

Restoration trajectories and food web linkages in San Francisco Bay's estuarine marshes: a manipulative translocation experiment

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ABSTRACT: We measured $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ signatures of natural and translocated mussels *Ischadium demissum* to identify food web source differences among estuarine marshes displaying various stages of restorative development. We hypothesized that mussels inhabiting younger marshes would be more dependent on allochthonous organic matter sources, while those inhabiting mature marshes would depend on autochthonous sources. Mussels collected from an undisturbed (reference) marsh located within the Napa River estuarine complex in San Francisco Bay were translocated to a series of restoring marsh sites located within the same river system. The isotopic composition of naturally growing mussels was compared with translocated mussels, which were incubated in restoring sites for 5 and 7 mo. Measurements of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ indicated differences in food web sources supporting *I. demissum* among the 4 marsh sites. A strong cage effect was detected during the initial 5 mo collection interval, indicating that translocated mussels had yet to equilibrate with their new environments. Multiple source mixing model analysis indicated that C_3 emergent vascular plants and brackish phytoplankton contributed most of the organic matter consumed by *I. demissum* over both time periods, but that mussels collected from the downstream sites exhibited higher dependence upon vascular plant detritus. Bay produced phytoplankton contributed very little to *I. demissum* diets, suggesting that the pelagic waters of San Francisco Bay have less influence on marsh food web dynamics than previously anticipated. The results of this experiment show that food web pathways are strongest at intermediate scales; they can be relatively short and unique to specific marshes along the estuarine gradient, but similarities in mussel diets among marshes in close proximity to one another suggests inter-marsh exchange of organic matter. It is, therefore, likely that food webs in young restoration sites depend upon organic matter subsidies from neighboring marshes, rather than from San Francisco Bay.

KEY WORDS: Restoration · Mussels · *Ischadium demissum* · Food web · Estuarine marshes · San Francisco Bay

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INTRODUCTION

Any animal's survival ultimately relies on effectively drawing energy and nutrients from the environment. Thus, identifying energy sources for consumers and consumer movement through natural systems has been a fundamental part of ecology since the herring food webs described by Hardy (1924). In estuaries consumers draw from more diverse food and energy sources compared with other ecosystems (Valiela et al.

2000), often reflecting the integrative nature of estuarine marshes, which serve as porous boundaries between watershed, river, wetland, open water and coastal ecosystems, each of which contributes organic and inorganic material to support estuarine metabolism (Odum 1980, Valiela et al. 2000). Estuaries benefit greatly from mixing that occurs at the land–water interface, manifesting energy subsidies in the form of highly productive food webs that in turn support production in adjacent ecosystems, such as the coastal

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ocean (Odum 2000). A primary goal of estuarine marsh restoration is to recover those ecological functions, such as cross boundary energy subsidies, which enhance coastal production of fish and wildlife (Kneib 2003, McCay et al. 2003). As recently noted by Wozniak et al. (2006), who directly examined carbon flows that run through restoring salt-marsh food webs, the majority of post-restoration studies limit their focus to structural differences between reference and restoring sites and do not address functional trajectories associated with marsh restoration.

In contrast to the estuarine outwelling hypothesis (Odum 2000), in which tidal waters export a mix of riverine, marine, terrestrial, and marsh derived particulate and dissolved organic matter (OM), recent evidence suggests that OM movement between ecosystems may occur on much finer scales than previously considered, and that the principal marsh export mechanism results from overlapping food webs (trophic transfer theory; Kneib 2000). Evidence for discreteness and spatial heterogeneity has been documented within the food webs of estuarine systems, suggesting that autochthonous inputs are more important than allochthonous inputs in supporting estuarine food webs, and that OM movement can occur on the scale of meters rather than kilometers (Grenier 2004, Guest & Connolly 2004, Guest et al. 2004, McMahan et al. 2005). In some cases food web pathways within the same marsh can be completely distinct, with virtually no overlap in prey consumption or primary producer assimilation occurring among aerial, marsh plain and channel feeding organisms (Grenier 2004). A similar pattern of autonomously functioning trophic webs has also been documented between bacterial and herbivorous pathways, the former of which exhibits limited connectivity with higher trophic levels (van Oevelen et al. 2006). These recent studies suggest that food web linkages and pathways among estuarine residents may not only be short, but also intimately linked to a specific habitat that a consumer occupies. In addition, because there is spatial heterogeneity in the abundance and distribution of the dominant food sources in estuarine marsh systems (Bertness & Pennings 2000), habitat specific food webs are accordingly discrete.

This does not mean that estuarine food webs do not interact with adjacent ecosystems, which also import and export organic nutrients and organisms (Valiela et al. 2000, Dean et al. 2005). Alterations in adjoining ecosystems can affect organic inputs to the estuarine food web, with many consequences for estuarine species (Polis et al. 1997, Puth & Wilson 2001). This includes constructed barriers (e.g. levees, shoreline armoring) that can truncate food web and other connections between neighboring systems. These factors can alter the flux of trophic energy, often removing

critical energy sources from otherwise highly subsidized food webs (Polis et al. 1997).

Restoration efforts in estuarine systems often target the reconnection of adjacent ecosystems by piercing artificially hardened shorelines, thereby reintroducing tidal inundation. While reconnecting ecosystems through levee breaches is easily achieved, it is much more difficult to recover lost ecological functions that depend upon connectivity between ecosystems and the reestablishment of physical and biological processes (Simenstad & Thom 1996, Simenstad et al. 2006). Furthermore, it is difficult to measure the performance of reconnections and trophic linkages between ecosystems, especially given the difficulty of establishing how a given organism actually uses a restored system. One way to assess linkages between organisms and ecosystems is through food web connections. Therefore, this study examines the functionality of restored estuarine marshes in supporting estuarine marsh organisms by determining the contribution of allochthonous versus autochthonous OM to the estuarine food web.

