Lessons from the past: isotopes of an endangered rail as indicators of underlying change to tidal marsh habitats

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ABSTRACT

Introduction: Tidal marsh systems along the Pacific coast of the United States have experienced substantial stress and loss of area and ecosystem function, which we examined by using the endangered California Ridgway’s Rail, Rallus obsoletus obsoletus (‘rail’) as an indicator of its tidal marsh habitat in the San Francisco Estuary. We organized a collection of historical (1885-1940) and modern (2005-2014) rail feathers and analyzed the feather isotope means for delta carbon (δ13C), sulfur (δ34S), and nitrogen (δ15N) by region and time period.

Outcomes: Feather isotopes represented the primary foraging habitat during historical then modern time periods. Neither individual nor regional rail feather isotopes suggested freshwater or terrestrial foraging by the rail. Three regions with both historic and modern feather isotopes revealed non-uniform spatial shifts in isotope levels consistent with a marine based food web and significant δ15N enrichment.

Discussion: Our results supported the rail’s status as a generalist forager and obligate tidal marsh species throughout the historic record. The variable isoscape trends generated from feather isotope means illustrated a modern loss of the isotopic homogeneity between regions of historical tidal marsh, which correlated with spatially-explicit habitat alterations such as increasing biological invasions and sewage effluent over time.

Conclusion: These findings have reinforced the importance of tidal marsh conservation in the face of ongoing underlying changes to these important ecosystems.

Introduction

The world’s wetlands have experienced substantial anthropogenic stress leading to significant habitat loss, estimated at 90% in recorded human history, approximately 50% of which occurred since 1900 (Zedler and Kercher 2005; Davidson 2014). Modern wetlands exhibit various degrees of degradation, especially tidal saltmarshes, a type of modern wetland covering 2% of the world’s land area. Tidal saltmarshes (or simply tidal marshes) have a documented loss approaching 95% around the world (McGranahan, Balk, and Anderson 2007). This habitat loss relates to coastal-centric anthropogenic impacts, one of the most recent and concerning being sea-level rise. Because of the critical ecosystem services provided by historically contiguous tidal saltmarshes, many coastal land management plans aim to restore or enhance contemporary tidal marshes (Zedler 2004; Zedler and Kercher 2005). However, scarce baselines exist for biotic or abiotic conditions of tidal marshes prior to their centuries of land loss, fragmentation, and degradation, despite the west coast tidal marshes having substantial historical records, specimens, and other data (Nichols et al. 1986; Garcia 1995; SFEI 1998; Takekawa et al. 2006). A deeper understanding of functioning tidal marsh, which historical baselines would provide, is needed to guide global conservation and restoration efforts before these threatened ecological communities reach limits in adaptability (Zedler 2004; Zedler and Kercher 2005; Takekawa et al. 2006).

The largest estuary on the Pacific coast of North America, the San Francisco Estuary (“Estuary,” hereafter), has a history of tidal marsh contraction across its landscape, resulting in large ecological changes. The San Francisco Estuary Institute (SFEI) has mapped and otherwise documented the extreme fragmentation of the tidal marsh, detailing a major habitat shift (Figure 1; SFEI 1998). The shift occurred in conjunction with over a century of increasing urbanization (Clarke, Hoppen, and Gaydos 1997). Settlement and expansion of the city...
of San Francisco began following the Gold Rush era in the 1850s and, by the early 1900s, tidal marsh reduction began (Takekawa et al. 2006). Modern restored tidal marshes (white) in which rails no longer exist are shown. The historical region nomenclature represents aggregates of historical documentation on rail specimen locations: NE (Northeast), E (East), SE (Southeast), S (South), SW (Southwest), and NW (Northwest). These historical regions include the modern tidal marsh areas, with some exception given modern sediment accumulation and invasive Spartina tidal marsh creation (McBroom 2012). The modern regions are fragments of tidal marsh associated with eight modern sampling sites (black), N (North, Petaluma), E (East, Arrowhead), SE (Southeast, Cogswell), S (South, Laumeister and Faber), W (West, Colma), and NW (Northwest, Gallinas Creek and Corte Madera). Not all modern tidal marsh with rails (gray) could be sampled.

Modern Estuary literature illustrates the geographic habitat diversity between tidal marsh fragments, presenting a contemporary patchwork of biotic habitat features (Cloern, Canuel, and Harris 2002), the most significant being vegetation zonation. Vegetation zonation commonly refers to the...
dominant primary production of a habitat’s food web, which for our purposes includes emergent vegetation or planktonic species. Primary producers utilize a carbon fixation pathway typically referred to as C3 or C4, and the underlying biochemical composition of food webs, the baseline nutrient inputs, depends first on the primary carbon fixation (Fry 2008). For this research, the biochemical composition of a tidal marsh is best defined by isotope values of elements such as carbon. For instance, in marine and tidal marsh food webs, depleted carbon isotopes relate to freshwater and/or pelagic nutrient sources, which typically link to C3 fixation; whereas enriched carbon isotopes relate to the littoral/tidal marsh systems, which more typically associate with C4 fixation (Fry 2002; Fry 2008). The contemporary Estuary has substantial documentation regarding vegetation zones of emergent plants and littoral or pelagic algae (Fry 2002; Cloern, Canuel, and Harris 2002; Moffett and Gorelick 2016). Monocot, C4, cordgrass species tend to be the most typical vegetation zonation of the Estuary and worldwide tidal marsh habitats (Fry 2008), but the Estuary has several tidal marshes with a dicot, C3, pickleweed species (Woo and Takekawa 2012). In addition, planktonic species (generally C3 phytoplankton) might overwhelm a food web, regardless of the emergent vegetation. To the contrary, invasive emergent vegetation might disrupt algal growth (Levin, Neira, and Grosholz 2006). In all, the Estuary’s biotic/isotopic patchwork is further altered and defined by a variety of invasive flora and fauna (Canuel et al. 1995; Cohen and Carlton 1998; Cloern, Canuel, and Harris 2002; Ayres et al. 2004). Moreover, the native and/or invasive vegetation zones of the Estuary link to several habitat features, as explored in the following paragraph and summarized in the list of tidal marsh regions of Table 1a.

Several abiotic factors that we refer to as geomorphology accompany and enhance the Estuary’s biotic “mosaic” (Cloern, Canuel, and Harris 2002). For our purposes, geomorphology signifies the Estuary’s two major watersheds, the Sacramento-San Joaquin and the Santa Clara (Ingram, Ingle, and Conrad 1996), each with variable freshwater inflows (Table 1a). Geomorphology also denotes to the elevations and channel structures, tides and currents, and sedimentation rates of tidal marshes, as summarized in Table 1a. We focused on the geomorphology attributes most likely to affect the carbon, sulfur, and nitrogen baselines of tidal marsh food webs. For instance, carbon and nitrogen values relate to nutrient sources such as watershed inflows, and sulfur values relate to the degree of nutrient cycling and sedimentation associated with variable tides and currents (Fry 2002; Fry 2008). Given that anthropogenic effects often disrupt systematic geomorphology, shifts in the carbon, sulfur, and/or nitrogen relate human impacts as well (Table 1a). Some of the more significant examples of such impacts include salt pan restoration, invasive species eradication, and sea-level rise (Takekawa et al. 2006; US Fish and Wildlife Service 2013).

