}}\) is hard to interpret because it is influenced by source nitrogen and trophic effects.

The difficulty in interpreting \(\delta^{15}\text{N}_{\text{aa}}\) underlies a major disagreement between Wiley et al. [15] and Sherwood et al. [11] regarding whether trophic-level shifts or, instead, changes in source nitrogen have influenced food webs in the oceanic realm of the North Pacific Ocean (NPO). Wiley et al. [15] and Sherwood et al. [11] built millennial-scale nitrogen isotope chronologies, each covering a different geographical region of the NPO and using different study organisms. These two studies drew opposing conclusions regarding whether trophic-level shifts or changes in source nitrogen at the base of the food web have influenced food webs in the NPO. To address this controversy, we developed amino-acid-specific \(\delta^{15}\text{N}\) chronologies from two breeding populations of the Hawaiian petrel (\(Pterodroma sandwichensis\)), one from the island of Hawaii and the other from Maui.

2. Material and methods

(a) Sample acquisition

Hawaiian petrel bones are from carcasses salvaged from Haleakala National Park (Maui) and Hawaii Volcanoes National Park (Hawaii) between 2001 and 2010 [15]. Subfossil bones from archaeological and palaeontological sites on Hawaii and Maui were also sampled [15]. While adult and hatch year birds had similar \(\delta^{15}\text{N}_{\text{aa}}\) values and are included in our Hawaii \(\delta^{15}\text{N}_{\text{aa}}\) dataset, owing to the disparity between adult and hatch year bird \(\delta^{15}\text{N}_{\text{aa}}\) values on the island of Maui, only adult Maui petrels were included in our \(\delta^{15}\text{N}_{\text{aa}}\) analyses.

(b) Protein isolation and radiocarbon dating

Gelatin from subfossil bones was previously prepared for radiocarbon and isotope analysis [15]. Radiocarbon dating, reported previously, was conducted at the accelerator mass spectrometry (AMS) facility at the University of California, Irvine (W. M. Keck Carbon Cycle AMS Laboratory) [15]. As described in Wiley et al. [15], conventional radiocarbon ages were calibrated using the programme CALIB 6.0 and assigned to time bins based on their median probability dates.

(c) Sample sizes

Bone samples derive from salvaged carcasses and palaeontological and archaeological bones. Samples fall within previously described time bins that mark development of the human population of the Hawaiian Islands: prehuman period (before human colonization of Hawaii; less than 1000 CE or more than 950 yr BP), foundation period (Polynesian colonization, small human population size; 1000–1400 CE; 550–950 yr BP), expansion period (increasing human population size; 1400–1800 CE; 150–550 yr BP) and the modern period (onset of industrialized fishing in the NPO; 1950–2010 CE) [15,16]. Samples from the island of Hawaii were chosen by randomly selecting samples within each of the three time periods (prehuman, late expansion, modern) that were within 1 standard deviation of the sample time-binned mean of our \(\delta^{15}\text{N}_{\text{aa}}\) data. This limited the influence of outliers. However, few samples were available from Maui. Consequently, all or the majority of the available samples from Maui were analysed. Sample sizes for our time bins were: modern Hawaii (\(n = 8\)), expansion Hawaii (\(n = 8\)), prehuman Hawaii (\(n = 5\)), modern Maui (\(n = 7\)), foundation Maui (\(n = 5\)), prehuman Maui (\(n = 7\)).

