

Identification and stable isotope analyses of flying fish scales from ornithogenic sediments at three islands in the South China Sea

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ABSTRACT: Flying fish scales were recovered from ornithogenic sediments at 3 coral islands in Qilian Yu, Xisha Islands, South China Sea, and identified based on species-specific morphological structures. Stable isotope analyses indicated insignificant changes in the ratio of ^{13}C to ^{12}C ($\delta^{13}\text{C}$) and the ratio of ^{15}N to ^{14}N ($\delta^{15}\text{N}$) in scales dating from the period ~1000 to 1850 AD, reflecting the stability of avian community structures and feeding habits of tropical seabirds on Qilian Yu where they have consistently preyed on flying fish. Compared with data from modern flying fish samples, we found $\delta^{15}\text{N}$ did not change, while $\delta^{13}\text{C}$ declined by about 1.9‰ from historical times to the present, but this change can be associated with the Suess effect (decrease of atmospheric $\delta^{13}\text{C}$ caused by fossil-fuel burning). A strong negative correlation was found between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the historical flying fish scales, which was attributed to the natural spawning behavior of the fish, i.e. adult fish move to inshore areas to spawn; the weak positive correlation previously observed in modern samples is a result of larger sampling areas and possible human disturbances.

KEY WORDS: $\delta^{13}\text{C}$ · $\delta^{15}\text{N}$ · Spawning behavior · Historical data · Suess effect

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INTRODUCTION

Paleoecological studies of seabirds, including nesting and feeding habits (e.g. Emslie & Patterson 2007, Huang et al. 2013, Tavares et al. 2016, Eerkes-Medrano et al. 2017) are valuable because seabirds often play a key role in the development of ecosystems in remote regions (e.g. Finney et al. 2000, Michelutti et al. 2010, Nie et al. 2012). Dietary characteristics of tropical seabirds, which can transfer nutrients to coral island ecosystems (Allaway & Ashford 1984, Xu et al. 2011), have received consider-

able attention (Erwin & Congdon 2007, Xu et al. 2014, Wu et al. 2017a). Here we focus on the South China Sea, which is located in a tropical region where there are numerous coral islands (Wang 2011). Many tropical seabirds, among which the red-footed booby *Sula sula* is the most important, inhabit the Xisha Islands, South China Sea (Exploration Group of Xisha Islands of Institute of Soil Science of Chinese Academy of Sciences 1977, Cao et al. 2007). Like tropical seabirds living in other regions in the world, they predominantly feed on flying fish (e.g. *Exocoetus volitans* in this study) and squid (e.g. *Loligo chi-*

nensis) from the surrounding waters (Cherel et al. 2008, Young et al. 2010, Xu et al. 2014). Cao (2005) completed a detailed study on the diet of red-footed boobies at Dongdao Island, Xisha Islands, through field observations. Xu et al. (2014) and Wu et al. (2017a) proposed that, in the past millennium, when seabirds were more abundant, they likely fed primarily on squid, in contrast to the situation today where they feed largely on flying fish. However, other than the relative proportion of modern prey, the past diet of tropical seabirds on the Xisha Islands has not been previously investigated, but it is worthy of study since it might reflect potential changes in the community structure of prey or a predator's population size.

Flying fish are recognized as an important food source for many tropical seabirds and can make up a larger part of the diet than alternative taxa such as squid (Villanueva 2000, Sukramongkol et al. 2007). However, flying fish have not been well studied. Flying fish are widely distributed in the South China Sea and are especially abundant around the Xisha Islands. They are important in the diet of not only tropical seabirds, but also epipelagic piscivorous fish (Wang 2011). Although we can collect fresh flying fish samples for analysis, we cannot obtain muscle samples from the fossil record due to the lack of preservation; hence, alternative materials are necessary. Fortunately, a large number of fish scales (residue buried after seabird feedings) were recovered from coral-sand ornithogenic sediments at the Xisha Islands (Xu et al. 2011, 2016). Prey are taken by adult birds from the ocean surface and brought to colonial tree and shrub nesting sites on the islands to consume or feed to their young (Cao 2005). The remains of flying fish, including scales buried in the ornithogenic sediments below nesting sites, provide an opportunity to investigate the paleoecology of flying fish and historical feeding habits of tropical seabirds on these islands.