The invasion of non-native cordgrass throughout the Estuary carries significance in the biotic and abiotic patchwork that is the modern Estuary. The distribution of California cordgrass (Spartina foliosa; hereafter, native Spartina) has decreased since the 1980s with the introduction of exotic smooth cordgrass (Spartina alterniflora). The exotic subsequently hybridized with native Spartina and resulted in invasive hybrid Spartina (S. alterniflora x S. foliosa; hereafter, hybrid Spartina) (Callaway and Josselyn 1992;
Ayres et al. 1999; Faber 2000). Invasive Spartina took over several tidal marsh regions, and researchers suspect that the invasive shifted habitat structures and ecological regimes among the fragmented regions (Ayres et al. 2004; Levin, Neira, and Grosholz 2006; Brusati and Grosholz 2009). In addition, invasive species removal or native plant and sediment transport – in the instance of hybrid Spartina control – has probably heightened modern differences (obviously vegetation differences but less obviously geomorphological differences) between tidal marsh fragments (US Fish and Wildlife Service 2013; McBroom 2012).

Based on the structure of food webs, the carbon, sulfur, and nitrogen isotopes analyzed from the tissues of plants and animals in a tidal marsh food web should reflect the biotic and abiotic mosaic of the Estuary (Cloern, Canuel, and Harris 2002; Levin, Neira, and Grosholz 2006; Brusati and Grosholz 2009). For the purpose of our study, we define Estuary isoscapes as regional isotope means analyzed from the tissue of a species belonging to a tidal marsh obligate species, and thus representative of water, soil, and vegetation sources (nutrients) cycling through community food webs (Bowen 2010). As we briefly explained with vegetation zonation, carbon isotopes analyzed from a species belonging to a tidal marsh food web will differentiate between freshwater and tidal marsh nutrient sources, as well as between tidal marsh and marine food webs. A delta carbon notation ($\delta^{13}C$) in "parts per thousand (‰)" incorporates ratios of "heavy" $^{13}C$ to "light" $^{12}C$. $\delta^{13}C$ will increase when food webs are based on cordgrass (C3) tidal marsh. Specifically, the flora and fauna of tidal marsh food webs approach a maximum carbon value of −12‰ whether on a landscape gradient from marine to tidal marsh or freshwater (also terrestrial) to tidal marsh. In other words, the $^{13}C$ of food webs will deplete diversity inputs from upland (freshwater/terrestrial) or pelagic (marine) sources are cycled into a food web, which should be evident in an isoscapes. For upland and less saline food webs – the more brackish and terrestrial systems – the average $\delta^{13}C$ (hereafter, $^{13}C$) for any trophic sample approaches −25‰, possibly −45‰ (in freshwater lakes). For the deeper littoral, more marine food webs, the average $^{13}C$ for any trophic sample will typically deplete and surpass −30‰, which is driven by several species of algae and sometimes local salinity levels (Canuel et al. 1995; Chmura and Aharon 1995; Fry 2008). A $^{13}C$ isoscapes have an amount of overlap and confusion in its ability to differentiate between wetland food webs or diet sources ([Marine/Tidal] and [Freshwater/Terrestrial] Table 1b; Fry 2008). Therefore, we created two other isoscapes, in conjunction with $^{13}C$, to best determine the origins (foraging ground) of a plant or animal diet (Fry 2002; 2008).

Our isoscapes of nitrogen and sulfur differ from that of carbon in their range of values associated with either marine, tidal, or freshwater/terrestrial food webs. As shown in Table 1b, sulfur and nitrogen isotopes use the same general delta notation, and so we abbreviate these isotopes and isoscapes as $^{34}S$ and $^{15}N$. When the $^{13}C$ from plant or animal samples provides no clear indication of foraging in tidal marsh food webs (i.e., tidal marsh diet close to −12‰ $^{13}C$), $^{34}S$ might then be analyzed. Based on a multifaceted process involving precipitation, sedimentation, and sulfate-reducing bacteria, $^{34}S$ tends to accumulate positive values in both tidal marsh and pelagic systems; often creating unique values well above +6‰ $^{34}S$ throughout tidal marsh and marine food webs ([Marine/Tidal] and [Freshwater/Terrestrial] Table 1b; Fry 2008). To summarize, if a $^{13}C$ value falls in the overlap between freshwater and marine tidal marsh values (e.g., ≥ −20‰; Fry 2008), a clearly enriched $^{34}S$ (e.g., 10–15‰ $^{34}S$) will persist in

| Regional isotope summaries | $\delta^{13}C$(‰) | $\delta^{34}S$(‰) | $\delta^{15}N$(‰) | N |
|----------------------------|-----------------|-----------------|-----------------|---|
| **Modern regions**         |                 |                 |                 |   |
| North                      | −17.6 ± 1.7*    | 15.4 ± 1.6      | 18.2 ± 1.5      | 2 |
| Northwest                  | −18.3 ± 0.6*    | 16.1 ± 0.7      | 16.7 ± 0.3      | 26|
| East                       | −16.0 ± 0.3     | 12.5 ± 0.5      | 17.2 ± 0.4      | 27|
| West                       | −14.6 ± 0.6     | 10.5 ± 0.8*     | 17.0 ± 0.5      | 20|
| Southeast                  | −14.5 ± 1.0     | 16.0 ± 0.9      | 19.5 ± 0.6      | 12|
| South                      | −17.4 ± 0.6     | 15.9 ± 0.9      | 21.0 ± 0.4      | 18|
| Modern total               | −16.4 ± 0.4     | 14.0 ± 0.5      | 18.1 ± 0.4      | 105|
| **Historical regions**     |                 |                 |                 |   |
| Northeast                  | −14.7 ± 1.3     | 14.8 ± 1.1      | 14.0 ± 1.0*     | 10|
| Northwest                  | −10.8           | 13.0            | 14.0*           | 1 |
| East                       | −14.4 ± 0.5     | 13.2 ± 0.6      | 14.7 ± 0.6*     | 29|
| Southeast                  | −15.4 ± 1.3     | 13.5 ± 1.5      | 14.3 ± 0.4*     | 6 |
| Southwest                  | −15.2 ± 0.7     | 14.1 ± 0.8      | 14.6 ± 0.7*     | 22|
| South                      | −15.6 ± 0.5     | 13.8 ± 0.6      | 13.9 ± 0.6*     | 34|
| Historical total           | −15.1 ± 0.3     | 13.8 ± 0.3      | 14.3 ± 0.3*     | 102|