The San Francisco Bay and Delta (SFBD) has endured many anthropogenic disturbances, but is also now experiencing substantial estuarine wetland restoration. Thus, it presents opportunities to explore the functional role restoration plays in subsidizing estuarine food webs. Extreme alterations to the hydrological regimes of the Sacramento and San Joaquin rivers, coupled with extensive diking and filling of wetland areas, have resulted in the cumulative loss of over 85% of the Bay's original 2200 km² of estuarine marshes during the past 150 yr (Nichols et al. 1986). Total internal primary production is considered small within SFBD in comparison with other estuaries (Cloern 2001) and has decreased 45% since 1975 (Jassby & Cloern 2000). Current literature suggests that the SFBD food web is primarily based on phytoplankton production, with little coupling to marsh derived detritus (Canuel et al. 1995, Jassby & Cloern 2000, Jassby et al. 2003). This finding probably represents a profound alteration in the SFBD food web base, as it contrasts with unaltered estuarine systems of comparable size where wetlands provide major supplementation to estuarine secondary production (Peterson et al. 1986, Stribling & Cornwell 1997, Kneib 2000, Odum 2000, Valiela et al. 2000). Our study is based on the prediction that restoration of estuarine wetlands in SFBD will result in overall increases in estuarine secondary production through detritus based pathways.

Conceptual model and objectives

The main objective of this study was to determine whether food web subsidization can be used to indi-

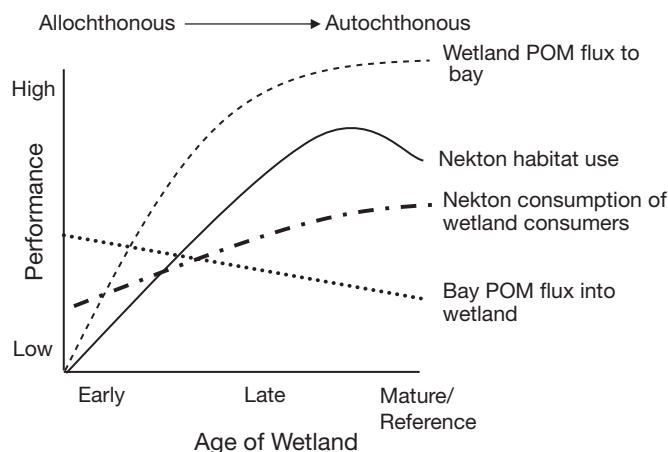


Fig. 1. Conceptual model of food web interactions and differential fluxes between San Francisco Bay and Delta waters and restoring wetlands as a function of restoration stage (age)

cate the stage of performance in a restoring wetland and to examine time trajectories describing the point at which restoration efforts begin supplementing the greater estuary (Fig. 1). The null hypothesis maintains that tidal marsh food web subsidization does not change from allochthonous to autochthonous OM sources with increasing development stage of a restoring wetland. However, given results from other estuaries with high marsh inputs, and the current reliance of SFBD consumers on phytoplankton, we predicted that SFBD pelagic POM (phytoplankton) and organisms supplement food web pathways in the early stages of development of wetland restoration sites, especially in young restoration sites where tidal inundation is the dominant source of POM to the base of the food web and autochthonous biomass is minimal. In intermediate stages of restoration the POM content exchange between open waters and wetlands was expected to shift, with increasing contributions from wetland processes resulting from the recruitment and establishment of marsh autotrophs. We expected ancient wetlands and the oldest restored wetlands to supply a net supplement of marsh derived OM to SFBD food webs, while the importance of bay POM flux into wetlands was expected to measurably decrease. This hypothesis was based on observations that biomass and productivity of autochthonous sources increase as restoration sites age.

Approach

We conducted an experimental translocation study using the marsh adapted filter feeding mussel, *Ischadium demissum* (also referred to as *Geukensia demissa*) (Charles & Newell 1997, Kreeger & Newell

2001, Huang et al. 2003), as a bioindicator to examine food web linkages within SFBD's restoring marshes. Although non-indigenous to the region, *I. demissum* was selected for this study because the species is known to feed seasonally on a variety of OM types, including phytoplankton, benthic microalgae, vascular plant and macro-algae detritus, micro-heterotrophs, and organic aggregates (Huang et al. 2003). We used stable isotopes to establish the relationship between restoration status and food web sources, with particular attention to allochthonous OM subsidizing the food webs of restoring marshes. The benefit of this experimental approach is that the study organism is sessile, thereby ensuring that the organism is dependent upon food sources available only in the collection site. Furthermore, the transplantation aspect of this experiment allowed us to track the reaction of a single population to new feeding habitats, which provides a baseline with which to compare any changes over time and space.

Stable isotope analysis is a relatively direct method of tracking energy between primary producers and estuarine consumers (Peterson et al. 1986, Peterson & Howarth 1987). This method has recently emerged as the leading technique in determining food web pathways in aquatic, terrestrial and marine systems (Peterson et al. 1986, Peterson & Howarth 1987, Hobson 1999), and has also been used to track organic matter subsidies between discretely defined ecosystems (Deegan & Garritt 1997, Guest et al. 2004, Wozniak et al. 2006). Because stable isotope distributions often vary among different ecosystems within the same estuary, it is possible to describe their relative contributions to the structure and function of food webs. While the aforementioned efforts contribute greatly to our current understanding of food web pathways, they remain descriptive in nature and, thus, lack experimental insights.

The study questions are: (1) Do isotope signatures of transplanted mussels change with translocation? (2) Do site based differences in the mussel signatures and diets exist? (3) Do site based differences align with patterns of marsh age? and (4) Does allochthonous material become less important to the diets of *Ischadium demissum* with increasing marsh age?

