Using isotope analysis of historical zooplankton samples to detect long-term changes in low salinity zone food webs of the San Francisco estuary, 1976-2010

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ABSTRACT

The San Francisco estuary is the largest estuary on the North American Pacific coast. The estuary has undergone rapid and extensive ecological change in response to water diversion, changing nutrients, and invasive species. Two possible causes for the decline in phytoplankton population in the late 1980's are (1) changes in nutrient concentrations from 1982-2012, or (2) the introduction of an invasive clam, *Potamocorbula amurensis* (*P. amurensis*), in 1986. The pelagic fish (i.e. delta smelt, striped bass, longfin smelt) population declined significantly in 2000 and potentially indicates a disruption in the food web. One approach to assessing the health of an ecosystem is investigating bottom-up effects on food webs, such as researching food and primary organic matter sources that support growth. Pelagic fish consume zooplankton during larval and juvenile life stages, and zooplankton can consume phytoplankton, other zooplankton, and detritus. Carbon and nitrogen isotopic signatures correspond to the organic matter the zooplankton ingest, and stable isotope analysis measures the ratios of different carbon and nitrogen tracers. For *Eurytemora affinis* (*E. affinis*) and *Sinocalanus doerrii* (*S. doerrii*), the $\delta^{13}C$ and $\delta^{15}N$ value significantly decreased after the invasion of the clam. The $\delta^{13}C$ and $\delta^{15}N$ value increased for *Neomysis mercedis* (*N. mercedis*) post-clam invasion, which could reflect a shift towards an allochthonous or carnivorous-dependent diet. Stable isotopes of zooplankton detected shifts in diet towards freshwater sources and from a different trophic level within the San Francisco estuary. This study provides evidence of a disruption in the San Francisco estuary where restoration and management practices should be implemented.

KEYWORDS

Estuarine ecosystem; invasive species; stable isotopic composition; freshwater flow; feeding relationships.

INTRODUCTION

Estuarine ecosystems are biologically diverse and susceptible to ecological disturbances. An estuary receives tidal ocean fluxes with extensive seasonal variations of freshwater river discharge (Potter et al. 2010). The freshwater and saltwater flow in an estuary physically mixes nutrients and organisms at different ranges of salinity concentrations (Kimmerer 2004). Varying salinities and temperatures provide suitable niches for aquatic communities. The presence or absence of a species affects the ecosystem food web (Vernberg and Vernberg 1972; Ambler et al. 1985). Food webs have feeding relationships (Fry 2006) that transfer energy in the form of nutrients and organic matter via consumption from primary producers to consumers (Vernberg and Vernberg 1972; Riera et al. 2000).

The San Francisco estuary is highly impacted by water diversion, changing nutrients, invasive species, and contaminants (Ball and Arthur 1979; Cloern et al. 1985; Kimmerer et al. 1994; Orsi and Mecum 1996; Jassby and Cloern 2000; Kimmerer 2004; Gilbert 2010). The San Francisco estuary is the largest estuary on the North American Pacific coast (Kimmerer 2004) and is increasingly urbanized (Ball and Arthur 1979; Kimmerer and Orsi 1996; Sommer et al. 2007). Human interventions in the San Francisco Bay include water diversion to southern California and the Central Valley, nutrient overload from wastewater treatment discharge, and introduction of competitive invasive species that can alter natural habitats (Kimmerer 2004). Estuarine species respond to changes in habitat conditions, such as change in freshwater flow, salinity gradient, temperature, and species introductions (Ball and Arthur 1979; Ambler et al. 1985; Cloern et al. 1985; Kimmerer 2004; Maguire et al. 2006; Winder and Jassby 2010; Gilbert 2010). The freshwater inflow to the San Francisco estuary is highly variable, both seasonally and inter-annually. From 1956 to 2000, 85% of the annual total flow of freshwater originated from the Sacramento River and 11% from the San Joaquin River (Kimmerer 2002). Physical movement of water has a higher effect on slow-growing biota and is less likely to affect species that have a high turnover rate (Kimmerer 2002).

Food webs are feeding relationships that link complex interactions among species (Fry 2006). Organic matter is transferred in the food web from prey in low trophic levels to predators in high trophic levels (Jassby and Cloern 2000). Studying lower trophic levels investigates the food resources that support consumers (Fry 2006). A decline in pelagic fish and invertebrate

communities in the late 1980's caused concern for the health and quality of the San Francisco estuary (Sommer et al. 2007). One proposed approach to investigating that collapse of San Francisco estuarine pelagic organisms is to detect changes in primary consumer populations (Kimmerer 2004; Sommer et al. 2007). Zooplankton are abundant in the San Francisco estuary, can be carnivores or omnivores, and are an essential food source for pelagic fish. Zooplankton consistently inhabit their zone along salinity gradients due to their incapability to swim against tidal currents (Kimmerer et al. 1998), the availability of food, and the presence or absence of predators. A low-salinity zone (LSZ) is a mixture of freshwater and saltwater that produces a low salinity range for numerous species to inhabit.

Gilbert (2010) investigated nutrient loads to measure the impact on fish populations. A primary producer's population growth and an estuary's food and energy production are dependent on the concentration of various nutrients (Cloern 2001). According to Gilbert (1998), an ecosystem with high NO^{3-} concentration primarily leads to high fish production, while NH^{4+} supports microbial food webs that are nutrient-depleted. Gilbert (2010) categorized three major food web types in the San Francisco estuary as dominated by diatoms, flagellates, or more recently cyanobacteria, which has changed depending on the shifts in algal composition, food availability for upper trophic levels, and nutrient levels at that time: i. the diatoms era, prior to 1982, was abundantly populated with *E. affinis* and delta smelts; ii. followed by a period of mixed phytoplankton of smaller size (cryptophytes and green algae), *Pseudodiaptomus* (a calanoid copepod of similar size and carbon content as *E. affinis*), and bass-shad in the flagellates era from 1982 to 2000; iii. and a cyanobacteria era from 2000 to present, with abundant *Limnoithona* spp. (a small cyclopoid copepod species), silverside, largemouth bass, and sunfish dominating the LSZ of the San Francisco estuary.