Stable isotope analysis is a useful tool for conducting ecological and food-web research (Martinez et al. 2014, da Silva et al. 2016). The ratio of ^{15}N to ^{14}N ($\delta^{15}\text{N}$) is typically used to provide information on consumer trophic level (DeNiro & Epstein 1981, Vander Zanden et al. 1997, Post 2002) or the size of organisms (e.g. Olsson et al. 2000), and the ratio of ^{13}C to ^{12}C ($\delta^{13}\text{C}$) can be used to infer habitat use and source of primary production (DeNiro & Epstein 1978, Cherel & Hobson 2007, Ronconi et al. 2014). In a previous study, we affirmed that flying fish scales act as an alternative to muscle samples, as in many other studies (e.g. Pruell et al. 2003, Kelly et al. 2006,

Roussel et al. 2014), and attributed variation in $\delta^{15}\text{N}$ to changing fish mass with age, while $\delta^{13}\text{C}$ varies with geographic location (Wu et al. 2017b). These results indicate that historical flying fish scales can provide valuable information about past ecosystems.

Before conducting stable isotope analyses of the fish scales, it is important to verify that identification to species is possible. We compared historical and modern scales to validate this study and found that additional information on fish size and geographic distributions of flying fish also can be investigated with these data.

MATERIALS AND METHODS

Sampling

The South China Sea ($3^{\circ}00' - 23^{\circ}37' \text{ N}$, $99^{\circ}10' - 122^{\circ}10' \text{ E}$), predominantly located in the tropics, is the third-largest marginal sea in the world, and is mainly surrounded by mainland, islands, and peninsulas. The Xisha Islands ($15^{\circ}47' - 17^{\circ}08' \text{ N}$, $110^{\circ}10' - 112^{\circ}55' \text{ E}$), most of which are coral islands, are situated in the northwest South China Sea (Fig. 1A), and have a tropical marine climate with year-round high temperatures and heavy rains. These natural conditions have allowed the Xisha Islands to develop a unique landscape. Trees (*Pisonia grandis* and *Guettarda speciosa*), bordered by shrubs (*Scaevola sericea*), cover the center area of some islands, providing habitat for more than 60 species of birds in historical times (Exploration Group of Xisha Islands of Institute of Soil Science of Chinese Academy of Sciences 1977, Cao et al. 2007).

The sampling methods used in this study are described in our earlier study (Xu et al. 2016). Profiles ZS2 ($16^{\circ}58'39.9'' \text{ N}$, $112^{\circ}16'15.5'' \text{ E}$), BD1 ($16^{\circ}57'45.0'' \text{ N}$, $112^{\circ}18'38.5'' \text{ E}$), and ND1 ($16^{\circ}56'47.7'' \text{ N}$, $112^{\circ}20'3.9'' \text{ E}$) were sampled at Zhaoshu, Beidao, and Nandao islands, Qilian Yu Islands (Fig. 1B), in 2015, respectively (Fig. 1C), and were all located beneath thick shrubs of *S. sericea*. PVC plastic pipes (11 cm diameter) were inserted into the soft substrate and the sediments were then excavated around the pipes to retrieve sample cores. At the same time, a coarse fraction of sediment sample from an adjacent duplicate pit (about $1 \times 1 \text{ m}$ square) was separated at intervals of 1 or 2 cm using a 10-mesh stainless steel sieve *in situ* to obtain sufficient sub-fossils, including bird and fish bones, bird guano, eggshells, and fish scales for analysis. Fish scales were then sorted from the coarse fraction of