MATERIALS AND METHODS

Study sites and design. The study was conducted from September through March 2005 in the Napa River estuary, a tributary to SFBD, where a series of 3 focal marshes of different ages created a chronosequence (Fig. 2). Marshes represented young (Pond 2A, P2A) and aging (Bull Island, BI) restoration sites and an

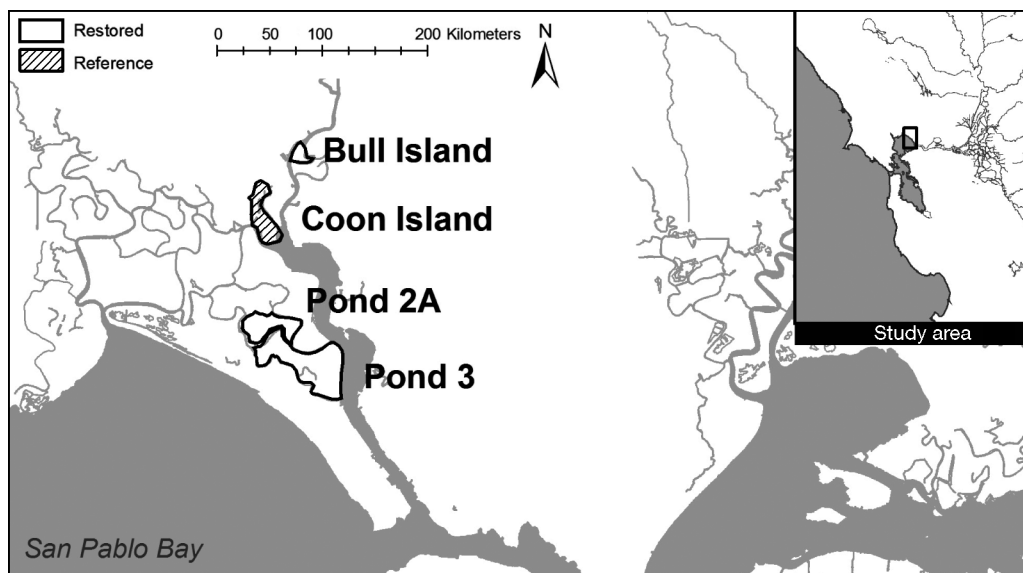


Fig. 2. Study site locations along the Napa River, San Francisco Bay, California

ancient marsh (Coon Island, CI), which was the reference site. A supplementary site, Pond 3 (P3), was included after an unexpected 2003 levee breach made this site partially viable for this study. The levee surrounding P2A was breached in 1995 and the levee at BI was breached 25 years ago. While the lower 3 marshes are within the spatially broad complex of estuarine marshes that characterizes the lower Napa estuary, BI is separated from the broader marsh complex by a narrow stretch of river that has been extensively armored. The study sites thus differ in both age and geographic position along the estuarine gradient. However, it was hypothesized that age would override other factors and was, thus, the highest priority factor in site selection. Unfortunately, alternative differences in environmental gradients governing marsh food webs could not be rigorously tested within the confines of this study.

We designed an experiment that enabled us to observe convergence and divergence of translocated and natural mussel isotope signatures and diet profiles. Fifty mussels between 60 and 90 mm in length were collected from channel walls within 10 m of channel mouths from the CI ancient marsh in September 2004. Mussels were then transplanted into small channels at each of the restoring marshes, as well as the ancient marsh itself. Mussels were harvested the following January and March, 2005. In each marsh, 10 mussels were placed in cages (10^3 cm rubber coated steel boxes with 1 cm mesh) and secured against channel walls with steel rebar within 10 m of channel mouths. Two mussels were placed in each cage, one of which was

collected during each harvest period and subsequently grouped with other transplanted mussels from the site. At the CI control site mussels were placed in cages that were then secured in the same channels from which the mussels were collected. This was done to test for overall cage effects. Because transplanted mussels were grouped by marsh for each date, individual cage effects could not be ascertained. Naturally growing mussels were also collected at each restoring site for comparison with translocated mussels. However, due to its young age, no naturally occurring mussels were available for collection in Pond 3.

An additional set of cages was placed in P2A to test for differences between organisms located at channel mouths and those inhabiting channels in the interior of the marsh. We hypothesized that, depending on OM transport distances, which have been observed to flow on the order of meters (Guest et al. 2004), channel mouth inhabitants would reflect a more general marsh isotopic signature, while organisms collected in an interior marsh channel may reflect a stronger site related signature.

Tissue preparation and isotope analysis. Adductor muscle and mantle tissue was extracted from individual mussels *Ischadium demissum*, with care taken to exclude stomach contents. Following the methods of Arrington & Winemiller (2002), samples were freeze-dried for 48 h and mechanically homogenized to a powder using a Wig-L-Bug® dental mill outfitted with a stainless steel vial and ball pestle. Powdered samples were weighed on a microbalance and placed in tin capsules for isotope processing. The isotopic composi-

tions for carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), and sulfur ($\delta^{34}\text{S}$) were determined for each sample. Carbon and nitrogen analyses were conducted at Oregon State University using a Carlo Erba NA-1500 elemental analyzer connected via a gas dilution to a Finnigan Delta Plus XL mass spectrometer, which measures concentrations within ± 0.1 and $\pm 0.2\%$. Sulfur isotope analysis was conducted at the Coastal Sciences Laboratory in Austin, Texas, using a VG (Micromass) isotope ratio mass spectrometer.