An invasive species introduces a competitor that can change feeding relationships and shift the cycle of nutrients within the food web. Zooplankton population abundances have responded to food web disruptions in the San Francisco estuary due to the invasive clam species, *Potamocorbula amurensis* (*P. amurensis*). In 1986, *P. amurensis*, which has a wide salinity tolerance, long larval period, and rapid reproductive and growth rates, successfully invaded the upper San Francisco estuary (Nicolini and Penry 2000). There are several mechanisms, not mutually exclusive, by which *P. amurensis* can impact zooplankton: direct predation (*P. amurensis* is a filter-feeding organism that has been shown to ingest planktonic larvae; Kimmerer 2004), competition for

phytoplankton, and causing a shift in phytoplankton species composition. For example, *E. affinis* and *A. sinensis* populations declined starting in 1987-1988 most likely due to the feeding effects of *P. amurensis* (Kimmerer et al. 1994).

Zooplankton are consumers, key indicators of shifts in food webs, and controlled by abiotic and biotic factors (Kimmerer 2004; Moderan et al. 2010; Moderan et al. 2012). Carbon and nitrogen stable isotope composition can be used as "signatures of organic matter" that can be measured in each trophic level and could thus trace dietary changes (Fry 2006). Carbon and nitrogen isotopes can help identify a consumer's food source and trophic status (Fry 2006). This research determines if (1) stable isotopes can be used to detect long-term changes in zooplankton diet in historic samples of the San Francisco estuary and (2) if stable isotopes provide evidence of shifts due to (a) changing nutrients and phytoplankton composition and abundance (potentially linked to wastewater treatment discharge), and/or (b) the clam invasion. I hypothesize that the carbon stable isotope composition ratios will shift toward more negative values to indicate food originating from the upstream or watershed freshwater inputs as a result of the lower availability of locally produced zooplankton food (phytoplankton); and that zooplankton nitrogen stable isotope composition may have changed accordingly to reflect either an increased reliance on a detritus food-base or anthropogenic sources of nitrogen.

METHODS

Study System

Low-salinity zone

The LSZ is generally midway between ocean and riverheads, and has a salinity range between freshwater and 5 (Kimmerer 2004). The study sites are located at stations NZ052 and NZ054 in the Sacramento River at the mouth of Mallard Slough 38°2'45" N 121°54'23" W (Figure 1). The Fish and Wildlife Service collected samples and preserved zooplankton at sites NZ052 and NZ054 from 1976 to 2012 on behalf of the Interagency Ecological Program (IEP). Data from this LSZ study will be compared to freshwater zones in the San Francisco estuary in a separate study.

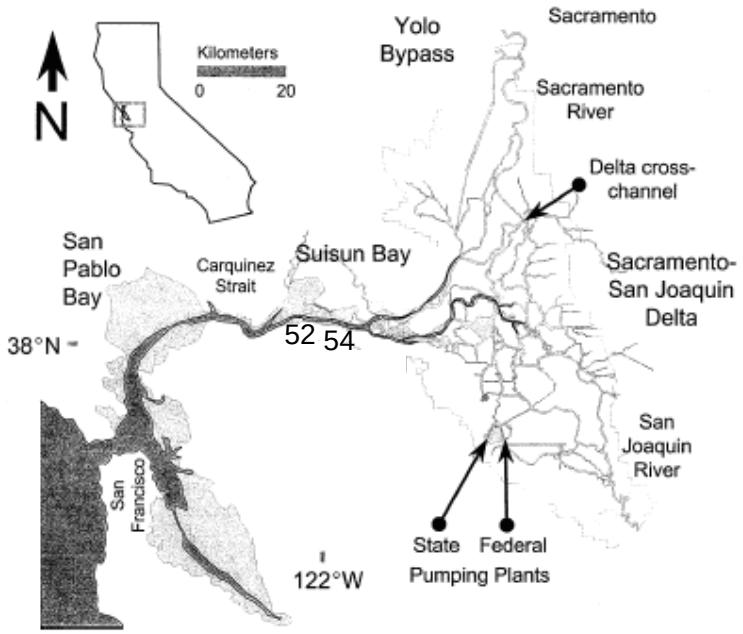

Figure 1. San Francisco estuary map. The zooplankton data collection sites NZ052 and NZ054 in the low-salinity zone (Source: Kimmerer 2002).

Species of interest

The copepod species commonly and abundantly found in the LSZ of the San Francisco estuary are *Acanthocyclops robustus* (*A. robustus*), *Acartiella sinensis* (*A. sinensis*), *Eurytemora affinis* (*E. affinis*), *Pseudodiaptomus forbesi* (*P. forbesi*), *Sinocalanus doerrii* (*S. doerrii*), and *Tortanus dextrilobatus* (*T. dextrilobatus*). Mysids are also in the crustacean subphylum as zooplankton, including *Hyperacanthomysis longirostris* (*H. longirostris*) and *Neomysis mercedis* (*N. mercedis*), and are commonly found in the LSZ. These zooplankton species can be carnivores or omnivores, and tolerate low ranges of salinity concentrations. All eight species are found in samples from 1976 to 2012 for a long-term comparison. Zooplankton have limited movement ability relative to its size and distance, primarily moving depending on the flow of water, and therefore the species consistently inhabit its zone over time.