*We marked the regional isotopes closest to a very broad terrestrial or freshwater indicator range (Fry 2002; Fry 2008). Depleted $^{15}N$ might indicate higher access to terrestrial or freshwater foraging inputs or sources within a region (Canuel et al. 1995; Fry 2008; Kwak and Zedler 1997).
supporting a tidal marsh diet source, regardless of the depleted $^{13}$C. In addition, $^{15}$N tends toward higher values when a diet or foraging guild has marine or tidal origins, as opposed to freshwater or terrestrial. This likely relates to the confluence of nutrients (e.g., increased agricultural run-off) in the oceans, which could inflate the $^{15}$N of food webs (Peterson and Howarth 1987; Canuel et al. 1995; Fry 2002). Increased salinity in a marine environment can inflate the $^{15}$N at the baseline production level and up through the food web (Canuel et al. 1995). Starvation in birds can also inflate the $^{15}$N in feather tissue (Hobson, Alisauskas, and Clark 1993; Winiarski, McWilliams, and Rockwell 2012). In conclusion, $^{13}$C, $^{34}$S, and $^{15}$N isoscapes created from obligate wetland species hold several caveats in their ability to determine diet or foraging sources within tidal marsh food webs, but overall these biochemical components persist as indicators of nutrient inputs and cycling throughout the Estuary.

The endangered California Ridgway’s Rail Rallus obsoletus obsoletus (hereafter “rail”) has museum specimens and/or records dating back 130 years as a west coast avian species resident to, and possibly restricted to, tidal marshes. The rail typically inhabits contiguous patches of cordgrass (native and/or hybrid Spartina), the dominant vegetation of the historical and modern Estuary. However, more sparse communities of rails inhabit pickleweed (Sarcocornia pacifica), the dominant vegetation of the historical and modern northern sections of tidal marsh (Table 1a, De Groot 1927; Moffitt 1941; Garcia 1995). The rail has compact individual home ranges (annual mean 2–4 ha; seasonal mean 1.2–1.8 ha) and limited evidence of long-range dispersal, and thus the rail has been called an Estuary tidal marsh obligate (Albertson 1995; Casazza et al. 2008; Rohmer 2010; Overton et al. 2014). Rails are considered opportunistic generalists, foraging for invertebrates as secretive marsh birds within the cover of tidal marsh vegetation (Horak 1970; Takekawa et al. 2011; Rush et al. 2012; Casazza et al. 2014). The wealth of rail specimens and/or feather samples from past and present times, combined with the low incidence of rail migration (Albertson 1995; Garcia 1995; Casazza et al. 2008; Overton et al. 2014), creates an opportunity for us to use rail feathers as isotopic indicators of the past and present nutrients and invertebrates available in the marsh. Other Rallus species with a similar diet and/or foraging strategy were used as indicators of estuarine marsh functionality such as carbon, nitrogen, and contaminant storage. The feather isotope and contaminant values provided the measure of marsh function and health (Novak et al. 2006; Fournier et al. 2016). Our rail’s feather isotopes, representing a probable diet of benthic invertebrates (Takekawa et al. 2011; Rush et al. 2012; Casazza et al. 2014), could thus indicate functional shifts at the baseline (isotopic) levels of isolated tidal marsh food webs (Fournier et al. 2016).

The objectives of this study were to conduct isotopic analyses of historical and modern collections of rail feathers to document changes in rail diet and therefore changes to their limited foraging habitat that is Estuary tidal marsh. We predicted that the vast majority of $^{13}$C, $^{34}$S, and $^{15}$N of historical and modern rail feathers would reflect a rail’s benthic tidal marsh diet, synonymous with foraging habitat throughout the Estuary. In other words, we predicted that the $^{13}$C and $^{15}$N of all rail feathers – as individual samples or regional means – would fall above the values commonly associated with Estuary benthos such as filter feeders and detritivores, and we predicted the $^{13}$C and $^{34}$S of the same individual rail samples or regional means would reflect tidal marsh or marine as opposed to freshwater or terrestrial rail diet. We predicted that some but very few individual rails or a region of rails might specialize and forage in more brackish or even freshwater habitats, which, if true, would alleviate concerns about impacts to rails from projected sea-level-rise. Because of the increasing marsh decline and reduction in total hectares of modern tidal marsh (San Francisco Estuary Institute (SFEI) 1998; US Fish and Wildlife Service 2013), we predicted that feather means from modern tidal marsh regions – essentially rail subpopulations – might reflect, on average, more deep-water marine isotope values, specifically depleted $^{13}$C and enriched $^{34}$S, when compared to feather means from comparable historical regions. This prediction would support the theory that rails were disinclined or prohibited from inland and upland migration in the face of sea-level rise and disappearing tidal marsh. Overall, we hypothesized that the Estuary’s tidal marsh fragmentation and degradation would create significant differences in the baseline foraging habitat of rails, which would reflect in feather isotopes between regions and between historical and modern time periods. We specifically predicted differences between the regional feather means both historically and modernly. Furthermore, differences between the $^{13}$C, $^{34}$S, and $^{15}$N of historical and modern feathers of a region would allow us to establish feather means as a baseline for Estuary tidal marsh composition. Such information would be valuable to guide the management of rails and their habitats and
support conclusions with respect to how climate driven variation such as sea-level rise may impact vulnerable populations.

Materials and methods

Overview

The following methods were based on relevant feather isotope literature dealing with diet, essential foraging habitat, and historical representations of diet (Thompson and Furness 1995; Kwak and Zedler 1997; Hatch 2011; Winiarski, McWilliams, and Rockwell 2012; Hall and Beissinger 2017). We isotopically analyzed feather samples of rails to investigate a tidal marsh obligate diet via carbon, sulfur, and nitrogen isotopes, scientifically noted as $\delta^{13}$C (‰), $\delta^{34}$S (‰), and $\delta^{15}$N (‰). We will reference the enrichment or depletion of $^{13}$C, $^{34}$S, and $^{15}$N isotopes hereafter. Changes in these three isotopes reflect biochemical changes to wetlands over time given changes in tidal marsh foraging sources. The $^{13}$C, $^{34}$S, and $^{15}$N from fish (Baker, Nelson, and Leslie 2016) and amphibian (Bishop, Drewes, and Vredenburg 2014) tissues have indicated wetland functionality such as nutrient transport and storage (Fry 2002; Bishop, Drewes, and Vredenburg 2014; Baker, Nelson, and Leslie 2016).