Isotope ratios. The δ notation indicates the enrichment (+) or depletion (–) of the heavy isotope relative to the light isotope of an element compared with the standard as defined by the formula:

$$\delta X (\%) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where $X = {}^{13}\text{C}$, ${}^{15}\text{N}$ or ${}^{34}\text{S}$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$, ${}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{34}\text{S}/{}^{32}\text{S}$. The standard substance used for carbon was the mass 45 to mass 44 ratio in CO_2 of carbonate from the fossil *Belemnitella americana* from the Peedee formation of South Carolina (Spiker & Schemel 1979). Canyon Diablo troilites were used for ${}^{34}\text{S}$ and atmospheric nitrogen was used as the standard for ${}^{15}\text{N}$ (Peterson & Howarth 1987).

Multiple source mixing model. We estimated the relative percent contribution of primary producer types to consumer base diets with SOURCE, a multiple source mixing model that uses linear programming techniques with multiple tracers to estimate the central tendency of a consumer's direct and indirect uptake of autotrophic sources (Lubetkin & Simenstad 2004). Primary producers incorporated into the mixing model included the live plant material of *Spartina foliosa*, *Scirpus maritimus*, *Salicornia virginica*, *Grindelia stricta*, *Typha* sp., benthic diatoms, filamentous green algae, brackish phytoplankton (collected at low salinity sites in marsh channels), and bay phytoplankton (collected in central San Francisco and San Pablo bays). Where occurring, average isotope values for

each species were obtained from 4 samples collected at each study site and combined across the lower Napa River system (Howe 2006). To comply with SOURCE requirements and minimize error magnitudes associated with center of mass estimates, each primary producer category must have a distinct isotopic tracer signature. For 2 sources to be considered distinct from one another, the squared normalized distance (NND^2) of their isotopic signatures must be > 0.1 (Lubetkin & Simenstad 2004). We therefore merged *S. maritimus*, *S. virginica*, and *Typha* sp. into 1 category: C_3 emergent vascular plants (EVP). All other primary producer categories were distinguishable using the NND^2 method (Table 1). Mixing model results were robust to variations in phytoplankton source signatures when upper and lower 95% CI limits were used in place of the mean.

Statistical analyses. Data were analyzed using SPSS 13.0[®] (univariate statistics), Microsoft Excel[®] (univariate statistics), and Primer 6[®] (multivariate statistics) software. We performed *F*-tests to test for equal variance and normality. We used analysis of variance (ANOVA, $\alpha = 0.05$) and 2-sample *t*-tests (assuming equal and unequal variance depending on requirements, $\alpha = 0.05$) to separately distinguish differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ signatures among organisms collected at each site. Type III ANOVA results for site and treatment comparisons were built using type II ANOVAs due to unequal sample sizes. Bonferroni post hoc tests were conducted to identify specific marsh or organism comparisons contributing to overall significant differences found with ANOVA results.

Multivariate data analyses were employed to compare overall consumer isotope signatures among sites and dates and consumer diet patterns derived from SOURCE mixing models. Consumer isotope and diet data were analyzed using Primer's nonmetric multidimensional scaling (NMDS) ordination, analysis of similarity (ANOSIM), and similarity percentage (SIMPER) analyses. Before NMDS ordination and similarity calculations, all mixing model derived data were square root transformed as recommended for percentage data (Schafer et al. 2002). Similarity matrices were constructed using the Bray–Curtis similarity coefficient (mixing model output data) and the Euclidean distance coefficient (isotope data) (Clarke & Warwick 2001). We performed 1-way ANOSIM on similarity matrices to determine whether site differences existed in the primary producer contributions to mussel diets. ANOSIM calculates an *R*-value that is scaled between -1 and $+1$, with the biological importance of the dif-

Table 1. Primary producer average isotope signatures (\pm SD) used for mixing model input after collapsing plant groups according to SOURCE's nearest neighbor distance requirements. Phytoplankton values are calculated via regression to the Redfield ratio of $6.6 \text{ g C g}^{-1} \text{ N}$ using samples with C:N ratios between 5 and $9 \text{ g C g}^{-1} \text{ N}$ (Kendall et al. 2001) and $\delta^{13}\text{C}$ (or $\delta^{15}\text{N}$) of samples (see Howe 2006); thus, they do not have SD values

Primary producer	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
Bay phytoplankton	–22.60	9.53	20.20
Brackish phytoplankton	–28.14	8.44	18.60
Benthic diatoms	-19.39 ± 2.72	7.42 ± 1.55	-1.18 ± 3.06
Filamentous algae	-24.19 ± 4.25	9.69 ± 3.16	9.73 ± 5.83
<i>Grindelia stricta</i>	-26.64 ± 0.60	3.89 ± 1.14	17.60 ± 1.00
C_3 emergent plants	-26.11 ± 1.08	7.33 ± 1.74	12.98 ± 2.67
<i>Spartina foliosa</i>	-13.54 ± 0.07	8.51 ± 4.86	17.25 ± 0.37

ference becoming greater as R approaches unity (Clarke & Warwick 2001). ANOSIM also provides a p-value similar to that of ANOVA, with values of $p < 0.05$ indicating significant differences. Significant differ-

ences found through ANOSIM were further examined using SIMPER analyses, which identify the primary producer categories that account for the principal differences seen in consumer diets among sites.