Data Collection

Collecting zooplankton

To measure the change in dietary composition of zooplankton, I processed the Fish and Wildlife Service historic samples and Wim Kimmerer Lab recent samples. Zooplankton samples were collected using 150 micron mesh nets from horizontal tows within 1 meter of water surface, which captured zooplankton of specific size and filtered smaller excess debris. Field tows occurred monthly to investigate changes over time. As part of a larger project aimed at studying planktonic food webs in the San Francisco estuary, I also processed recently collected samples of zooplankton that had been allowed to clear their gut in filtered local water, and had then been frozen; as well as live specimen collected and temporarily stored in water of similar salinity. Historical samples from the IEP (Fish and Wildlife Service) were preserved with 5% buffered formaldehyde and 95% deionized water in 50 ml sample bottles. Although some previous research claimed that preservatives could prevent the use of such samples for stable isotope analysis, recent evidence supports that formaldehyde preservation has a stable and predictable impact on the stable isotope composition of zooplankton (Moderan, unpublished data 2014). Stable isotope analysis could thus be reliably applied to study long-term temporal trends and could potentially be used in food web studies after applying an experimentally determined correction factor (Moderan, unpublished data 2014).

Processing samples

I identified, sorted, counted, and processed zooplankton for stable isotope analysis. Historic samples were retrieved using a 100 micron mesh sieve, rinsed with deionized water to clean excess chemicals, and transferred to a counting tray. Under a dissecting microscope, I identified zooplankton to the species level and handpicked individual zooplankton of adult and late copepodite stages using tweezers. To meet the mass requirements for stable isotope analysis using the mass spectrometer, I transferred between 5 to 300 individuals of each species, depending on their size and carbon and nitrogen content, from the counting dish to tins. Samples in the tin capsules were dried at 60°C, then folded and sealed using tweezers. The packaged tin boxes were

sent to University of California Davis Stable Isotope Facility for mass spectrometry processing. The mass spectrometry has been calibrated against NIST Standard Reference Materials and sample values are corrected based on known laboratory standards (long term standard deviation for $\delta^{13}C$ at 0.2 permil and $\delta^{15}N$ at 0.3 permil) (UC Davis Stable Isotope Facility).

Mass spectrometry

Samples undergo combustion until presented as a simple gas (Fry 2006). Gas molecules are ionized, lose electrons and therefore become positively charged, and are accelerated via electric fields (Fry 2006). A magnetic field, utilizing inertia, separates ions according to their atomic mass and isotope, and a computer tallies the counts to calculate the final isotope values (Fry 2006).

Data Analysis

Stable isotope analysis

Stable isotopes can be used to study the origins and cycling of organic matter (Fry 2006). The isotope ratios, $^{13}C/^{12}C$ and $^{15}N/^{14}N$, can be traced through the food web as signatures reflecting consumer's diets (Maguire and Grey 2006). Although $\delta^{13}C$ is often more useful to distinguish different primary producers in the diet of consumers, the $\delta^{15}N$ increases linearly along food webs, and therefore is often used to estimate the trophic levels of a given organism. The δ (delta) notation signifies the difference between isotope values. The difference is calculated using the following equation: $\delta^{H}X = [(R_{SAMPLE}/R_{STANDARD} - 1)]\times 1000$, where H represents the heavy isotope mass of element X, R is the ratio of heavy to light isotope of the element (i.e. $^{13}C/^{12}C$ and $^{15}N/^{14}N$), and multiplying by 1000 amplifies small differences (Fry 2006). The difference in stable isotopes is measured in units of permil (‰) or parts per thousand. The natural abundance range of δ values is between -100 and +50‰. When the δ value is negative, there is less heavy isotope than the standard value, and therefore if δ is 0‰, then there is no difference from the standard. Higher δ values indicated samples enriched in heavy isotopes and lower δ values represent samples enriched in lighter isotopes (Fry 2006).

R statistical analysis

The R software was used to determine the yearly and monthly carbon and nitrogen isotope ratio trends (R Core Development Team 2014). Field sampling data from the IEP monitoring program recorded changes in zooplankton abundance and diversity, salinity, temperature, and stable isotopes $\delta^{13}C$ and $\delta^{15}N$. I plotted temperature, salinity, and chlorophyll *a* (chl *a*) concentrations from 1975 to 2010 to determine trends in environmental conditions. I tested the normality of data with a Shapiro-Wilk test. Most of the data was normally distributed. I calculated the variation from normal distribution. Boxplots and t-tests (for comparison between 2 or more groups, or non-parametric equivalents when the assumptions for use of parametric tests were not met) compared the carbon and nitrogen isotope compositions for each species before and after the clam invasion, and Kruskal-Wallis tests (for comparison between 3 or more groups) compared those between the three nutrient eras proposed by Gilbert (2010).

RESULTS

Historical record of samples

Out of the 241 samples taken from the NZ052 and NZ054 LSZ sites combined, 33 samples were collected prior to clam invasion and the remaining 208 samples following the invasion. Samples processed from the diatom, flagellate, and cyanobacteria eras were 17, 31, and 193 respectively. Out of the 8 zooplankton species present at both low-salinity sites, three species (*E. affinis*, *N. mercedis*, and *S. doerrii*) were present in the LSZ pre- and post-clam invasion. The remaining species were found only post-clam invasion. Divided among the diatom, flagellate, and cyanobacteria eras, *E. affinis* and *S. doerrii* were found in all three, and *A. sinensis*, *H. longirostris*, *N. mercedis*, and *P. forbesi* were collected in two of the three eras.