Temporal design

Our study used rail feathers collected across the Estuary, dating from 1885 to 2014. USGS provided us with feather samples taken from rails during radiotelemetry studies in the San Francisco Estuary (Figure 1, modern sampling sites) between 2005 and 2014 (Casazza et al. 2008; Rohmer 2010; Overton et al. 2014). We then sampled historical feathers from mounted rail specimens preserved and stored at the University of California at Berkeley Museum of Vertebrate Zoology, the California Academy of Sciences, the University of California at Davis Museum of Wildlife and Fish Biology, and the National Fish and Wildlife Forensics Laboratory. These museum specimen collections lacked study skins from 1945 to present day; the collections were likely not expanded post-1945 for reasons such as the rail’s federal protection status in 1970 (Garcia 1995). We thus adapted our study to incorporate a historical time period from 1885 to 1940 and a modern time period from 2005 to 2014.

Sampling techniques

We sampled two types of feathers from individual rails, plucking breast feathers from ventral tracts along the upper thorax, and clipping 4.0 cm cross-sections from the third primary (the flight feather commonly labeled “P8” (Pyle 2008)). However, our historical primary samples were extremely limited due to museum restrictions. To correct for this limitation, we compared the breast and primary isotopes from several individual rails, specifically 32 rails from random regions of the Estuary. A paired Student t-test ($n = 32$: 29 modern, 3 historical) determined that three different isotopes were consistent in value across feather types, meaning we found no significant differences ($\delta^{13}$C: $t_{31} = 0.11$, $p = 0.91$; $\delta^{34}$S: $t_{31} = 0.04$; $p = 0.97$; and $\delta^{15}$N: $t_{31} = 1.21$, $p = 0.27$) between the breast and primary isotope values of individual rails. While atypical for most avian species, this result was anticipated given the breast and primary isotope comparisons reported on black rails, a sister species with similar molt patterns and foraging environments (Pyle 2008; Hall and Beissinger 2017). After confirming our expectations, we randomly used either a breast or primary feather for all further analyses, maximizing our regional collections with 110 historical and 105 modern rail feather samples.

Regional design

We aggregated our feathers into regions outlined by the isotope and rail literature of the Estuary (Canuel et al. 1995; Cloern, Canuel, and Harris 2002; Overton et al. 2014; Hall and Beissinger 2017), in addition to an extensive mapping project of the past and present Estuary tidal marsh extent. All locations of collection were mapped within the expansive historical (circa 1820) and fragmented modern (1985–1996) Estuary boundary data (Figure 1, San Francisco Estuary Institute (SFEI) 1998). Most museum (historical) rail specimens were accompanied by brief colloquial notes providing a nearby collection landmark within a county (Garcia 1995). Due to confusing notations, 10 historical locations of collection could not be reconciled with our historical tidal marsh boundary data, and the corresponding feathers were excluded from further analyses. We then buffered the historical and modern boundaries to better accommodate some regional delineations for both historical and modern feathers. Upon further review, our regions were supported by the genetic population structure of Estuary rails (Wood et al. 2016), and were further supported by habitat variation based on vegetation zonation, geomorphology, tidal regime, salinity, and human impacts (Table 1a, Rossio, Ustin, and Hastings 2006; Moffett, Robinson, and Gorelick 2010; McBroom 2012; Woo and Takekawa 2012; Moffett and Gorelick 2016).

Several historical and modern regions (Figure 1, see tidal marsh with rails) were either partially or completely lacking in feather sample representation. Some rail subpopulations were relatively unavailable – such as our historical Northwest region with only
one sample, and other subpopulations were relatively difficult to sample – such as our modern North region with only two samples (Table 1b). We also merged the USGS feathers from two northern San Pablo Bay sampling sites, Gallinas Creek and Corte Madera (modern Northwest, Table 1), into one modern Northwest region (NW, Figure 1). We then merged USGS feathers from two adjacent southern sampling sites, Laumeister and Faber (modern South, Table 1). We ultimately established three regions containing both modern and historical feathers (South, Southeast, South, Table 1), and five regional feather collections lacking either a historical or modern analog (historical: Northeast and Southwest; modern: North, Northwest, and West, Table 1), for a total of eight study regions (Figure 1, N, NE, E, SE, S, SW, W, NW). We considered five additional historical regions beyond our study shoreline. Eight rail museum specimens from regions geographically distant to our study shoreline, modernly lacking in rails, were explored for possible variations in the historical rail diet or foraging ground within the outer historic boundaries of the Estuary.

Isotope analyses

All feather samples were prepared for isotope analysis at the USGS Mercury Laboratory, Western Ecological Research Center, Dixon, California. Samples were prepared by washing in 1% Alconox solution and drying at 40°C for a minimum of 24 h following protocols of the UC Davis Stable Isotope Facility (Mathews 2014). We finely chopped two to three breast feathers or a portion of one primary feather with dissection scissors for each sample. Feather material (2.5–3.5 µg) was weighed and packaged for isotopic analysis. Feather samples were analyzed using an Elemental vario ISOTOPE Cube interfaced to a SerCon 20–22 IRMS (Sercon Ltd., Cheshire, UK) for 13C, 34S, and 15N simultaneously, reported in the delta notation. Isotope profile correction was based on lab standards calibrated to international references (Mathews 2014).

Data overview

Laboratory-reported results from the feathers of individual rails were reviewed prior to the process of grouping results into regions. We compared 13C, 34S, and 15N of each individual rail to published standards regarding the typical marine/tidal values (Table 1b), which generally range between −12.0 to −30.0‰ 13C, and/or +2 to +6.0‰ 34S, with relatively enriched, >14‰, 15N (Peterson and Fry 1987; Kwak and Zedler 1997; Fry 2002). We then proceeded to map and group the individual feathers with the regional design described above Table 1b, and we assigned a color scale to the map of regional 13C, 34S, and 15N means, producing our isoscapes.

Table 2. Feather 13C and 15N means for three regions (Figure 1, NW, E, SE; Table 1b, Modern Northwest, East, Southeast), subtracted by the dunlin discrimination factor (2.2‰ 13C, 3.4‰ 15N; Ogden, Hobson, and Lank 2004), as our best approximation of 13C and 15N rail diet values.