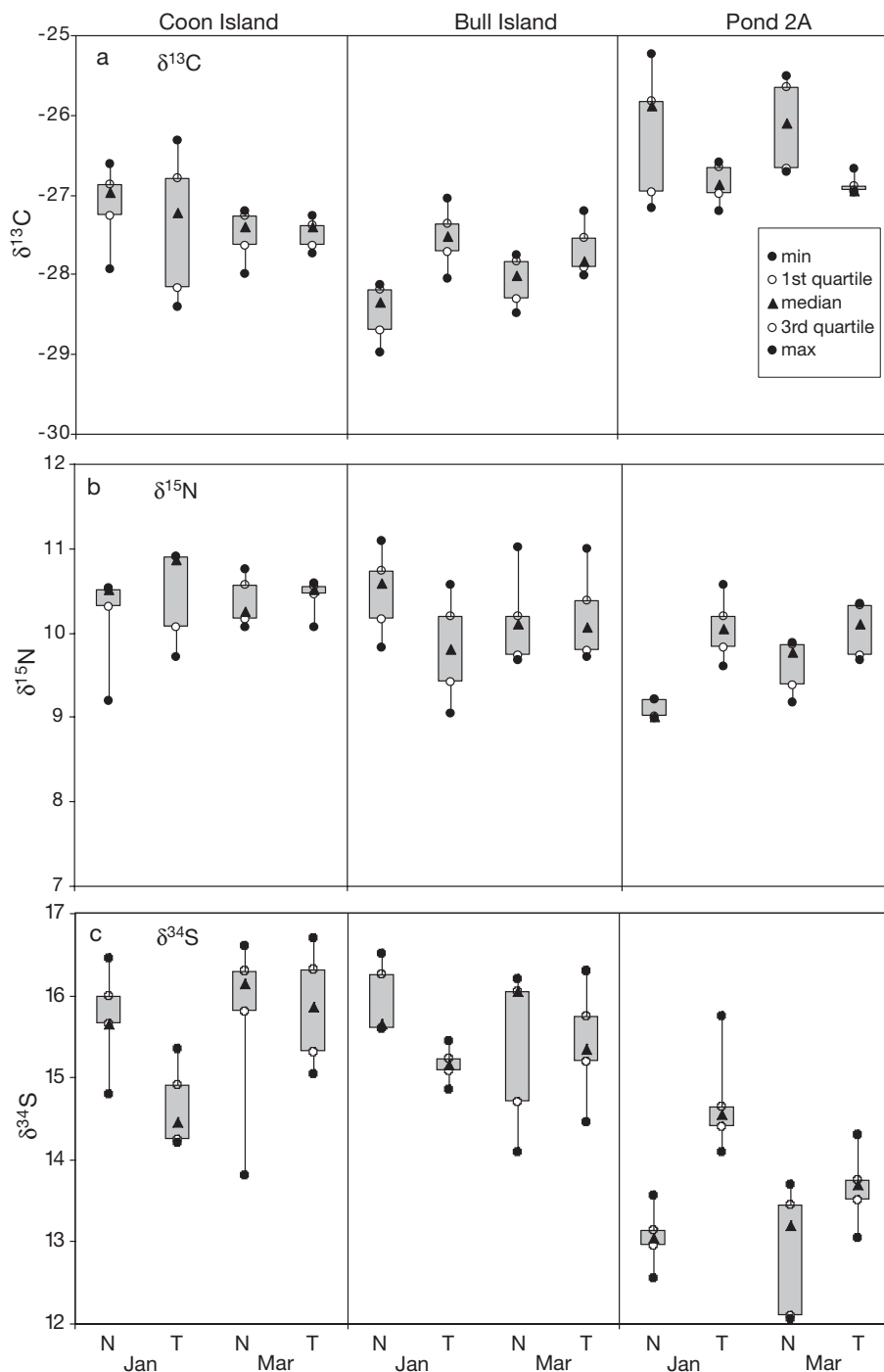


Fig. 3. *Ischadium demissum*. Comparison of measured (a) $\delta^{13}\text{C}$, (b) $\delta^{15}\text{N}$ and (c) $\delta^{34}\text{S}$ stable isotope signatures across marsh sites and between naturally growing (N) and transplanted (T) mussels at each site in January (Jan) and March (Mar) 2005. Control site occurs at Coon Island

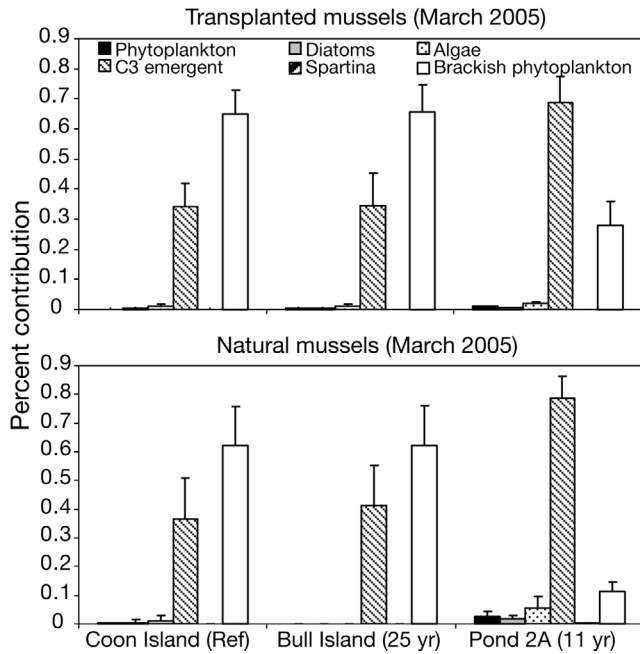


Fig. 4. *Ischadium demissum*. Percent diet contributions of primary producers to transplanted and naturally growing mussels, March 2005

RESULTS

Isotope signatures

In January 2005 isotopic signatures of transplanted and naturally growing mussels collected at CI indicated a strong cage effect for $\delta^{34}\text{S}$ values ($t = -3.10$, $p = 0.014$), but not for $\delta^{13}\text{C}$ ($t = -0.545$, $p = 0.49$) or $\delta^{15}\text{N}$ ($t = 0.77$, $p = 0.46$). The cage effect also occurred at BI and P2A, with large differences in $\delta^{34}\text{S}$ values between transplanted and naturally growing mussels (Fig. 3). Transplanted mussel $\delta^{34}\text{S}$ values were depleted in comparison with natural mussel $\delta^{34}\text{S}$ values at BI. Conversely, the $\delta^{34}\text{S}$ of transplanted mussels was more enriched than the naturally occurring mussels at P2A. The cage effect probably leads to the lack of significant site differences in mussel isotope signatures among the marshes studied in January 2005 ($\delta^{13}\text{C}$: $F = 1.636$, $p = 0.217$; $\delta^{15}\text{N}$: $F = 2.280$, $p = 0.125$; $\delta^{34}\text{S}$: $F = 1.475$, $p = 0.249$).