Normality of data

The Shapiro-Wilk normality test concluded that 7 of the 8 species' carbon isotopic values follow a normal distribution. The carbon isotope values for *A. robustus*, *A. sinensis*, *E. affinis*, *H. longirostris*, *N. mercedis*, and *T. dextrilobatus* (p-value < 0.05, respectively), and *P. forbesi* (p-value < 0.01) came from a normally distributed dataset (Table 1). The p-value for *S. doerrii* rejects the null hypothesis and concludes that the data did not come from a normally distributed set. The Shapiro-Wilk test concluded that nitrogen isotope values for all 8 species came from a normal distribution (p-value < 0.05; Table 1). The variance of $\delta^{13}C$ and $\delta^{15}N$ was unequal for all species (Table 1), which concludes to use the Welch t-test to test if there is a difference between the means of two or more groups.

Table 1. Normality of zooplankton species. Shapiro-Wilk p-values and variances testing normality of data.

Zooplankton Species	$\delta^{13}C$ P-Value	$\delta^{15}N$ P-Value	$\delta^{13}C$ Variance	$\delta^{15}N$ Variance
A. robustus	0.2962	0.193	4.3353	6.3488
A. sinensis	0.175	0.3391	1.0722	1.8870
E. affinis	0.1781	0.3545	4.7561	2.8209
H. longirostris	0.2585	0.9413	1.5473	4.9103
N. mercedis	0.1287	0.1764	3.0090	4.1544
P. forbesi	0.02691	0.8729	1.7157	3.5941
S. doerrii	0.004817	0.1483	3.2588	2.8388
T. dextrilobatus	0.3724	0.06635	0.6573	0.9820

Environmental conditions in the LSZ

The environmental conditions varied monthly. Surface water temperature reached a maximum mean temperature in the month of July and minimum temperature in January from 1975 to 2012. Annual mean temperature relatively declined from 1975 to 2012 (Appendix A). Temperature steadily increased from 1975, peaked in 1988, and continued to decline in 2007 until reached a minimum in 2012 (Figure 2). The freshwater river discharge was low from May to November and high from December to March. Higher freshwater river discharge corresponds to low salinity values downstream over time, which includes low salinity means of 3.65 to 2.77 from January to May, respectively, and high salinity means of 5.27 to 5.59 from September to December, respectively. The maximum mean salinity was in 1977, dropped to a relatively consistent low salinity in 1979, and rose in 1990, followed by a drop in 1995, and finally was relatively stable until 2012 (Figure 3; Appendix A). High mean chlorophyll *a* values ranged from

6.76 to 8.59 µg/L in May to August, respectively, and low mean chlorophyll *a* values ranged from 2.98 to 2.48 µg/L in November to February, respectively. Chlorophyll *a* values declined after year 1987 corresponding to the introduction of invasive clams *P. amurensis* (Table 2). Mean chlorophyll *a* peaked in 1979, and significantly dropped in 1987, where it relatively held consistently low until 2012 (Figure 3).

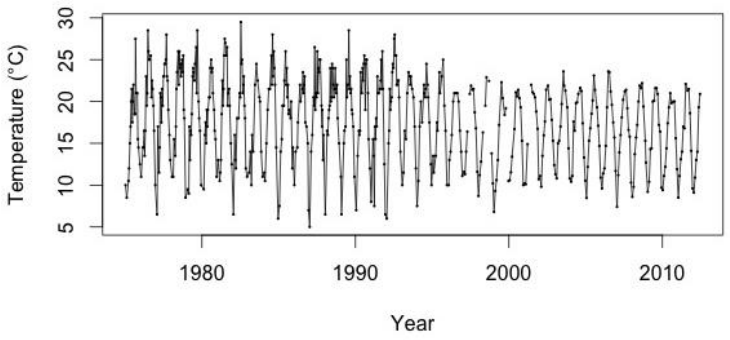

Figure 2. Temperature in the LSZ. Temperature variation yearly from 1975 to 2012. Every temperature recording is indicated by a black dot.

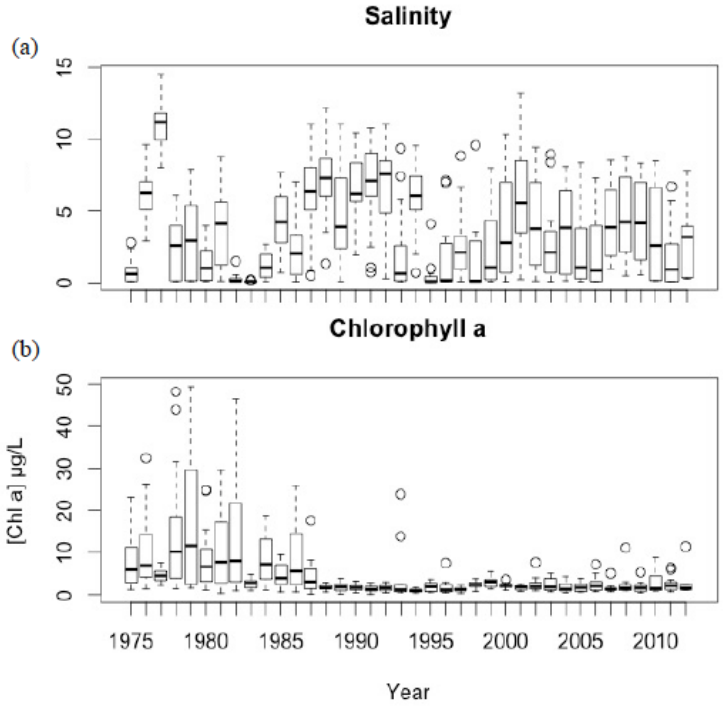

Figure 3. Salinity and chlorophyll *a* recorded yearly. The IEP measured (a) salinity and (b) chlorophyll *a* at the low salinity zone sites from 1975 to 2012.