| Tissues analyzed by region | δ13C(‰) | δ15N(‰) | N |
|----------------------------|----------|----------|---|
| **Northwest feathers (2012–2014), prey (China Camp – adjacent to GCM: 2001–2003)** | | | |
| Rail diet calculation (rails in Northwest pickleweed) | −20.5 ± 0.6 | 13.3 ± 0.3 | 26 |
| Hemigrapsus oregonensis (crabs in native Spartina) | −17.6 ± 0.7 | 14.0 ± 1.5 | 10 |
| Hemigrapsus oregonensis (crabs in mudflat) | −19.1 ± 0.3 | 16.7 ± 1.8 | 5 |
| Carcinus maenas (crabs in native Spartina) | −15.5 ± 0.8 | 16.3 ± 0.8 | 6 |
| Carcinus maenas (crabs in mudflat) | −17.5 ± 1.3 | 15.4 ± 0.7 | 5 |
| Balanus balanoides (barnacles in native Spartina) | −20.6 ± 0.9 | 12.1 ± 0.2 | 5 |
| Balanus balanoides (barnacles in mudflat) | −20.6 ± 0.6 | 15.5 ± 0.9 | 4 |
| **East feathers (2009–2013), prey (Alameda – adjacent to arrowhead: 2001–2003)** | | | |
| Rail diet calculation (rails in East hybrid Spartina) | −18.2 ± 0.3 | 13.8 ± 0.4 | 27 |
| Urosalpinx cinera (snails in hybrid Spartina) | −17.4 ± 0.2 | 14.9 ± 0.4 | 11 |
| Urosalpinx cinera (snails in mudflat) | −16.0 ± 0.7 | 15.2 ± 0.4 | 13 |
| Balanus balanoides (barnacles in hybrid Spartina) | −15.9 ± 1.4 | 14.4 ± 0.3 | 10 |
| Balanus balanoides (barnacles in mudflat) | −18.3 ± 0.8 | 14.6 ± 0.2 | 9 |
| **Southeast feathers (2006–2008), prey (San Lorenzo – adjacent to Cogswell: 2001–2003)** | | | |
| Rail diet calculation (rails in Southeast hybrid Spartina) | −16.7 ± 1.0 | 16.1 ± 0.6 | 12 |
| Hemigrapsus oregonensis (crabs in native Spartina) | −19.1 ± 1.3 0.617.19 | 12.9 ± 0.7 | 5 |
| Hemigrapsus oregonensis (crabs in mudflat) | −17.8 ± 0.4 | 14.8 ± 0.4 | 5 |
| Carcinus maenas (crabs in hybrid Spartina) | −16.3 ± 0.6 | 17.1 ± 0.3 | 8 |
| Carcinus maenas (crabs in mudflat) | −17.6 ± 0.6 | 17.3 ± 0.6 | 5 |
| Cerithidea californica (snails in native Spartina) | −14.4 ± 0.2 | 11.8 ± 0.2 | 9 |
| Cerithidea californica (snails in mudflat) | −14.4 ± 0.4 | 11.9 ± 0.7 | 6 |
| Balanus balanoides (barnacles in native Spartina) | −19.3 ± 1.7 | 17.4 ± 0.7 | 8 |
| Balanus balanoides (barnacles in mudflat) | −16.8 ± 2.2 | 16.6 ± 0.4 | 17 |
| Geukensia demissa (mussels in native Spartina) | −21.5 ± 0.4* | 13.3 ± 0.4* | 7* |
| Geukensia demissa (mussels in mudflat) | −22.1 ± 0.5* | 13.3 ± 0.1* | 5* |

Below each diet calculation: potential prey, all invertebrates collected in 2001 and 2003 from these regions. Each invertebrate species was isotopically analyzed and reported for a food web study designed by Brusati and Grosholz (2009).

*discrepancy with values originally reported for mussels from San Lorenzo by Brusati and Grosholz (2009), which was corrected here, provided by Brusati pers. comm (2017).
We next examined the regional $^{13}$C and $^{15}$N for evidence of trophic level and benthic foraging. In general, the carbon will fractionate and enrich in $^{13}$C in the higher (consumer) trophic levels of food webs, but the difference between the consumer and prey will be low, $\sim 1\%$. This difference equates to the isotope discrimination factor. Nitrogen also has a much greater discrimination factor, generally $3\text{–}4\%$ (Fry 2008). We wanted to ascertain the $^{13}$C and $^{15}$N for predicted prey or foraging items, subtracting $^{13}$C and $^{15}$N discrimination factors from rail feather isotope values (Table 2). Since rail-specific discrimination factors do not exist, we used the discrimination factor for a dunlin, another avian tidal marsh forager (Hall and Beissinger 2017). We took our modern Northwest, East, and Southeast rail feather $^{13}$C and $^{15}$N values from Table 1b, and subtracted the discrimination factor calculated for dunlins: $\pm 2.2$, $\delta ^{13}$C; $\pm 3.4$, $\delta ^{15}$N (Ogden, Hobson, and Lank 2004). These modern rail diet calculations were compared to the $^{13}$C and $^{15}$N values of invertebrates (Table 2) that were sampled in 2001 and 2003 within the Northwest, East, and Southeast mudflats and vegetation plains, as reported by Brusati and Grosholz (2009).

Spatial and temporal analyses

We used the wilcox.test function and the Mann–Whitney U statistic to test differences among historical regions, modern regions, and historical-to-modern regions, with a minimum of six samples (historical Southeast, $n = 6$, Table 1b) per region. We performed a Bonferroni correction for each comparative analysis with the p.adjust function in the car package (R Development Core Team 2014).

Results

Isotopes analyzed from individual rail samples ranged from approximately $-22$ to $-10\%$ for $^{13}$C, $7$ to $20\%$ for $^{34}$S, and $-10$ to $23\%$ for $^{15}$N. The most enriched $^{13}$C, $-10.8\%$, was the sole sample from the historical Northwest ($n = 1$, Table 1). Our most depleted $^{13}$C sample ($-21.6\%$) was linked to a sole rail from Gallinas Creek, our site in the modern Northwest. A historical specimen from an outer region (Morro Bay, Salicornia Island) provided our most enriched $^{34}$S, $19.4\%$, and also a relatively high $^{13}$C, $-13.6\%$. Our most depleted $^{34}$S, $7.5\%$, was linked to a sole rail from Colma Creek, our site in the modern West. Both our most depleted and most enriched $^{15}$N were linked to rails in the historical and modern South. A historical South rail from 1907 produced a $^{15}$N of $10.0\%$, while a modern South rail from 2013 had a $^{15}$N of $22.5\%$. The $^{13}$C and $^{34}$S of individual rails within and without (the five additional regions analyzed for unique or variable diet isotopes) our study site boundary fell within a reasonable marine range. Because all of the individual rail isotopes were within reasonable marine or tidal marsh ranges (Table 1b), we proceeded with the regional and time period analyses.

As summarized in Table 1b, the feather isotope means reported for historical and modern regions fell between $-19.0\%$ and $-14.0\%$ $^{13}$C; the $^{34}$S means (historical or modern) fell between $10.0$ and $17.0\%$, and the $^{15}$N means (historical and modern) fell between $12.0$ and $21.0\%$. The overall maximum mean $^{13}$C, $-14.4 \pm 0.5\%$, was from historical East feathers. The $^{13}$C mean from the modern Southeast feathers (Table 1b) presented a similar value, $-14.5 \pm 1.0\%$. The minimum mean $^{13}$C of $-18.3 \pm 0.6\%$ was from modern Northwest feathers, and these feathers also provided the overall maximum mean $^{34}$S of $16.1 \pm 0.7\%$. The most depleted range of mean $^{34}$S was from modern West feathers, $10.5 \pm 0.8\%$. Our absolute low mean $^{15}$N, $12.8 \pm 2.9\%$ was produced by two rails sampled from the historical outer region of Tomales. Our absolute high mean $^{15}$N of $21.0 \pm 0.4\%$ was from modern South feathers collected between 2007 and 2013 (see Table 1b).