By March 2005 there were no cage effects observed at CI ($\delta^{34}\text{S}$: $t = -0.107$, $p = 0.978$, $\delta^{15}\text{N}$: $t = 0.123$, $p = 0.906$, $\delta^{13}\text{C}$: $t = 0.123$, $p = 0.906$), and transplanted and natural mussel sig-

natures had converged at each restoration site (Fig. 3). Site effects were significant for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ ($\delta^{13}\text{C}$: $F = 8.939$, $p = 0.001$; $\delta^{15}\text{N}$: $F = 5.587$, $p = 0.009$; $\delta^{34}\text{S}$: $F = 27.950$, $p = 0.0001$), while treatment effects were not significant for any of the 3 isotopes. However, a significant site by treatment interaction was observed for $\delta^{13}\text{C}$ values across all 3 marshes ($F = 5.457$, $p = 0.005$).

Mussel diet profiles

Due to the lack of converging isotope signatures between translocated and naturally growing mussels, mixing model analyses of January mussel isotope data are not presented. Mixing model results for March indicated common diet sources between translocated and naturally growing mussels, similar to the convergence in isotope signatures (Fig. 4). Brackish phytoplankton contributed the most to mussel diets at both CI (CI) (mean \pm SD: transplant, $64.9 \pm 7.9\%$; natural, $62.2 \pm 7.9\%$) and BI (BI) (transplant, $65.7 \pm 8.8\%$; natural, $62.2 \pm 14\%$), with EVP contributing the only other significant source of organic material (transplant CI, $34.2 \pm 7.8\%$; transplant BI, $34.7 \pm 10.6\%$; natural CI, $36.8 \pm 14\%$; natural BI, $41.4 \pm 14\%$). The opposite trend was observed at P2A, where EVP (transplant, $68.7 \pm 8.7\%$; natural, $78.5 \pm 7.8\%$) provided a greater contribution to mussel diets than brackish phytoplankton (transplant, $28.0 \pm 8.0\%$; natural, $11.2\% \pm 3.6$).

Strong site differences were detected using 2-way ANOSIM based on site and treatment (Site: $R = 0.561$, $p = 0.001$; Treatment: $R = 0.070$, $p = 0.100$). Pair-wise site comparisons and MDS results (Fig. 5) indicated that the diets of mussels inhabiting P2A (P2A) were significantly different from both Bull and Coon islands,

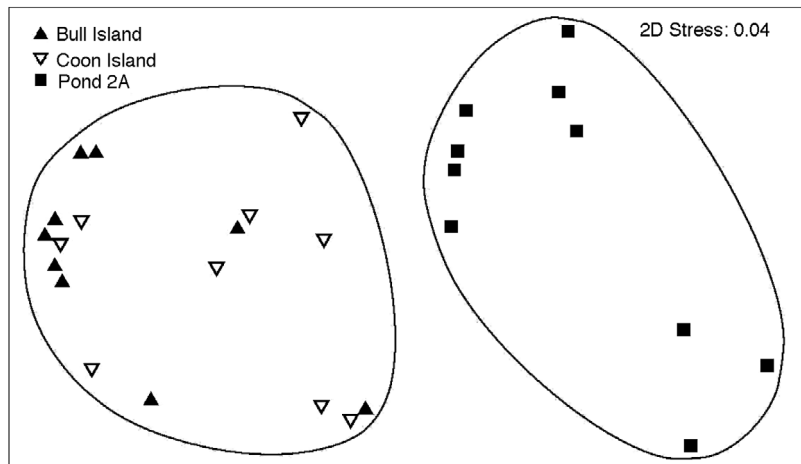


Fig. 5. *Ischadium demissum*. MDS plot based on percent contribution of primary producers to the diets of mussels collected in March 2005

Table 2. Results (based on square root transformed data) of between-site SIMPER analysis for mussels collected in March 2005. SIMPER analysis explains the primary producers that most contribute to significant differences in mussel diets observed between sites. Contrib. = contribution

	Mean contrib. to diet (% contrib. to difference)		
	Pond 2A	Bull Island	Coon Island
Brackish phytoplankton	0.43	0.81 (21.40)	0.80 (23.54)
<i>Grindelia stricta</i>	0.33	0.02 (17.38)	0.07 (16.92)
<i>Salicornia virginica</i> ^a	0.29	0.03 (14.61)	0.07 (13.68)
<i>Scirpus maritimus</i> ^a	0.26	0.05 (12.23)	0.12 (10.83)
Filamentous algae	0.18	0.05 (8.24)	0.55 (10.04)
<i>Typha</i> sp. ^a	0.65	0.57 (8.16)	0.08 (7.74)
Bay phytoplankton	0.13	0.01 (6.27)	0.04 (5.46)

^aSeparated for the purposes of SIMPER analysis, but collapsed into one category (emergent vascular plants, EVP) for all other mixing model analyses

but that the older restoration site (BI) was not significantly different from the reference site (BI × CI: $R = 0.072$, $p = 0.167$; BI × P2A: $R = 0.879$, $p = 0.001$; CI × P2A: $R = 0.701$, $p = 0.001$).