Table 2. Significant decline in chlorophyll *a*. Chlorophyll *a* mean, minimum (Min), and maximum (Max) every other year from 1975 to 1995.

Year	Mean Chl *a* (µg/L)	Min Chl *a* (µg/L)	Max Chl *a* (µg/L)
1975	8.046	1.170	23.160
1977	4.544	2.160	7.560
1979	16.48	1.55	49.40
1981	10.96	0.31	29.64
1983	2.561	0.930	4.790
1985	4.692	0.620	9.420
1987	4.228	0.150	17.600
1989	1.7080	0.1500	3.8600
1991	1.410	0.050	2.840
1993	4.158	0.540	23.820
1995	1.8480	0.5600	3.6400

Zooplankton $\delta^{13}C$ and $\delta^{15}N$ isotope compositions

Nutrients and phytoplankton hypothesis. The carbon and nitrogen isotope values were higher during the diatom era before 1982 than the following eras for *E. affinis*, *P. forbesi*, and *S. doerrii* (Figure 4). The mean $\delta^{13}C$ and $\delta^{15}N$ for *E. affinis* decreased between the diatom era and the cyanobacteria era (Appendix B). *E. affinis* and *S. doerrii* are the only species with complete data for all three eras. The mean $\delta^{13}C$ for *S. doerrii* decreased between diatom, flagellate, and cyanobacteria eras from -28.33 to -30.11‰ (Appendix B). Similarly, the mean $\delta^{15}N$ for *S. doerrii* slightly increased after the diatom era to 12.67‰ in the flagellate era, and significantly decreased to 11.06‰ during the cyanobacteria era. *P. forbesi* mean $\delta^{13}C$ and $\delta^{15}N$ decreased from the flagellate to cyanobacteria era (no data for diatom era). For comparison, the mean $\delta^{13}C$ decreased and the $\delta^{15}N$ increased for *N. mercedis* from the diatom to flagellate eras (no data for cyanobacteria era). In contrast, the mean $\delta^{13}C$ increased and $\delta^{15}N$ decreased for *A. sinensis* from flagellate to cyanobacteria era (no data for diatom era).

The carbon isotope composition of 2 out of 4 species (*E. affinis* and *S. doerrii*) differs significantly between the three nutrient eras (Kruskal-Wallis t-test; p-value < 0.05; Table 3). All 4 species' nitrogen isotope values are significantly different between the three eras (Kruskal-Wallis t-test; p-value < 0.05; Table 3).