(d) \(\delta^{15}\text{N}\) amino acid analysis and estimates of trophic-level decline

Amino-acid-specific nitrogen isotope analyses were conducted at the Japan Agency for Marine-Earth Science and Technology. Data are expressed as \(\delta^{15}\text{N}_{\text{aa}} = \left(\left(\frac{^{15}\text{N}_{\text{sample}}}{^{14}\text{N}_{\text{sample}}} / \frac{^{15}\text{N}_{\text{standard}}}{^{14}\text{N}_{\text{standard}}}\right)-1\right) \times 10^{3}\) relative to the standard atmospheric Nh and units are per mil (‰). \(\delta^{15}\text{N}\) analysis of N-pivaloyl/isopropyl (PV/iPR) amino acid derivatives of gelatin (ca 0.1 mg hydrolyzates (12 N HCl, 110°C, 12 h) was performed as described by Chikaraishi et al. [17]. Derivatives were analysed on a 6890N GC (Agilent Technologies, USA) fitted with a HP Ultra-2 capillary column (50 m, 0.32 mm i.d., 0.52 μm film thickness) in line with oxidation (950°C) and reduction (550°C) furnaces interfaced to a Delta Plus XP IRMS (Thermo Fisher Scientific, Bremen, Germany). Accuracy was evaluated by analysis of an external standard consisting of PV/iPR derivatives of nine isotopically characterized amino acids (alanine, glycine, leucine, norleucine, aspartic acid, methionine, glutamic acid, phenylalanine and hydroxyproline) [18]. Reproducibility was better than 0.5‰.

The trophic proxy, \(\delta^{15}\text{N}_{\text{PV/iPR}}\) was used as an indicator of trophic level. In addition, trophic position was calculated using a multi-TDF model [19], necessitated by the observation that the trophic discrimination factor for birds (3.6‰) is lower than that of aquatic and other lower trophic level organisms (7.6‰) [17,19,20]. The model is: \(\text{TP} = (\delta^{15}\text{N}_{\text{PV/iPR}} - \delta^{15}\text{N}_{\text{PV/iPR}} - \text{TDF}_{\text{typical}} - 3.4)/\text{TDF}_{\text{typical}} + 2\), where the trophic discrimination factor (TDF) for birds (\(\text{TDF}_{\text{bird}}\)) is 3.6‰, and \(\text{TDF}_{\text{typical}}\) is the TDF for lower trophic levels = 7.6. We also evaluated our results with a simple step model using a TDF = 7.6‰, but, as illustrated in the electronic supplementary material, table S1, this did not alter our conclusions regarding changes in trophic level between ancient and modern time periods.

(e) Diet analysis

Analyses were conducted on regurgitations collected from 12 Hawaiian petrels from Maui in 2007 and 2009. Toothed fishes, primarily lantern fish and flying fish, and ommastrephid squid comprised over 90% of the items in the diet, similar to the findings of Simons [21].

(f) Statistical approach

The population means (and variance) for \(\delta^{15}\text{N}_{\text{PV/iPR}}\) and \(\delta^{15}\text{N}_{\text{PV/iPR}} - \delta^{15}\text{N}_{\text{PV/iPR}}\) for each time period in each location were estimated using analysis of variance (ANOVA). Data from Hawaii and Maui were analysed separately as there is no reason to believe that changes in \(\delta^{15}\text{N}_{\text{PV/iPR}}\) and \(\delta^{15}\text{N}_{\text{PV/iPR}} - \delta^{15}\text{N}_{\text{PV/iPR}}\) are the same across time periods in the two locations, especially, because late expansion data (only available from Hawaii) and foundation data (only available from Maui) represent different time periods. Parameter values in the ANOVAs were estimated using a Bayesian approach, which produces unbiased estimates at small sample sizes and allows for the estimation of explicit probability statements [22]. Specifically, we were able to calculate: (i) the probability that \(\delta^{15}\text{N}_{\text{PV/iPR}}\) decreased from ancient to modern time periods, and (ii) the probability that \(\delta^{15}\text{N}_{\text{PV/iPR}} - \delta^{15}\text{N}_{\text{PV/iPR}}\) (i.e. trophic position) decreased over the same time period. Average
time-specific trophic position was calculated as a derived quantity using $\delta^{15}$N$_{Glu} - \delta^{15}$N$_{Phe}$ values according to the formula in the electronic supplementary material, table S1.