SIMPER results indicated that significant diet differences seen between P2A and the other 2 sites are largely caused by brackish phytoplankton (Table 2). At least 3 times as much consumption of brackish phytoplankton occurred in the older CI and BI sites (CI, $63.7 \pm 10.1\%$; BI, $64.3 \pm 10.6\%$) compared with that in the younger P2A site ($19.5 \pm 10.6\%$). The opposite pattern occurred with bay phytoplankton, which comprised roughly 13% of mussel diets in P2A, but only 1 and 4% of mussel diets at BI and CI, respectively. In addition to the small contribution of bay phytoplankton to the overall diets of the mussels, this category had little effect on diet differences observed among sites. While the influence of bay phytoplankton on mussel diets was highest in the youngest marsh, no clear relationship between marsh age and the percent contribution of bay phytoplankton was evident, as mussels collected in the BI restoration site consumed less bay-produced phytoplankton than mussels obtained in the reference site, CI. Concurrently, the amount of autochthonous EVP consumption increased with decreasing marsh age, also in contradiction to the predictions of the conceptual model. Mussels sampled at the youngest site, P2A, reflected a diet composed of $73.6 \pm 9.4\%$ EVP, nearly twice as much as the contribution of EVP to mussels inhabiting Coon ($35.4 \pm 10.3\%$) and Bull islands ($37.4 \pm 11.8\%$).

Channel location and spatially proximate marshes

Supplemental comparisons of mussel diets estimated for mussels collected in the interior and the mouth of P2A in March 2005 revealed no significant difference between the 2 areas ($R = 0.104$, $p = 19.8\%$). Isotope sig-

natures mirrored this finding, indicating no significant differences between mussels transplanted to the interior of P2A and those located at the mouth ($R = 0.008$, $p = 36.5\%$). Also, mussel diets from P2A and Pond 3 were not significantly different in March 2005 ($R = 0.144$, $p = 14.8\%$).

DISCUSSION

This transplant experiment demonstrates that inter-site similarities and differences in mussel isotope signatures and diet profiles are detectable using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ over a 7 mo time period. The experiment was also successful in tracking the amount of change in isotope signatures that occurred when mussel populations were translocated into new marsh sites, indicating that differences in food web bases do exist on the scale of individual marshes and that consumer organism tissues can rapidly equilibrate to new conditions. With respect to food web subsidization of young restoring sites, our results indicate that bay-produced phytoplankton contributes very little to the diets of generalist marsh consumers, such as *Ischadium demissum*, and that inter-marsh exchange of OM may play an important role in supporting food webs associated with marshes in the early stages of restoration. However, care must be taken when interpreting diet patterns of transplanted organisms, as insufficient transition periods for muscle turnover coupled with cage effects can cloud the relationship between the isotopic signatures of food resources and consumer organisms.

Cage and temporal effects

The convergence between natural and transplanted mussels at all sites in March 2005 indicates that 7 mo was sufficient to account for muscle turnover rates. The mismatch in sulfur isotope signatures in January probably reflects a transition period when translocated mussels were adapting to their new environments. For example, enriched $\delta^{34}\text{S}$ values in January P2A transplants may have resulted from the fact that transplanted mussels originated from CI, where mussels were observed to have more enriched sulfur signatures in comparison with those found naturally in P2A. Transitional isotope signatures could have occurred via 2 separate mechanisms. First, the 5 mo period may not have afforded mussels enough time for full muscle turnover. Muscle tissue turnover rates are uncertain and depend on size class. Six-year-old blue mussels,

Mytilus edulis, can take 3 yr for full muscle turnover (Hawkins 1985), while ribbed mussels, *Geukensia demissa*, can reach equilibrium with their food after 206 to 397 d (McKinney et al. 2001). The isotope composition of mussels from our 5 mo (150 d) time period, thus, reflects transition values intermediate to the isotopic values of natural mussels inhabiting the original and new marsh environments. The convergence of isotope signatures of the transplanted mussels in March indicates they were able to reach equilibrium with their new environments after 210 d. The second potential mechanism leading to the January treatment effect of isotope signatures and diet bases may result from the physical lifting of mussels out of the substrate into cages on the channel wall surfaces. In January cages were largely devoid of sedimentation and the entire mussel surface was exposed. In contrast, heavy accumulation of sediments had occurred by March and mussel exposure above the substrate largely matched that of naturally growing mussels. Exposure to air is known to affect the assimilation efficiency by *Ischadium demissum* of organic materials (Charles & Newell 1997). Thus, exposed mussels may feed differently than buried mussels, which could alter the isotopic signatures of mussels elevated in cages. It is possible that both physiological muscle turnover rates and physical environmental conditions were responsible for the January cage effect.

Diet patterns: mechanisms for site similarities

This study found that mussels in the 2 upstream sites, Bull and Coon islands, had very similar diets that differed significantly from those in the more downstream P2A site. Significant differences in the diets of mussels inhabiting different marshes indicate that food webs within a single estuarine complex can be discrete on the scale of several kilometers. However, there can also be similarities among marshes separated at the kilometer scale, and this may indicate connectivity within mussel habitats that are adjacent to each other along the estuarine gradient.

Similarities in POM composition among sites may result from 2 dominant sources that depend on the scale of organic matter exchange in the estuary. Firstly, local plant assemblages contribute to the POM pool and typically vary along the estuarine gradient (Spiker & Schemel 1979, Bertness & Pennings 2000). Thus, sites located at the same position along the gradient are more likely to have POM pools from the same plant sources. Sites in different areas of the estuary probably have POM pools originating from different plant assemblages, as salinity and elevation are major determinants of plant assemblage structure in estuaries (Bertness & Pennings

2000). Coon and Bull islands are located 8 and 11 km upstream, respectively, from P2A. Thus, these sites are closer to one another than either marsh is to P2A. Plant assemblage compositions at the 2 upstream sites are accordingly more similar to one another, as opposed to the plant assemblage contributing to the POM pool at P2A (K. Tuxin, unpubl. data). For example, *Spartina foliosa* comprises 10% of the total areal coverage of P2A, as opposed to 2 and 0.4% of CI and BI sites, respectively. This pattern is reflected in the percent contribution of *S. foliosa* to mussel diets, with the greatest contribution of 0.13% at P2A, and decreasing contributions at CI (0.06%) and BI (0.01%). Also, both upstream sites have more freshwater vegetation, such as bulrushes and cattails, while the vegetation at P2A consists of salt marsh vegetation. Therefore, diet overlap of *Ischadium demissum* at the 2 upstream sites may result from their similar location on the estuarine gradient.