None of the $^{13}$C or $^{15}$N values from prey species analyzed by Brusati and Grosholz (2009) matched our rail diet calculations. As a possible sole prey species, barnacles best matched the rail diet calculations for all three regions with prey data (Table 2), but this imprecise matching supports a more a generalist forager diet. As reported in Table 2, rail diet calculations in our modern Northwest region produced a modern rail diet mean of $-20.5 \pm 0.6\%$ $^{13}$C and $13.3 \pm 0.3\%$ $^{15}$N. Diet calculations from modern East feather isotopes produced a mean of $-18.2 \pm 0.3\%$ $^{13}$C and $13.8 \pm 0.4\%$ $^{15}$N. Modern East snails in hybrid Spartina produced $^{13}$C and $^{15}$N means (Brusati and Grosholz 2009) fairly close to the rail diet means in the modern East. Diet calculations from modern Southeast feather isotopes produced a mean of $-16.7 \pm 1.0\%$ $^{13}$C and $16.1 \pm 0.6\%$ $^{15}$N. Based on the reported $^{13}$C and $^{15}$N mean values for a variety of Southeast invertebrates (Brusati and Grosholz 2009), several combinations of these invertebrates would produce $^{13}$C and $^{15}$N values within our modern Southeast diet range (see Table 2).

The mean $^{13}$C, $^{34}$S, and $^{15}$N (Table 1b) showed no significant difference between historical regions ($p \geq 0.10$, $n = 15$) but showed significant differences among modern regions (Figure 2, Table 3). Modern $^{13}$C was depleted in the Northwest and South rails (Figure 2a); $^{13}$C values were not significantly different between these regions (Table 3). The modern mid-latitude marshes were relatively $^{13}$C enriched (Figure 2a). The modern West and East were not
Figure 2. Isoscapes for regional isotope means superimposed on the original SFEI (1998; Figure 1); marsh map. The modern means (solid) were overlaid on historical means (textured) for $^{13}$C (a), $^{34}$S (b), and $^{15}$N (c).
Table 3. Pairwise table of modern significant (p-values significant* if < 0.01) differences associated with $^{13}$C, $^{34}$S, and $^{15}$N of feather samples from paired regional groups.

| Modern geographic comparisons | $\delta^{13}$C | $\delta^{15}$N |
|------------------------------|---------------|--------------|
| North Northwest: West        | 0.06, <0.01*  | 0.01*        |
| North Northwest: East        | 0.00, <0.01*  | 0.01*        |
| North Southeast: West        | 0.00, <0.01*  | 0.01*        |
| North Southeast: East        | 0.00, <0.01*  | 0.01*        |
| North South: West            | 0.00, <0.01*  | 0.01*        |
| North South: East            | 0.00, <0.01*  | 0.01*        |
| North Northwest: Southeast   | 0.00, <0.01*  | 0.01*        |
| North Southeast: Southeast   | 0.00, <0.01*  | 0.01*        |
| North South: Southeast       | 0.00, <0.01*  | 0.01*        |
| North Northwest: Northeast   | 0.00, <0.01*  | 0.01*        |
| North Southeast: Northeast   | 0.00, <0.01*  | 0.01*        |
| North South: Northeast       | 0.00, <0.01*  | 0.01*        |

Regional samples were analyzed through a series of Mann-Whitney U-tests on n rails of each modern region (Table 1b). Results were arranged from North–South latitude. These same pairwise tests were run between feather samples from paired historical regions. We found no significance (p < 0.01).

Table 4. Intra-marsh isotope significance (p-values* significant if < 0.05) associated with historical–modern periods.

| Regions                  | Isotope | W-Value | p-Value |
|--------------------------|---------|---------|---------|
| Historical East/modern East | $\delta^{13}$C | 661.00 | <0.01 |
| Historical East/modern East | $\delta^{15}$N | 497.00 | <0.01 |
| Historical Southeast/modern Southeast | $\delta^{34}$S | 68.50 | <0.01 |
| Historical South/modern South | $\delta^{34}$S | 12.25 | <0.01 |
| Historical South/modern South | $\delta^{15}$N | 0.00 | <0.01 |

Historical $^{13}$C, $^{34}$S, and $^{15}$N of feather samples were compared to their modern analogs through a series of Mann–Whitney U tests on n rails from the historical and modern East, Southeast, and South regions sharing borders (Table 1).

Discussion

Regional $^{13}$C, $^{34}$S, and $^{15}$N means indicate that rails regularly foraged upon the benthic community at the tidal marsh marine interface. As predicted, changes in these isotope means across time follow several known or presumed changes in nutrients and nutrient-filtering benthic communities across the Estuary. Our results do not support our prediction that one rail, or subpopulation of rails, foraged at the freshwater interface. In addition, only one of our three tidal marshes with historical and modern representation follow our prediction of a historical to modern gradient from littoral to more marine isotopes in rail feathers. Congruent with our initial hypothesis, we did find significant regional and temporal differences for all three of our feather isotopes. However, our modern isoscapes reflect an isotopic heterogeneity, whereas our historical isoscapes reflect homogeneity (Figure 2). Modern regions have isotopically diverged (Table 3), indicating that regional food webs have changed over time (Table 4; Figures 2 and 3). We attribute this divergence to two main modifications of the Bay: (1) interruptions of nutrient cycles caused by the invasion of hybrid Spartina and filter-feeding invertebrates (Brusati and Grosholz 2009; McBroom 2012; Overton et al. 2014) and (2) the flux of excess ammonia caused by the treatment of human effluent (Pacific Institute 2009; Takekawa et al. 2006; McKee et al. 2011). Both of these modifications might impact ecosystem function and therefore health.

Our $^{13}$C and $^{15}$N of historical and modern feathers (individual and Table 1b regional), combined with our discrimination factor calculations (Table 2), supported a modern rail diet indicative of benthic tidal marsh foraging. Our feather $^{13}$C and $^{15}$N were appropriate for tidal marsh food webs, as reported in modern tidal marsh literature for samples of organic matter, vegetation, benthic prey guilds, and benthic consumers such as subspecies of our rail (Kwak and Zedler 1997; Canuel et al. 1995; Cloern, Canuel, and
We examined three individual rail feathers with relatively depleted $^{13}$C (below $-20.0\%$), all from the modern Northwest (NW, Figure 1). While we first suspected terrestrial or freshwater foraging among these three rails (see Terrestrial/Freshwater, Table 1b), these low $^{13}$C values were more likely a product of the lower $^{13}$C range associated with C3 vegetation zonation common in the Northwest pickleweed habitats. In addition, the strong tides and nutrient cycling in the Northwest, along with major seasonal inflows from freshwater river inlets (Table 1a), might have depleted the $^{13}$C values from more typical tidal marsh foraging values (Canuel et al. 1995; Cloern, Canuel, and Harris 2002; Fry 2002). Interestingly, the historical Northwest analog (historical Northwest, $n = 1$, Table 1b) produced our most enriched $^{13}$C result (our absolute maximum $^{13}$C, $-10.8\%$). Unfortunately, our low historical sample size prevents temporal analyses in the Northwest (Table 1b).