Separate ANOVAs were run on $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Glu} - \delta^{15}$N$_{Phe}$ for both Hawaii and Maui, estimating mean values of each quantity in the three time periods (prehuman, late expansion or foundation, modern). Equal variances among time periods within each analysis were assumed, and parameter values for each ANOVA were obtained using Markov chain Monte Carlo (MCMC) executed with the programs R [23] and WinBUGS [24]. Vague priors were used for each of the means (normal distributions centred around 0 with a variance of 1000) and the variance (uniform distribution between 0 and 100). Three chains each for 100,000 iterations and a burn in of 10,000 were used. The remaining samples were thinned by saving only every third value, thus creating a posterior distribution for each parameter that consisted of 90,000 independent values. The derived probabilities (e.g. decreases in $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Glu} - \delta^{15}$N$_{Phe}$ from ancient to modern times) were calculated by subtracting the estimated mean values in the early time period (prehuman and late expansion or foundation) from the values in the modern time period for each iteration of the MCMC. The probabilities were then calculated as the proportion of those subtracted values that are positive (electronic supplementary material, table S1).

3. Results and discussion

Wiley et al. [15] and Sherwood et al. [11] built millennial-scale nitrogen isotope chronologies, each covering a different geographical region of the NPO. In a study of a wide-ranging oceanic predator, the Hawaiian petrel (figure 1), Wiley et al. [15] found a species-wide decline in $\delta^{15}$N$_N$ in a millennial-scale time series of modern and ancient bones assembled from modern ornithological, archaeological and palaeontological collections, and circumscribed the timing of the decline to the past 100 years. This trend was interpreted as evidence of a significant species-wide decline in trophic level, most likely associated with industrial fishing. Sherwood et al. [11] found a similar decline in $\delta^{15}$N$_N$ values of three long-lived, deep-sea corals (Kulamanamana haumeaae) in the vicinity of the main Hawaiian Islands (figure 1) and interpreted this trend as evidence of a temporal increase in nitrogen fixation. Based on the rough similarity in temporal patterns in the two studies, Sherwood et al. [11] reinterpreted the Hawaiian petrel $\delta^{15}$N$_N$ data as reflecting an increase in nitrogen fixation in the North Pacific subtropical gyre, and argued against a trophic effect associated with fishing, thus laying down a gauntlet for all $\delta^{15}$N$_N$ studies that have claimed to show trophic effects of marine fishing [10,28,29].

To bolster their $\delta^{15}$N$_N$ dataset, Sherwood et al. [11] also analysed $\delta^{15}$N$_N$ from two corals, yielding one record from about 700 yr BP (AD 1300) to present and another from about 800 yr BP (AD 1400) to 165 yr BP (AD 1850). The $\delta^{15}$N$_N$ data offer a means to disentangle the signal of source nitrogen and trophic position from a single sample [17]. Certain amino acids such as phenylalanine are principally influenced by changes in source nitrogen, whereas others such as glutamic acid undergo significant increases in $\delta^{15}$N with each trophic step. Further, the difference between the $\delta^{15}$N values of glutamic acid and phenylalanine, $\delta^{15}$N$_{Glu} - \delta^{15}$N$_{Phe}$, is a proxy for trophic position. Data from the coral specimen that dated from 700 yr BP to present showed a decline in average $\delta^{15}$N of both source and trophic amino acids, and no change in $\delta^{15}$N$_{Glu} - \delta^{15}$N$_{Phe}$ supporting the interpretation that the $\delta^{15}$N$_N$ coral data reflect a shift in source nitrogen. Yet, the sinking particles that support the deep-sea corals and the surface particles at the base of the Hawaiian petrel food web differ substantially in $\delta^{15}$N$_N$ [13,30], and no Hawaiian petrel $\delta^{15}$N$_N$ data were available to support Sherwood et al.‘s [11] interpretation that a temporal trend in nitrogen fixation was widespread across the North Pacific subtropical gyre.