The second potential explanation for similarities in POM compositions among sites involves inter-marsh exchange of organic material. Marsh islands in close proximity to one another are more likely to exchange organic detritus than are sites that are more distant from each other. Also, marsh sites in similar locations are more likely to receive common allochthonous inputs to the POM pool in similar quantities. For example, mussels at Bull and Coon islands derived 81 and 80% of their diets from brackish phytoplankton, respectively, compared with mussels inhabiting P2A where diets consisted of about 43% brackish phytoplankton. Likewise, higher proportions of San Francisco Bay phytoplankton occurred in P2A diets (13%) compared with the 2 upriver sites (CI, 4%; BI, 1%). This pattern also agrees with our conceptual model that contends that younger sites will have more allochthonous subsidies to the internal food web than will older, more mature marshes. However, because mussels inhabiting the ancient site consumed more bay derived phytoplankton than those mussels inhabiting the 25 yr-old BI restoration site, the pattern of bay influence conflicts with the original hypothesis that the developmental stage of a marsh strongly influences the magnitude of bay derived subsidies. Instead, these data suggest that the pattern of bay phytoplankton contributions aligns better with the spatial positioning of a marsh along the estuarine gradient and that marshes in close proximity will have similar sources supporting the base of the food web.

Diet patterns: evidence for marsh-derived OM subsidies to estuarine food webs

The fact that some filter feeding mussels assimilated up to 89% of their diet from autochthonous estuarine

plants is unexpected considering numerous studies that document the dominance of phytoplankton in SFBD consumer diets, particularly among suspension feeders (Canuel et al. 1995, Jassby et al. 2003, Sobczak et al. 2005). However, these examples are limited in their ability to generalize about food web metabolism in all of the estuary's ecosystems as they only examined pelagic food webs associated with the open waters of SFBD. Thus, although these studies effectively characterized mid-channel, open water food webs in the Bay, they did not address estuarine marsh specific food webs. In this light, the consumption of estuarine marsh derived detritus by a marsh adapted species is perhaps less surprising that we originally thought. Nevertheless, the results of this study remain unexpected given that, despite being a generalist feeder, *Ischadium demissum* can selectively and more profitably feed upon live seston cells, such as benthic microalgae and phytoplankton particles (Kreeger & Newell 2001). Assimilation of EVP material by these mussels indicates that marsh derived detritus may be able to support a wide range of estuarine species during the fall and winter months (Kreeger & Newell 2000) and that this food source is available in all marsh areas regardless of developmental stage. The composition of EVP detritus, however, may vary among marshes located along the estuarine gradient.

While our original conceptual model of bay produced phytoplankton subsidizing food webs in young restoration sites does not coincide with our findings, evidence suggests that subsidies from tidal marsh ecosystems to early restoring marshes are both possible and likely. This is best exemplified by the similarity in mussel diets between P2A and the unvegetated restoration site, Pond 3, which was located nearby and breached only 1 yr before this study. Because of apparently high inter-marsh exchange, restoration trajectories based on food web function cannot be assessed for these marshes using percent allochthonous material in the diets of *Ischadium demissum* as an indicator. Allochthonous material fluxing into a particular marsh site occurs in the form of bay produced phytoplankton as well as marsh derived EVP detritus, the latter of which is not traceable to a particular marsh with the 3 isotopes we used. It is, therefore, possible that a 10 yr-old marsh produces enough organic matter to support itself and also that young restoration sites are supplemented by the exports of more mature marshes in close proximity. The lack of a significant difference in the March diets of mussels inhabiting Pond 3 and P2A supports both scenarios. The mussels at these 2 adjacent marshes exemplify the premise that inter-marsh exchange can certainly occur and that the 11 yr-old restoration site is capable of producing enough organic material to provide that subsidy. Therefore, with

regard to restoration design, positioning restoration sites in close proximity to mature marsh ecosystems may enhance the function of food webs in the early stages of restoration. However, our data also suggest that food webs in restoring marshes quickly become autochthonously driven and may not require external subsidies for long periods of time.

CONCLUSIONS

The results of this study indicate that both young restoring and ancient tidal marshes contribute extensively to the food web of shallow water ecosystems in San Francisco Bay, and that similarities in isotope signatures and diet profiles between consumers inhabiting different sites are more likely due to the spatial positioning of sites than to marsh age or restoration status. Furthermore, because differences in mussel diets were detectable along the estuarine gradient, this study provides evidence that, despite differences in tidal range and flora, west coast estuaries function similarly to those on the east coast of North America and elsewhere in that food web linkages are shorter and more spatially discrete than expected under the estuarine outwelling hypothesis. This suggests that a more robust study of restoration trajectories with respect to food web subsidies could be achieved if conducted within a more restricted spatial scale, as landscape location, as opposed to restoration status, is the more dominating environmental gradient regulating organic matter source availability. Also, this study demonstrates the importance of inter-marsh connectivity, as inter-marsh exchange appears to play an important role in subsidizing the food webs of marshes in the early stages of development. Future studies seeking to address the ability of marsh restoration to supplement open water estuarine food webs should focus on the role of trophic transfers and overlapping food webs between the marsh complex and open water systems, as this export mechanism may be a critical link between emergent marshes and the aggregate estuarine productivity.

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