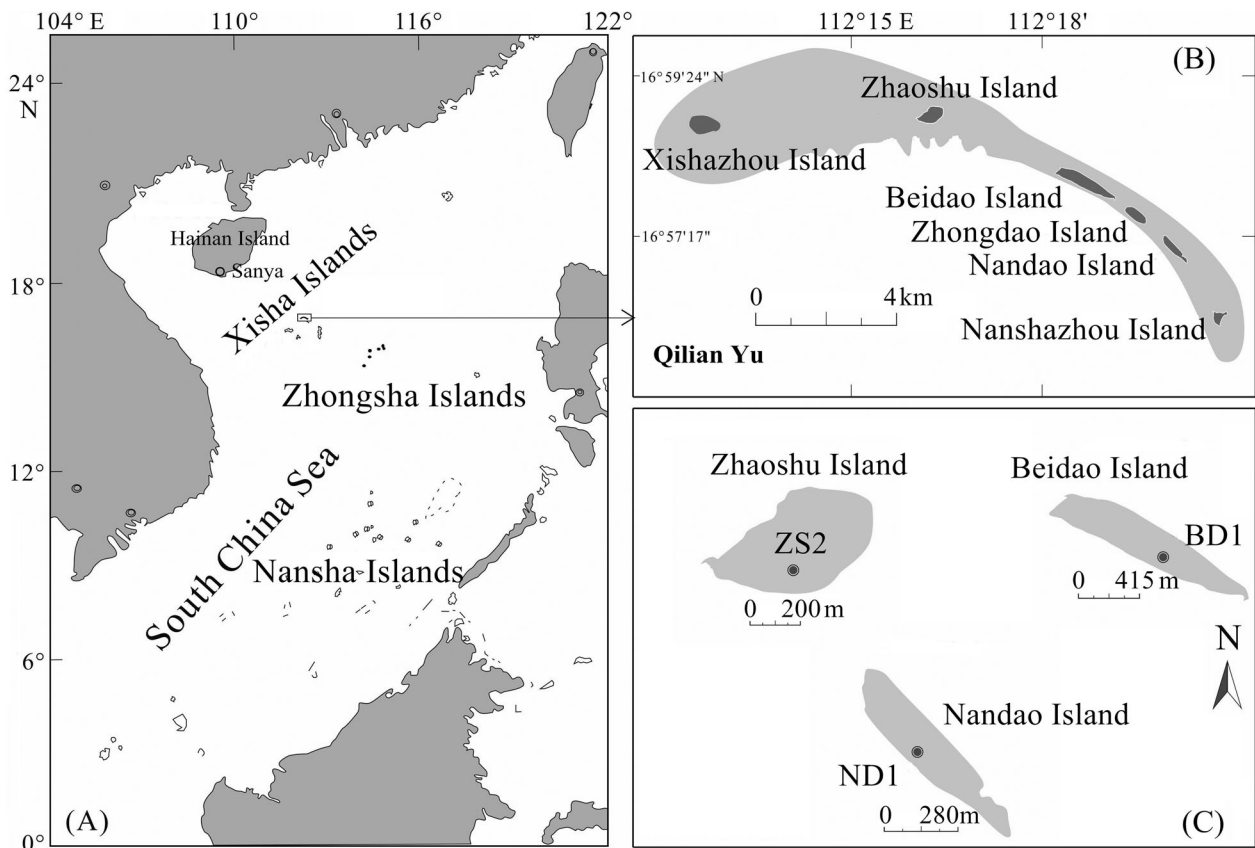


Fig. 1. (A) Location of study area in the South China Sea. (B) Qilian Yu Islands. Profiles ZS2, BD1, and ND1 were collected from Zhaoshu, Beidao, and Nandao islands, respectively. The grey area indicates submerged reefs. (C) Sampling sites on the 3 islands

sediment samples in the laboratory. Chronology of the profiles was determined by ^{210}Pb and AMS (accelerator mass spectrometry) ^{14}C dating, and the results were reported in detail by Xu et al. (2016). For comparative studies and to verify identifications to species, a total of 82 samples of modern flying fish of different masses were collected from the South China Sea (9° – 19°N , 111° – 115°E) near the islands in this study, and the results of stable isotope analyses were reported in our previous study (Wu et al. 2017b).

Species identification

A typical teleost scale comprises 2 portions: a hard upper, well-mineralized layer (the external layer) composed of calcium phosphate, similar to the mineral apatite, overlying a poorly mineralized layer, known as the basal or fibrillar plate, which is composed largely of collagen (Hutchinson & Trueman 2006). Scales of bony fish have consistent morpholog-

ical structures that are species specific, and can also be used to age individual fish (Poulet et al. 2005, Huang et al. 2015). Flying fish have never been identified using this method, but comparative analysis revealed that scale focus is apparent in the tail scale of flying fish (Fig. 2). Uniformly distributed around the scale focus, circuli are distinct, varying from fine to thick in the lateral field. There are 2–8 radii in the anterior field, radiating from the focus to the margin of the anterior field, with no radii in the lateral or posterior field. Only one or no minor radii appear between 2 major radii. The partition of fields is not obvious. Based on these features, more than 300 flying fish scales were recovered and identified from the 3 profiles. AMS ^{14}C dating of both bird and fish bones (Xu et al. 2016) indicated that they were almost all deposited after 1000 AD and before 1950 AD, with few human disturbances. Here, 103 scales (35 from BD1, 37 from ND1, and 31 from ZS2; 94 before 1850 AD) with different ages and depths in the sediments were selected for stable isotope analysis and are hereafter referred to as historical scales.