Due to the overlapping $^{13}$C for individual and regional rail feathers, we considered the $^{34}$S for all individual and regional feathers, as suggested in tidal marsh food web literature (Peterson and Fry 1987; Kwak and Zedler 1997; Baker, Nelson, and Leslie 2016). We found enriched, marine/tidal values of $^{34}$S in all historical and modern rail feathers with the exception of feathers from the modern West. West feathers approached the freshwater range of values for $^{34}$S, but did so with values that could still be attributed to tidal marsh (Table 1b). All other results supported a tidal marsh diet, as opposed to a terrestrial or freshwater diet, for both historical and modern rails. We anticipated that one of the eight museum feathers from the locations beyond our regional perimeter might exhibit either a terrestrial or freshwater diet. However, none of the individual or regional

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**Figure 3.** Boxplots of historical (white) and modern (gray) regional isotope means for $^{13}$C (a), $^{34}$S (b), and $^{15}$N (c) associated with the three adjacent tidal marsh regions, East, Southeast, and South, for which we had comparative samples.
feathers was analyzed within the most common continental (freshwater or terrestrial) range of $^{34}$S – approximately 2.0–6.0‰ $^{34}$S (Table 1b, Fry 2008). This indicated a lack of historical or modern terrestrial/freshwater foraging among the rails sampled (Bishop, Drewes, and Vredenburg 2014; Baker, Nelson, and Leslie 2016), although results were limited to the time of feather growth (Kwak and Zedler 1997; Pyle 2008; Blomberg et al. 2013).

In general, both historical and modern rail feathers presented relatively enriched $^{13}$C, which when paired with enriched $^{34}$S suggested a diet from and foraging within tidal marsh systems. However, as we noted, the feathers of our modern West region presented a $^{34}$S mean (10.5‰ $^{34}$S, Table 1b) that approached the terrestrial or freshwater $^{34}$S range (Fry 2008), and was significantly different from all other regional $^{34}$S means. The modern West had a number of individual feather samples with distinctly different $^{34}$S, while still paired with an enriched $^{13}$C value indicative of tidal marsh. This region had a unique tidal marsh habitat that had undergone an extensive hybrid *Spartina* eradication effort while rails still inhabited the marsh (McBroom 2012). This human-caused impact may have presented a rare and favored prey species with depleted $^{34}$S, an exclusive foraging area with a unique distribution of depleted $^{34}$S sources, or a lack of sulfate-reducing bacteria (Peterson and Fry 1987).

All of these possibilities would be restricted to the time of rail feather growth (Pyle 2008; Winiarski, McWilliams, and Rockwell 2012).

We noted a very broad historical-to-modern depletion of $^{13}$C of rail feathers, which was not always significantly different between time periods (Table 1b; Figure 2a), but which overall supported our expectation of depleting $^{13}$C values in response to rising sea levels. The regions of the historical Estuary might have contained pockets of vegetation associated with higher salinity, which could create historically enriched $^{13}$C throughout a local food web and thereby rail feathers (Canuel et al. 1995). However, the carbon fixation method at the base of the food web typically overwhelms or at least drives the $^{13}$C values in food webs (Fry 2008). Additionally, pickleweed, associated with C3 fixation and depleted $^{13}$C, has dominated the northern landscape of the modern Estuary (Fry 2002; Cloern, Canuel, and Harris 2002; Moffett and Gorelick 2016). It is unlikely that pickleweed dominated any of the historical Estuary (Chmura and Aharon 1995; Watson and Byrne 2012; Woo and Takekawa 2012). Therefore, we consider it more likely that the enriched $^{13}$C of historical feathers (Table 1b) resulted from historical rails foraging in C4 native *Spartina*. Historical rails probably had better access to tidal marsh with a C4 native *Spartina* zonation during the historical time period (1885–1940) (Garcia 1995; De Groot 1927; Moffitt 1941). Thus, historical availability of native *Spartina* for rails to forage in most likely caused the less variable $^{13}$C isoscape of historical tidal marsh. We had not predicted this historically homogenous $^{13}$C isoscape (Figure 2a). While a generalist benthic forager such as the rail might benefit from a biodiverse landscape, the rail probably benefited from contiguous native *Spartina* cover in the historical tidal marsh in the same way the rail benefited from increased hybrid *Spartina* cover in the modern tidal marsh (Overton et al. 2014).

While the historical $^{13}$C showed an unexpected loss in the regional homogeneity (Table 3), the regional heterogeneity in the modern $^{13}$C (Figure 2a) was expected given some of the seminal isotope literature of the Estuary (Canuel et al. 1995; Cloern, Canuel, and Harris 2002). We attributed our record of historical to modern divergence to hybrid *Spartina* invasions in the modern East and modern Southeast Estuary. These invasions extended North from the modern Southeast region, although a native *Spartina* stronghold was maintained in the modern South region (McBroom 2012). The East and Southeast regions showed divergent $^{13}$C shifts in historical to modern feathers with regards to direction and significance (Figure 3a, Table 4), even though both regions were with some amount of hybrid *Spartina* (Overton et al. 2014). The South and East regions showed similar depletions in feather $^{13}$C with regards to direction (historical to modern) and significance (Figure 3a, Table 4). Initially, we found such similarities surprising since the South and East contained different *Spartina* spp and geographical separation (Figure 1). However, the respective cove and lagoon geomorphologies of the East and South may have created a relatively more marine (depleted) $^{13}$C in each region due to eutrophication and algae-based food webs (Canuel et al. 1995; Cloern, Canuel, and Harris 2002). In addition, our rail feathers were likely grown close to winter (Pyle 2008), a season with more run-off and agricultural inflow to these areas of the Estuary. This run-off increased with human settlement over time (Luoma and Cloern 1982; Takekawa et al. 2006; Clarke, Hoppen, and Gaydos 1997).