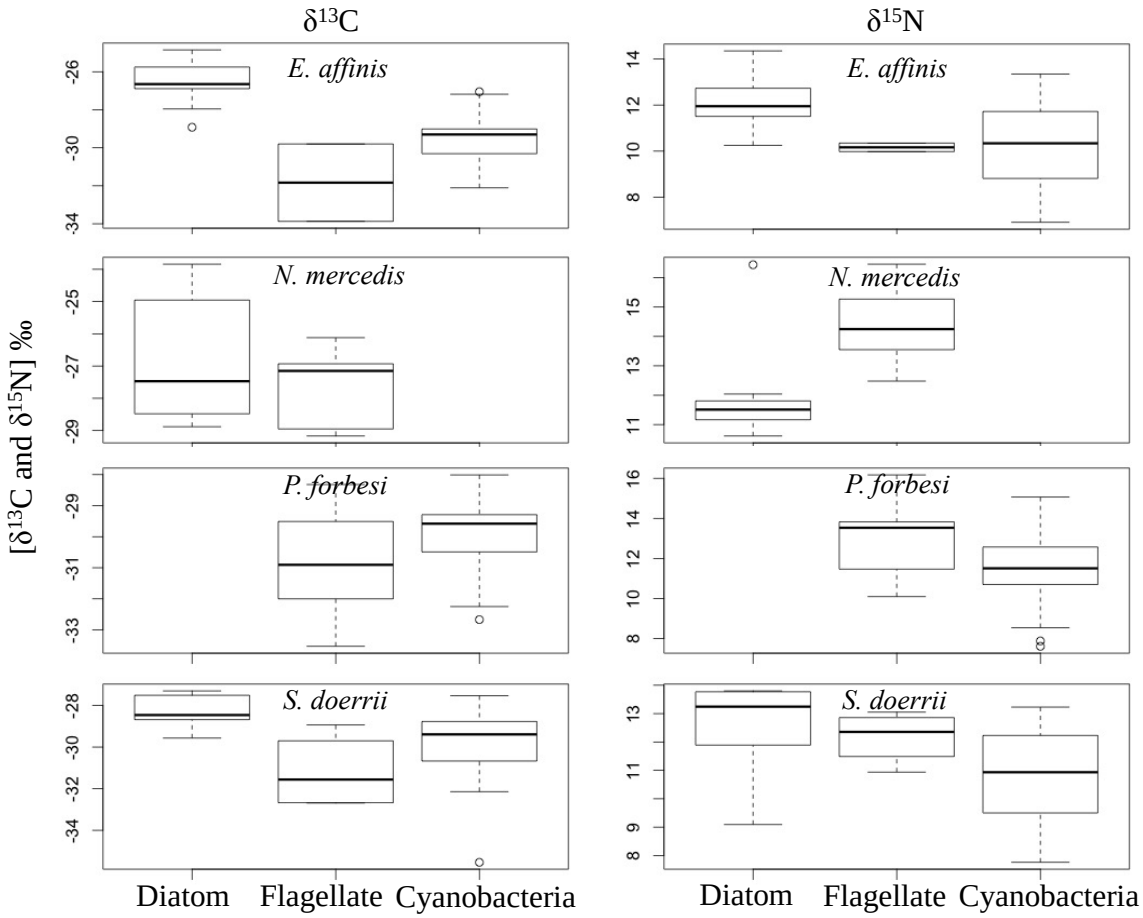

Figure 4. $\delta^{13}C$ and $\delta^{15}N$ values during nutrient eras. Boxplots of $\delta^{13}C$ and $\delta^{15}N$ for each zooplankton species in the diatom, flagellates, and cyanobacteria dominated eras. Please note the different scales.

Table 3. Testing significant different between nutrient eras. Kruskal-Wallis p-values for four species' $\delta^{13}C$ and $\delta^{15}N$ data for the diatom, flagellate, and cyanobacteria eras.

Species	$\delta^{13}C$ P-value	$\delta^{15}N$ P-value
E. affinis	1.313e-05	0.01288
N. mercedis	0.2774	0.01809
P. forbesi	0.09618	0.03144
S. doerrii	0.01812	0.04687

Clam invasion hypothesis. After 1986, *E. affinis* mean $\delta^{13}C$ value decreased from -26.55 pre-calm to -29.84‰ post-clam invasion (Table 4). Similarly, the mean $\delta^{13}C$ for *S. doerrii* dropped after clam invasion from -28.42 to -30.11‰. In contrast, *N. mercedis* mean $\delta^{13}C$ increased slightly after the clam invasion from -27 to -27.54‰. The $\delta^{15}N$ responded in similar trends as the $\delta^{13}C$ before and after *P. amurensis* introduction (Figure 5). The mean $\delta^{15}N$ decreased after clam

invasion for *E. affinis* and *S. doerrii*. Similar to $\delta^{13}C$ increasing for *N. mercedis*, the mean $\delta^{15}N$ increased after clam invasion from 12.12 to 14.72‰ (Table 4).

The carbon isotope composition of 2 out of 3 species (*E. affinis* and *S. doerrii*) differs significantly before and after the clam invasion (Welch two-sample t-test; p-value < 0.05; Table 5). The nitrogen isotope composition of 2 out of 3 species (*E. affinis* and *N. mercedis*) differs significantly pre- and post-clam invasion (Welch two-sample t-test; p-value < 0.05; Table 5).

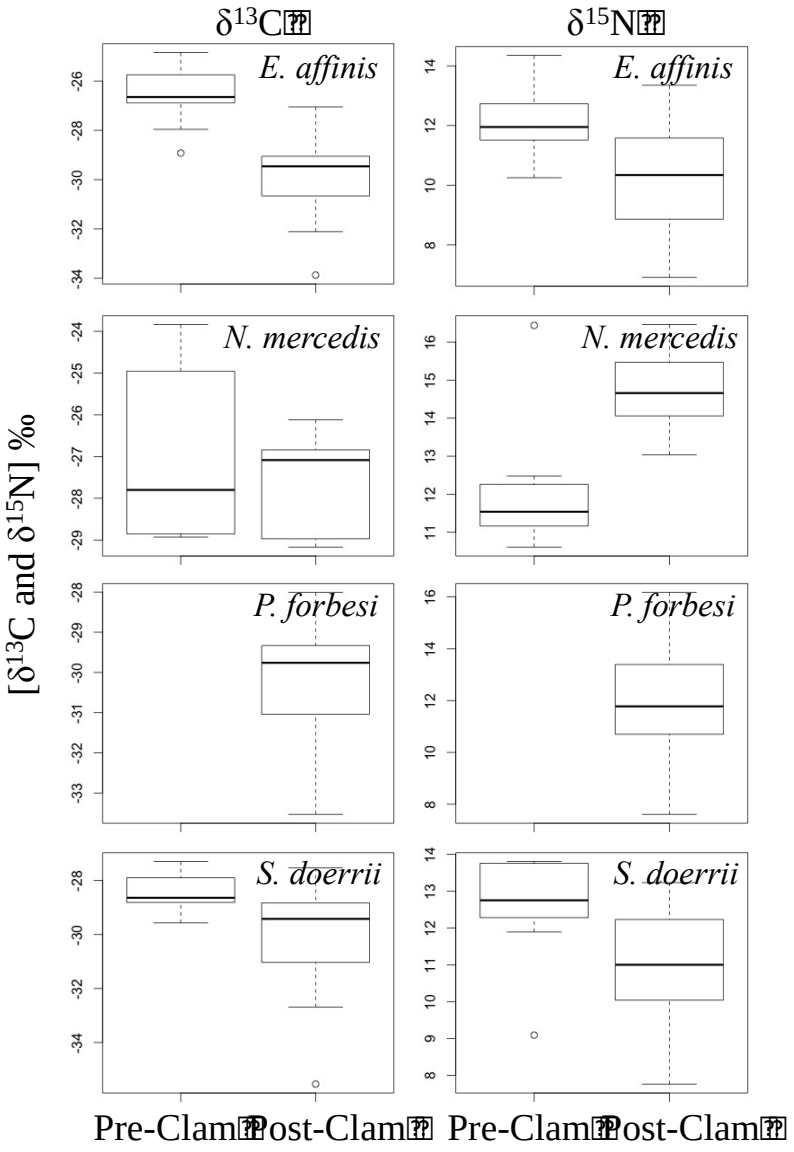

Figure 5. Change in $\delta^{13}C$ and $\delta^{15}N$ after clam invasion. Boxplots of $\delta^{13}C$ and $\delta^{15}N$ for zooplankton species pre- and post-clam invasion.