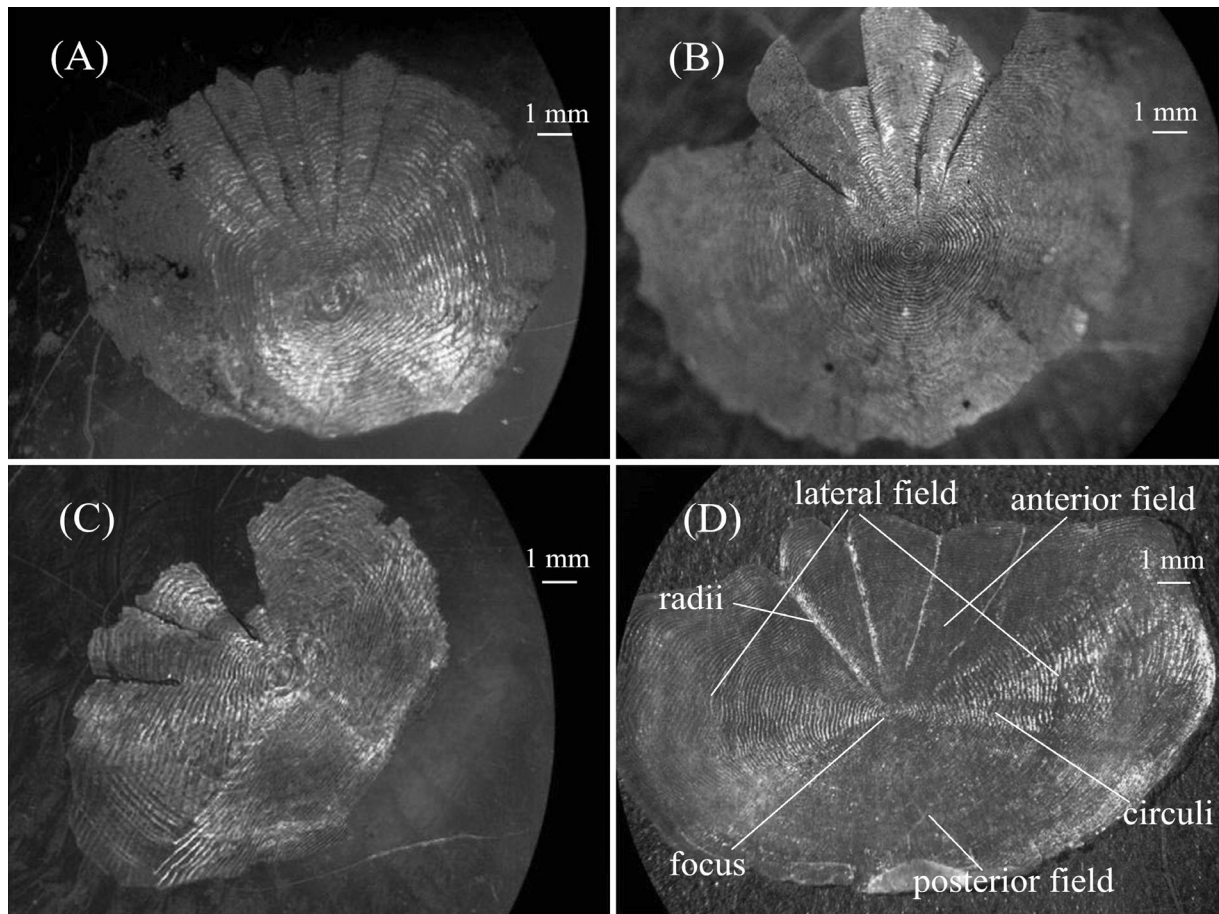


Fig. 2. Scales of (A,B) historical flying fish, (C) historical sample but not flying fish, and (D) modern flying fish, as viewed with an optical microscope

Stable isotope analysis

The pretreatment of historical fish scale samples followed the methods of Estep & Vigg (1985) and Sinatamby et al. (2007). They were all treated with 1.2 N HCl for 2 min to remove carbonate before being air dried. Well-treated samples and standards were fully combusted at 1000°C using a FLASH 2000 HT Elemental Analyzer (Thermo Fisher), the carbon and nitrogen contents were measured, and then the gases were separated by a 'purge and trap' adsorption column and sent to an isotope ratio mass spectrometry (IRMS MAT 253) for isotope analysis. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰): $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R refers to the ratio $^{13}\text{C}/^{12}\text{C}$ and the R_{standard} value is based on Vienna PeeDee Belemnite (VPDB); $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R represents the ratio $^{15}\text{N}/^{14}\text{N}$ and the R_{standard} value is based on atmospheric nitrogen