In an independent laboratory from the original study, we generated two separate $\delta^{15}$N$_N$ chronologies, representing Hawaiian petrel populations from the islands of Hawaii and Maui, which are known to be genetically distinct and are known to differ in foraging habits [15,31]. We compared the $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Glu} - \delta^{15}$N$_{Phe}$ of Hawaiian petrel collagen from prehuman, archaeological and modern time periods from Hawaii and Maui using a Bayesian ANOVA, which allowed us to explicitly quantify the probability of changes in $\delta^{15}$N between ancient and modern time periods (figure 2 and the electronic supplementary material, table S2). We also calculated point estimates (means) and 95% credible intervals for both quantities in each time period (electronic supplementary material, table S3). The datasets

Figure 1. At-sea distribution of Hawaiian petrel and N* distribution. Curved line delineates Hawaiian petrel distribution [25]. Filled and open red circles are locations of corals with declining $\delta^{15}$N$_N$ ca 1850-present and declining $\delta^{15}$N$_{Phe}$ ca 1850-present, respectively [11]. The anticyclonic North Pacific subtropical gyre extends west of 120° W between 10° N and 40° N [26]. Colour gradient is the distribution of N* defined and gridded by Sherwood et al. [11], where N* = N = 16P + 2.9 μmol kg$^{-1}$, where P = phosphorus) [27]. Positive values are interpreted as an increase in nitrogen fixation and negative values as net denitrification by Sherwood et al. [11]. (Online version in colour.)
from each island were analysed separately with strikingly similar results. Between Hawaii’s late expansion or prehuman and modern time periods and Maui’s foundation or prehuman and modern time period, the probability that $\delta^{15}N_{\text{Phe}}$ declined was less than or equal to 0.13 for both islands and the probability that $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$ and trophic position declined was more than 0.99 for both islands. While $\delta^{15}N_{\text{Phe}}$ provides no evidence that a change in nutrient regime (e.g. an increase in nitrogen fixation) influenced the Hawaiian petrel food web, declines in both $\delta^{15}N_{\text{Glu}}$ and $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$ are consistent with a decline in trophic position between prehuman and modern periods of 0.4 and 0.3 for the Hawaiian petrel population on Hawaii and Maui, respectively (electronic supplementary material, table S1).

We explored reasons for the disparity in $\delta^{15}N_{\text{Phe}}$ trends between the coral and petrel data, looking for mechanisms that could explain the differences between the two datasets. The coral data are derived from waters near Hawaii where nitrogen fixation rates are elevated and the nitrogen to phosphorus ratio (N:P) is greater than the expected Redfield stoichiometry of 16:1 [4,5]. This results in elevated values for the N* parameter (nitrogen concentration in excess or deficit to phosphorus relative to Redfield stoichiometry $N^* = N - 16P + 2.9 \mu\text{mol kg}^{-1}$) [27]. Elsewhere, anthropogenic atmospheric nitrogen deposition is the major cause of temporal increases in N:P [5]. Even so, large areas of the NPO are characterized by a nitrogen deficit and negative N* values (figure 1). The deep-sea corals studied by Sherwood et al. [11], whereas the absence of a shift in the $\delta^{15}N_{\text{Phe}}$ of Hawaiian petrels is indicative of the absence of a pronounced increase in nitrogen fixation or atmospheric nitrogen deposition across their broad foraging range. Thus, the disparity between coral and petrel $\delta^{15}N_{\text{Phe}}$ trends is not surprising, leading us to ask: what is the significance of the $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$ decline in the Hawaiian petrel?