Table 4. Affect of clam invasion on zooplankton species' $\delta^{13}C$ and $\delta^{15}N$ values. Mean $\delta^{13}C$ and $\delta^{15}N$ concentrations for each zooplankton species pre- and post-clam invasion.

	$\delta^{13}C$ Pre-Clam Invasion						$\delta^{13}C$ Post-Clam Invasion					
Species	Min	1st Qu.	Median	Mean	3rd Qu.	Max	Min	1st Qu.	Median	Mean	3rd Qu.	Max
E. affinis	-28.92	-26.89	-26.65	-26.55	-25.75	-24.85	-33.87	-30.66	-29.46	-29.84	-29.05	-27.05
N. mercedis	-28.93	-28.84	-27.80	-27.00	-24.99	-23.84	-29.17	-28.52	-27.08	-27.54	-26.88	-26.12
P. forbesi	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	-33.53	-30.94	-29.76	-30.19	-29.36	-28.01
S. doerrii	-29.57	-28.81	-28.64	-28.42	-27.90	-27.30	-35.54	-31.03	-29.42	-30.11	-28.83	-27.53

	$\delta^{15}N$ Pre-Clam Invasion						$\delta^{15}N$ Post-Clam Invasion					
Species	Min	1st Qu.	Median	Mean	3rd Qu.	Max	Min	1st Qu.	Median	Mean	3rd Qu.	Max
E. affinis	10.25	11.51	11.95	12.05	12.73	14.35	6.91	8.86	10.34	10.32	11.58	13.35
N. mercedis	10.61	11.21	11.54	12.12	12.15	16.44	13.04	14.11	14.66	14.72	15.37	16.46
P. forbesi	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	7.61	10.72	11.77	11.91	13.38	16.17
S. doerrii	9.09	12.28	12.75	12.53	13.76	13.80	7.76	10.04	11.00	11.06	12.23	13.23

Table 5. Significant difference after clam invasion. Welch two-sample t-test for $\delta^{13}C$ and $\delta^{15}N$ values comparing means before and after the clam invasion.

Species	$\delta^{13}C$ P-value	$\delta^{15}N$ P-value
E. affinis	3.445e-07	0.001858
N. mercedis	0.5528	0.007726
S. doerrii	0.001562	0.06698

Trends in stable isotopes and correlations with potential environmental drivers

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ analyzed shifts in dietary organic matter. From 1976 to 2012, $\delta^{13}C$ values ranged between -24 to -34‰ and $\delta^{15}N$ values ranged between 6 to 16‰ for *E. affinis*, *N. mercedis*, *P. forbesi*, and *S. doerrii* (Figure 6). *E. affinis* and *S. doerrii* have the largest range of $\delta^{13}C$ values with a minimum and maximum of -33.87 to -24.85‰ and -35.54 to -27.3‰, respectively (Table 6). *T. dextrilobatus* has the smallest $\delta^{13}C$ range of values (Table 6). *H. longirostris*, *P. forbesi*, and *A. robustus* have the largest range of $\delta^{15}N$ values, and *S. doerrii* and *T. dextrilobatus* have the smallest $\delta^{15}N$ value range (Table 6).

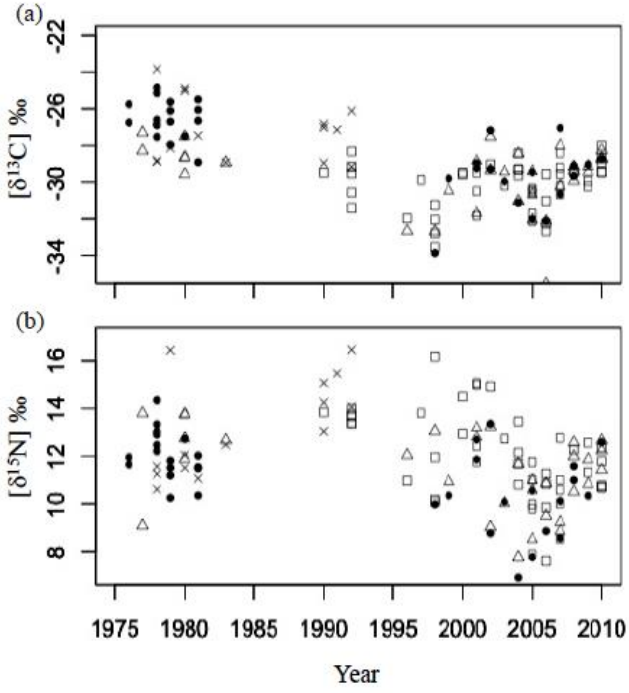

Figure 6. Zooplankton isotope trends over time. Scatterplots of (a) carbon and (b) nitrogen isotope values for each zooplankton species over time. *E. affinis* (filled dot), *N. mercedis* (cross), *P. forbesi* (square), and *S. doerrii* (triangle) plotted by year.

Table 6. Comparing zooplankton $\delta^{13}C$ and $\delta^{15}N$ values. Minimum (Min), median, mean, and maximum (Max) $\delta^{13}C$ and $\delta^{15}N$ values for all eight zooplankton species.