(N_2 -atm). Analytical precision (the standard deviation) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was less than $\pm 0.1\text{‰}$ and $\pm 0.2\text{‰}$, respectively. Stable isotope analyses were determined at the State Key Laboratory of Atmospheric Boundary Layer Physics and Atmospheric Chemistry (LAPC), Institute of Atmospheric Physics, Chinese Academy of Sciences (Beijing, China). Both the pretreatment and isotopic test methods (Tieszen et al. 1983) were followed for the stable isotope analysis of modern flying fish scale samples (Wu et al. 2017b).

RESULTS

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes in flying fish scales recovered from the 3 profiles BD1, ND1, and ZS2 versus their buried ages (year of burial) are shown in Fig. 3, with related statistics in Tables 1 & 2. We analyzed data from each profile and for different ages separately, but also pooled data from all 3 profiles for

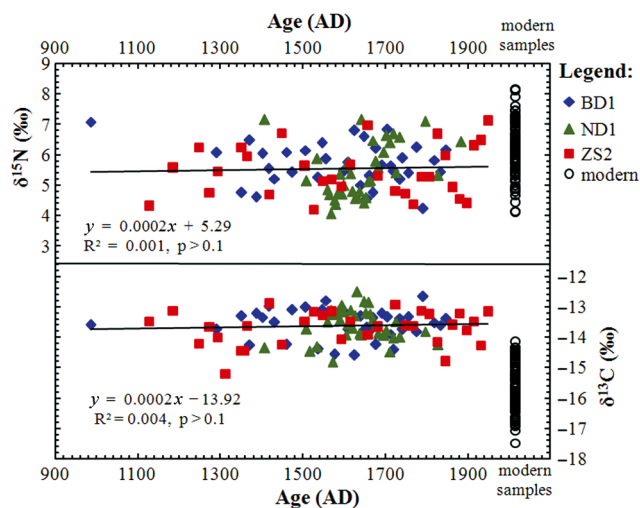


Fig. 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in historical flying fish scales versus corresponding buried ages for profiles BD1, ND1, and ZS2. The results of modern samples are from Wu et al. (2017b). The black lines are the results of linear fitting for historical samples over time

historical samples. For comparison and further analysis, we collected published data on modern flying fish from the South China Sea (Wu et al. 2017b). C/N ratios of samples are also presented in Table 1 with those of modern samples from Wu et al. (2017a). For $\delta^{15}\text{N}$, the small discrepancy between the 2 averages for historical ($5.6 \pm 0.8\text{‰}$, mean \pm SD, $n = 103$) and modern ($6.3 \pm 0.8\text{‰}$, $n = 82$) samples is likely due to a higher mass in modern fish (for $\delta^{15}\text{N}$ in historical and modern samples, 1-way ANOVA, $F_{1,183} = 31.66$, $p < 0.001$; for $\delta^{15}\text{N}$ in historical samples from different

time bins before 1850 AD, 1-way ANOVA, $F_{6,87} = 1.65$, $p = 0.144$). However, $\delta^{13}\text{C}$ in the historical scales ranged from -15.2‰ to -12.5‰ , with an average of $-13.7 \pm 0.6\text{‰}$ ($n = 103$), and obviously differed from modern fish scales $\delta^{13}\text{C}$ (-17.5‰ to -14.2‰ , with an average of $-15.6 \pm 0.8\text{‰}$); for $\delta^{13}\text{C}$ in historical and modern samples, 1-way ANOVA, $F_{1,183} = 454.56$, $p < 0.001$; for $\delta^{13}\text{C}$ in historical samples from different time bins before 1850 AD, 1-way ANOVA, $F_{6,87} = 1.05$, $p = 0.399$), although there was no difference in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ among the samples of BD1, ND1, and ZS2 (for $\delta^{13}\text{C}$, 1-way ANOVA, $F_{2,100} = 0.60$, $p = 0.551$; for $\delta^{15}\text{N}$, 1-way ANOVA, $F_{2,100} = 1.42$, $p = 0.246$).