Our new data are consistent with our earlier claim that the Hawaiian petrel experienced a trophic shift, a phenomenon that may have broad implications for the NPO. The decrease in $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$ implies a trophic-level decline but could also reflect a change in diet quality (e.g. variation in protein, fat or carbohydrate content) [19,32]. Considering that our diet analyses and those of others show that Hawaiian petrels are top predators that feed on purely proteinaceous diets of teleost fishes and squid [21], any changes in protein content are likely to be inconsequential. A decline in magnitude of trophic discrimination factors associated with prey might also influence our $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$ data. Recent literature shows more variation in amino acid-specific trophic discrimination factors among organisms than previously described, probably resulting from differences in feeding ecology, physiology and as discussed earlier, the balance of protein, fat and carbohydrates in an organism’s diet [19,32–34]. Owing to the paucity of species-specific trophic discrimination factors in the literature, the extent to which variation in trophic discrimination factors influenced the Hawaiian petrel’s prey and our $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$ data are difficult to assess. If a change in trophic discrimination factors is responsible for the decline in $\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}}$
between ancient and modern Hawaiian petrels, this would imply that the prey of Hawaiian petrels changed over time. While we do not know that a decline in trophic level was the sole factor driving the observed decrease in Hawaiian petrel $\delta^{15}$N$_{Phe}$, independent lines of evidence show a decrease in the mean size of fishes in the NPO, a phenomenon consistent with a trophic-level decline [35]. A trophic-level decline occurring within a vast open ocean basin is indicative of large-scale ecological change. Wiley et al. [15,36] did not find a correlation between climate change indices (e.g. El Niño Southern Oscillation) and our isotope chronologies. Another major agent of ecological change in the NPO is industrial fishing. Ecosystem responses to industrial fishing offers one scenario where ocean-wide effects of industrial fishing could produce a trophic-level decline in the Hawaiian petrel and probably in other oceanic predators.

Sherwood et al. [11] emphasized changes in nutrient regimes within a large region of the NPO, the North Pacific subtropical gyre. The low average $\delta^{15}$N$_{Phe}$ of 1.9% for Hawaiian petrels from Maui is consistent with a substantive contribution of nitrogen fixation to the base of the food web for the population of Hawaiian petrels that breeds on Maui. Yet, even in the Maui population, $\delta^{15}$N$_{C_{40}} - \delta^{15}$N$_{Phe}$ values are consistent with a trophic decline and not with a temporal increase in nitrogen fixation. Thus, a dietary shift, most likely a trophic-level decline, affected Hawaiian petrels broadly, regardless of where populations fed.

If indeed a trophic shift occurred in the food web of the Hawaiian petrel, particularly if related to fisheries, the implications could be ecologically and economically far-reaching. Fisheries have been described as a vast uncontrolled experiment with unforetold ecological consequences [1], yet an observation of a trophic-level shift implies changes to trophic structure that can lead to deteriorating ecosystem function [38]. Fishery-related alterations to open ocean food webs are an international issue with implications for food supplies and cultures [39,40]. As human population grows, increased fishery production may spur further trophic-level shifts similar to that observed in our retrospective data. This possibility, coupled with climate change, suggests an uncertain future for the oceanic NPO [41]. Although our millennial-scale data do not decisively implicate fisheries, they offer important evidence of a trophic shift and a rare opportunity to gauge the full magnitude of potential ecological stress by providing a baseline prior to human intervention, a perspective that is critical for predicting the future of marine ecosystems.

Data accessibility. The data for this paper are tabulated in the electronic supplementary material. After publication, a digital archive of our data is available in Dryad: http://dx.doi.org/10.5061/dryad.66jd4 [42].

Authors’ contributions. P.H.O., A.W. and H.F.J. conceived the project and provided gelatin samples and associated radiocarbon data. The manuscript was written primarily by P.H.O. with contribution from A.E.W. and H.F.J. E.Z. and S.R. designed and conducted statistical analysis and assisted with writing. W.W. identified prey and interpreted prey data. Y.C. prepared and analysed amino acid derivatives, provided intellectual context and assisted with writing. P.H.O., A.W., H.F.J. and Y.C. contributed equally to this work.

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References

8. FAO Fisheries. 2013 FAO global capture production database updated to 2013 summary information Fisheries and Aquaculture Department. 1 – 5.