Interestingly, like our $^{13}$C isoscapes, our historical $^{34}$S isoscapes was homogenous, and the modern $^{34}$S isoscape was heterogeneous, showing some significant differences between regional $^{34}$S (Table 3, Figure 2b). This loss of historical homogeneity was again attributed to the hybrid *Spartina* invasions in the modern East and modern Southeast (McBroom 2012). But the insignificant depletion of $^{34}$S in historical to modern East rail feathers was surprising given the $^{13}$C results in the East and South, and also given the $^{34}$S results in the South – significant modern enrichment (Figure 3b, Table 4). We thought the tidal protection associated with the East cove and South lagoon geomorphologies might allow for algea-
based food webs, which typically produce enriched $^{34}$S values (Canuel et al. 1995; Cloern, Canuel, and Harris 2002; Fry 2002). We had also anticipated that inclines in the eroding Estuary tidal marsh, paired with sea-level rise (US Fish and Wildlife Service 2013), would lead to more marine inputs across the Estuary and would enrich the $^{34}$S in any given tidal marsh. However, we did not see this trend in the East. We saw this trend in the Southeast (although the temporal difference was not significant) and South (Figure 3b, Table 4). According to modern Estuary literature, the dense mat created by hybrid Spartina (versus native Spartina) can interrupt algae growth, which would explain the low $^{34}$S in the East (Levin, Neira, and Grosholz 2006). We believe that hybrid Spartina caused the insignificant temporal difference in the East $^{34}$S (Figure 3b, Table 4), which otherwise would have significantly enriched over time, in the same way the historical and modern feathers of the Southeast and South did (Figure 3b, Table 4).

The regional divergence of modern tidal marsh feathers (Table 3) was helped along by the significant and surprisingly extreme enrichment of modern feather $^{15}$N when compared to the low and historically homogenous $^{15}$N (Table 4, Figures 2c and 3c). We offer several possible explanations of this significant $^{15}$N enrichment and divergence across the Estuary:

1. Starvation can inflate the $^{15}$N in the feathers of a bird. However, our modern rails were of an appropriate mass when weighed for a radio-marking study conducted from 2006 to 2014 (Casazza et al. 2008; Overton et al. 2014). Because the Estuary provides ubiquitous prey species year round (US Fish and Wildlife 2013), we presumed that rail weight would be stable from the previous winter’s feather growth period (Pyle 2008).

2. Major invertebrate species invasions over the past century shifted species composition of the benthic communities of the Estuary tidal marsh (Cohen and Carlton 1998). This could have presented modern rails with a preferred prey with distinctly enriched $^{15}$N values. However, significant enrichment of modern $^{15}$N means (Table 4) was not uniform in the three regions for which we had both historical and modern $^{15}$N results (East, Southeast, South, Table 1b, Figure 3c). Also, when species invasions have occurred in the past, they have typically been invertebrates highly unlikely to exhibit extremely enriched $^{15}$N within a tidal marsh food web (Canuel et al. 1995; Kwak and Zedler 1997). Furthermore, these invertebrate invasions often spread estuary-wide (Cohen and Carlton 1998; Canuel et al. 1995), which, if preferred by rails, would uniformly enrich feather $^{15}$N from the historically homogenous values, creating homogenous modern $^{15}$N, which was not our resulting isoscape (Figure 2c).

3. There may have been a complete trophic shift in rail diet from historical to modern times, which would cause significant enrichment in $^{15}$N. For instance, if rails had switched their prey base from snails and insects to fish, this would be a significant trophic-level shift (Kwak and Zedler 1997). However, the difference between historical and modern rail feathers (Table 1b) equated to more than two trophic levels, based on the fractionation rate associated with dunlin feathers (Table 2, Brusati and Grosholz 2009) – our closest surrogate to rails with regards to shoreline foraging (Ogden, Hobson, and Lank 2004). Thus, historical rails would have been foraging at two trophic levels beneath benthic invertebrates. This is highly unlikely, given that Moffitt (1941) report several different invertebrates in his dissection of stomachs from 18 rails collected near our South region during the winter of 1938 – a year within our historic period. In addition we know that modern rails consume at least some benthic invertebrates (Rush et al. 2012; Casazza et al. 2014). Therefore, the enrichment cannot be a result of a trophic shift in diet.

We think the most likely explanation for the significant $^{15}$N enrichment is nutrient loading from human settlement and effluent. The lagoon South aligns with an epicenter of urbanization (Clarke, Hoppen, and Gaydos 1997; Takekawa et al. 2006), and the South produced our most depleted (historical) and enriched (modern) mean $^{15}$N (Figures 2c and 3c). While the South had the greatest enrichment, we reported peripheral enrichment in the East and Southeast as well, from historical to modern times. Hybrid Spartina in both the East and Southeast might have removed some of the $^{15}$N from the food webs since hybrid Spartina uptakes nitrogen at greater rates than native Spartina (Tyler, Lambrinos, and Grosholz 2007). However, no vegetation structure could correct for the impact of three large wastewater treatment plants in the southern Estuary, built in the interim between our historical and modern sampling periods (Takekawa et al. 2006; Luoma and Cloern 1982). These treatment plants are near or adjacent to each of our tidal marsh regions with historical and modern feathers (Figure 1, E, SE, S). Two wastewater treatment plants approach, and one plant surpasses, 30 million gallons of treated sewage discharge released into the Estuary every day (Luoma and Cloern 1982; Pacific Institute 2009). This localized flux in ammonia certainly represents one major contributing factor to the baseline alterations we documented across the Estuary (McKee et al. 2011; Deegan et al. 2002; Takekawa et al. 2006; Tyler, Lambrinos, and Grosholz 2007).
Our $^{15}$N isoscapes raised our concern with regards to the large enrichment of $^{15}$N in modern rails, especially those occupying the southern regions of the Estuary. Nitrate, a side effect of watershed run-off and sewage, have biological consequences in both animals and humans when an upper threshold is reached (Porter, Jaeger, and Carlson 1999), raising our concern for the compound’s unknown effect on rails. Improved water treatment methods from more recent decades have lowered the historical concern over too much nitrogen in the Estuary (Takekawa et al. 2006). However, the level of $^{15}$N enrichment in the rails warrants future assessment. Nutrient loading associated with enriched $^{15}$N puts aquatic systems at risk of eutrophication, which impacts an ecosystem’s ability to function, causing die-offs and biodiversity losses during seasonal heat, which may increase with global climate change (Takekawa et al. 2006; McGranahan, Balk, and Anderson 2007; Battisti and Naylor 2009).

**Conclusion**

We produced $^{13}$C, $^{34}$S, and $^{15}$N isoscapes to illustrate a loss in isotopic homogeneity from historical to modern times. While the finding does not necessarily represent a direct threat to the rail, the evidence of changes linked to human effluent and invasive species warrant further research. Such research should focus on the foraging requirements and limitations of rails. This is especially true with regards to upstream sites in the Estuary, which will be examined as potential relocation sites for rails as sea-level rise threatens to inundate tidal marsh habitats (Casazza et al. 2016).

Ultimately, historical isotopic baselines for tidal marsh vegetation and geomorphology, prior to the decades of global habitat degradation, are in most part lacking from the literature. Such baselines would provide a benchmark to measure certain long-standing changes to the Estuary. Our historical isoscapes serve as surrogates for that baseline isotopic data and provide insight into environmental change over time in the Estuary. In this way, our study can enhance the models employed by future researchers in order to inform conservation and management of endangered tidal marsh wetlands, particularly those impacted in highly urbanized estuaries.

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