For historical scale samples from the 3 profiles, the C/N ratios were similar (Table 1) with a mean of 2.90 ± 0.10 ($n = 103$), close to that of modern scale samples 2.87 ± 0.05 ($n = 25$; Table 1, 1-way ANOVA, $F_{3,124} = 0.53$, $p = 0.660$). These results indicate that the historical scales are well preserved and diagenetic processes in the soil did not affect the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

DISCUSSION

Isotope characteristics of historical flying fish scales

Our previous study showed that flying fish scale $\delta^{15}\text{N}$ co-varies with individual size, while $\delta^{13}\text{C}$ reflects the marine habitat, such as shallow neritic versus off-shore pelagic (Wu et al. 2017b). As Beidao, Nandao,

Table 1. Statistics of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and C/N in flying fish scales. Note: the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in modern samples are from Wu et al. (2017b), and the C/N values of modern samples are from Wu et al. (2017a)

	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)			C/N
	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD	
BD1	-14.6	-12.7	-13.6 ± 0.5 ($n = 35$)	4.2	7.1	5.7 ± 0.7 ($n = 35$)	2.92 ± 0.09 ($n = 35$)
ND1	-14.8	-12.5	-13.6 ± 0.5 ($n = 37$)	4.1	7.2	5.4 ± 0.9 ($n = 37$)	2.90 ± 0.12 ($n = 37$)
ZS2	-15.2	-12.9	-13.7 ± 0.5 ($n = 31$)	4.2	7.1	5.4 ± 0.8 ($n = 31$)	2.90 ± 0.10 ($n = 31$)
Historical samples	-15.2	-12.5	-13.7 ± 0.6 ($n = 103$)	4.1	7.2	5.6 ± 0.8 ($n = 103$)	2.90 ± 0.10 ($n = 103$)
Modern samples	-17.5	-14.2	-15.6 ± 0.8 ($n = 82$)	3.8	9.2	6.3 ± 0.8 ($n = 82$)	2.87 ± 0.05 ($n = 25$)

Table 2. Statistical results (‰, mean \pm SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the historical (before 1850 AD) flying fish scale samples in different time bins

	900–1300	1300–1400	1400–1500	1500–1600	1600–1700	1700–1800	1800–1850
$\delta^{13}\text{C}$	-13.7 ± 0.3 ($n = 7$)	-13.8 ± 0.6 ($n = 5$)	-13.6 ± 0.6 ($n = 8$)	-13.4 ± 0.4 ($n = 21$)	-13.6 ± 0.4 ($n = 28$)	-13.6 ± 0.5 ($n = 18$)	-13.7 ± 0.4 ($n = 7$)
$\delta^{15}\text{N}$	5.6 ± 0.9 ($n = 7$)	5.6 ± 0.9 ($n = 5$)	5.9 ± 0.8 ($n = 8$)	5.1 ± 0.6 ($n = 21$)	5.5 ± 0.8 ($n = 28$)	5.7 ± 0.9 ($n = 18$)	5.8 ± 0.5 ($n = 7$)

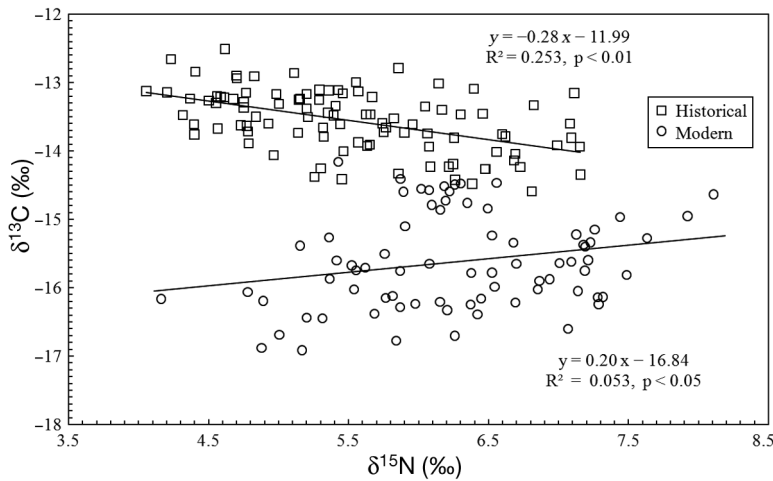


Fig. 4. Relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in historical (before 1850 AD) and modern flying fish scales. The results for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in modern samples are from Wu et al. (2017b)

and Zhaoshu islands are representative of Qilian Yu and contribute the majority of the vegetation cover there, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in historical flying fish scales showed little change with age in profiles BD1, ND1, and ZS2 (Fig. 3, Table 2). Furthermore, ANOVA indicated that there was no statistical difference in either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ for the historical fish scale samples among the different time bins prior to 1850 AD (Table 2). This result indicates that the size and geographical distribution of flying fish have not varied over time, which is in agreement with a previous study on Guangjin Island, also in the Xisha Islands (Wu et al. 2017a). It is likely that the diversity of tropical seabird species there also had no major changes as different tropical seabird species might differ slightly in feeding preferences (Diamond 1978), although seabird population size in Qilian Yu has evidently fluctuated over the past 1000 yr (Xu et al. 2016). An additional study showed that tropical seabirds may change their diet and prey to include more squid than flying fish when there is a larger seabird population size (Wu et al. 2017a). Compared with the size of certain food sources (mostly flying fish), tropical seabirds tend to alter their diet composition when their population size changes, an important strategy for adapting to major ecological and environment changes.

Differences between historical and modern isotope values

There was no evident difference in $\delta^{15}\text{N}$ among the historical scale samples from BD1, ND1, and ZS2

(1-way ANOVA, $F_{2,100} = 1.42$, $p = 0.246$, Fig. 3, Table 1), indicating no evident changes in seabird ecology for the 3 islands during the past 1000 yr. $\delta^{15}\text{N}$ in the historical scales ranged from 4.1‰ to 7.2‰, nearly equal to the range of modern fish scales except for several individuals with a higher mass and higher $\delta^{15}\text{N}$ (Wu et al. 2017b). According to a field survey (Cao 2005), 71% of flying fish that are prey of seabirds are less than 100 g, but only 54% of our modern samples are in this category. Thus, it is likely that the historical flying fish had a relatively lower mass than our modern samples. There is no difference between historical and modern fish scale $\delta^{15}\text{N}$ even after the effect of fish mass is eliminated, as also found in the sea around Guangjin Island (Wu et al. 2017a).

The difference between $\delta^{13}\text{C}$ in modern and historical fish scales is $\Delta\delta^{13}\text{C} = -1.9\text{‰}$, nearly equal to the local atmospheric ^{13}C Suess effect of -1.8‰ (in 2014), caused by fossil fuel combustion and carbon emissions with fewer ^{13}C isotopes, and -1.8‰ is calculated using a model from Schelske & Hodell (1995) and Jia et al. (2013). Thus, we believe the atmospheric ^{13}C Suess effect is reflected in our historical and modern flying fish scale samples.

Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in historical scales

When we examined the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in historical flying fish scales before 1850 AD, we found a strong negative correlation, i.e. $\delta^{13}\text{C}_{\text{scales}} (\text{‰}) = -0.28 \times \delta^{15}\text{N}_{\text{scales}} (\text{‰}) - 11.99$ ($R^2 = 0.253$, $n = 94$, $p < 0.01$). This result is quite different from that of modern flying fish, which have a weak positive relationship: $\delta^{13}\text{C}_{\text{scales}} (\text{‰}) = 0.20 \times \delta^{15}\text{N}_{\text{scales}} (\text{‰}) - 16.84$ ($R^2 = 0.053$, $n = 82$, $p < 0.05$; Wu et al. 2017b) (Fig. 4). Comparing the 2 groups, there are 2 differences between modern and historical scales, i.e. historical flying fish were all derived from waters close to Qilian Yu, as scales were retrieved from the 3 coral-sand ornithogenic sediments collected in Beidao, Nandao, and Zhaoshu islands, while modern flying fish samples were collected across a greater geographical range—the Xisha Sea around Yongxing Island, Southern Hainan Sea, and within the western South China Sea. Additionally, flying fish from the historical record were all captured by seabirds living at the 3 islands, but some modern flying

fish were likely not potential prey for modern seabirds due to the long distance from Qilian Yu.

Previous research may help us understand the discrepancy between past and present stable isotope values. Xu et al. (2014) reported a significant negative correlation between fossil collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of tropical seabirds on Ganquan Island, and attributed this to variation in trophic levels and foraging areas. It is known that some migratory birds exhibit feather isotope values, indicating exploitation of both marine and freshwater ecosystems (Kline et al. 1998, Lott et al. 2003). As shown by Wu et al. (2017b), we associate variations in $\delta^{15}\text{N}$ with changes in fish mass with size and variations in $\delta^{13}\text{C}$ with geographical location. In this case, we assume that the strong negative relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in historical scales is related to the spawning habitat of flying fish, in that adult fish schools move to inshore areas and spawn on seaweed and suspended matter, then forage separately and return to open waters (Zhang 1956). Also, as juvenile fish gradually move to open waters as they age, $\delta^{15}\text{N}$ of fish scales will evidently increase during this time (Wu et al. 2017b), and $\delta^{13}\text{C}$ of fish scales will increasingly reflect a more pelagic habitat based more on offshore plankton and less on inshore plankton. In addition, $\delta^{13}\text{C}$ of eupelagic plankton is typically much lower than that of coastal species (Hobson 1999, Kaehler et al. 2000), so $\delta^{13}\text{C}$ of fish will decrease as they age and move to more open waters. Thus, as flying fish age and move from Qilian Yu coastal waters to the outer open sea, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values will decrease and increase in their scales, respectively, as indicated by those recovered from the coral-sand ornithogenic sediments collected at Qilian Yu Islands. However, more research is needed to support this observation, including stable isotope analysis of plankton collected near the islands.

Qilian Yu, the Yongle Islands, and Yongxing Island have all been inhabited historically by seabirds (Exploration Group of Xisha Islands of Institute of Soil Science of Chinese Academy of Sciences 1977, Cao et al. 2007). Dongdao Island is located ca. 50 km southeastward of Qilian Yu, and is still occupied by thousands of tropical seabirds, especially red-footed boobies (Cao 2005). Weimerskirch et al. (2005) reported that red-footed boobies breeding on Europa Island in the Mozambique Channel have a maximum foraging range of ca. 150 km. We assume there is no great difference in foraging range of seabirds breeding on Europa Island and Xisha Islands as well (Cao 2005). It is inferred that seabirds on Qilian Yu mainly prey on food derived from its northern, northwestern, and northeastern ocean areas, where there are no

islands within several hundreds of kilometers and thus less competition with other seabird colonies. Seabirds preferentially prey on flying fish coastally and with appropriate mass and size, but they must prey on fish with larger weight and high $\delta^{15}\text{N}$ and low $\delta^{13}\text{C}$ in the open waters since inshore food sources are not sufficient for them. The likelihood of whether individual birds capture small inshore flying fish with low $\delta^{15}\text{N}$ and high $\delta^{13}\text{C}$ or offshore ones with high $\delta^{15}\text{N}$ and low $\delta^{13}\text{C}$ depends on the age of the individual.

As for modern flying fish, they were collected from larger regions, including the Xisha Sea, Southern Hainan Sea, and within the western South China Sea. A negative correlation between fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ reflects a pristine state because of similar reproductive behaviors of these flying fish, but it must be in a small region with consistent background values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Flying fish in the South China Sea likely have many different localized spawning habitats resulting in different background values for $\delta^{13}\text{C}$ (for example, latitude effect, Goericke & Fry 1994) and $\delta^{15}\text{N}$ values (Somes et al. 2010). Any negative correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ will be weakened if flying fish are collected from different spawning habitats. However, human disturbances may help disrupt this pattern to some extent and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ could change under human interference. The ^{13}C Suess effect has decreased $\delta^{13}\text{C}$ in atmosphere carbon dioxide and in marine organisms (for Qilian Yu, it is -1.9‰), but the amount varies in different regions (Sonnerup et al. 1999, Swart et al. 2010). Tens of millions of tons of chemical fertilizers are produced and discharged into the environment every year in the world, probably altering natural stable isotope composition as they have different $\delta^{15}\text{N}$ values (Peterson & Fry 1987). Although our results here show that the sea area around Qilian Yu has been little impacted by pollution, the entire South China Sea has. Such human impacts may also have an effect on flying fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, but this requires additional investigation.

CONCLUSION

Size and geographical distributions of flying fish depredated by tropical seabirds from Qilian Yu exhibited no obvious changes over historical time. There was no difference between historical and modern flying fish $\delta^{15}\text{N}$, but $\delta^{13}\text{C}$ declined 1.9‰ from the past to present, likely reflecting the Suess effect. A strong negative correlation between historical flying

fish scale $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ is attributed here to their spawning behavior, probably resulting from adult fish schooling in inshore areas where they also spawn and juvenile fish moving to outer open waters as they age, accompanied by a decrease in $\delta^{13}\text{C}$ and increase in $\delta^{15}\text{N}$. Seabirds inhabiting the islands can prey on inshore young fish with high $\delta^{13}\text{C}$ and low $\delta^{15}\text{N}$, or relatively large fish far from the islands with low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$. The weak positive correlation for modern flying fish scales implies that flying fish in the South China Sea have many different spawning habitats and the sea around Qilian Yu is just one of them. The increase in modern human disturbances in the South China Sea may contribute to these fluctuations in flying fish ecology.

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