Species	$\delta^{13}C$ Min	$\delta^{13}C$ Median	$\delta^{13}C$ Mean	$\delta^{13}C$ Max	$\delta^{15}N$ Min	$\delta^{15}N$ Median	$\delta^{15}N$ Mean	$\delta^{15}N$ Max
A. robustus	-34.31	-31.74	-31.34	-28.41	8.70	10.82	11.49	16.85
A. sinensis	-31.35	-28.88	-29.09	-25.89	10.15	13.22	13.19	17.14
E. affinis	-33.87	-27.75	-28.20	-24.85	6.91	11.52	11.18	14.35
H. longitostris	-30.66	-27.93	-27.84	-25.49	8.23	13.08	13.19	18.21
N. mercedis	-29.17	-27.31	-27.23	-23.84	10.61	12.76	13.24	16.46
P. forbesi	-33.53	-29.76	-30.19	-28.01	7.61	11.77	11.91	16.17
S. doerrii	-35.54	-29.37	-29.74	-27.30	7.76	11.76	11.38	13.80
T. dextrilobatus	-29.16	-27.43	-27.39	-26.25	12.44	14.81	14.83	17.67

DISCUSSION

Historical findings in the LSZ

Analyzing stable isotopes can help detect changes in diet. The study provided 24 years of data after the clam invasion and 10 years before the clam invasion. Since the majority of the samples were collected during the cyanobacteria era, it is possible the limited number of samples from the diatom and flagellate era somewhat impaired our ability to detect changes between eras. Overall, we found a shift in carbon and nitrogen isotope signatures, probably indicating a change in organic matter sources supporting some zooplankton species over the 34-year period. The clam invasion in 1986 marked the shift in diet (Sommer et al. 2007). The grazing effects of clams (Kimmerer 2004) resulted in a decreased abundance of phytoplankton in the low-salinity zone and an apparent shift toward more $\delta^{13}C$-depleted and $\delta^{15}N$-depleted samples for *E. affinis* and *S. doerrii*. This study measured some trophic effects of modifications in the San Francisco estuary ecosystem, which is important for future water management planning (Gilbert 2010).

Zooplankton species as indicators of changes

Zooplankton are an average 1 mm in length and commonly categorized into freshwater, low-, and high-saline zones depending on the species' salinity tolerance (Kimmerer and Orsi 1996; Kimmerer 2004). The most abundant species found in the LSZ samples for which we found significant differences in stable isotope composition were *E. affinis*, *N. mercedis*, *P. forbesi*, and *S. doerrii*. *P. forbesi* expanded in the LSZ in 1989 and tolerates wider salinity ranges than *E. affinis*

in the San Francisco estuary (Orsi and Walter 1991; Kimmerer and Orsi 1996). *S. doerrii* was first detected in the San Francisco estuary in 1978 in estuarine zones between freshwater and low-salinity (Kimmerer and Orsi 1996). *E. affinis* and *S. doerrii* are present all year (Ambler et al. 1985) and therefore the changes in species abundance reflects modifications in the environmental conditions. Kimmerer (2004) observed a significant decline in *E. affinis* after 1970. Potential causes for the population decline includes less organic input to the estuary, decline in phytoplankton populations due to increased export pumping and benthic grazing, and effects of pesticides (Kimmerer 2004). This data showed that *E. affinis* had significant changes in diet. In a comparable study of an increasingly urbanized estuary, the Chesapeake Bay, *E. affinis* has been found to be able to obtain some of its nutrition from detritus (Heinle et al. 1977). In this study, the $\delta^{13}C$ and $\delta^{15}N$ values for *E. affinis* decreased, which corresponds to a diet from a lower trophic level (or from an area where the phytoplankton nitrogen isotopic baseline was slightly lower) and a higher contribution from freshwater or terrestrial-derived food sources. Similarly, *P. forbesi* populations declined in summer months possibly due to limited food available (Kimmerer 2004). Low chlorophyll *a* values after 1986 correspond to low phytoplankton biomass, particularly during high flows in the LSZ because fewer phytoplankton are present (Jassby and Cloern 2000).

Mysids are less than 20 mm in length and common in the LSZ (Kimmerer 2004). Juvenile mysids mostly eat plants, although some mysid species larger than 3 mm can be carnivorous and consume copepods (Siegfried and Kopache 1980). Previous research found that *H. longirostris* and other introduced mysid species (Modlin and Orsi 1997) became more abundant than *N. mercedis* in the San Francisco estuary. This study found few samples of *N. mercedis* possibly due to the species' small salinity range and high variation (Heubach 1969; Kimmerer et al. 1998). In addition to salinity tolerance ranges, *N. mercedis* population declined in late 1980s due to food limitation (Orsi and Mecum 1996) and increased temperature in summer months (Heubach 1969). The mean carbon and nitrogen isotope signature for *N. mercedis* increased from 1978 to 1992 (Appendix C), which potentially corresponds to adapting to newly available food.

Correlation with environmental conditions

The surface water temperature values were high in the summer months and low in winter months. Salinity increased during summer months and declined in winter, which is potentially due to high precipitation during months September to December that flushes freshwater through the LSZ. Salinity fluctuated over 36 years, which reflects wet and dry years that leads to longitudinal shifts of the salinity gradient. Increased salinity input moves the LSZ upstream, similar to moving the LSZ downstream during wet years; however, the NZ052 and NZ054 stations did not relocate and the data collected were consistently at these two sites. The change in salinity can have implications on the habitat for aquatic species.

The data reflects a decline in chlorophyll *a* values, which supports the hypothesis that the lack of phytoplankton biomass caused a shift in zooplankton diet due to a change in food source. Kimmerer (2004) provides supporting evidence that chlorophyll *a* in the San Francisco estuary declined sharply in late 1980s in low-salinity areas. Possible explanations for decline in phytoplankton biomass can be caused by seasonal changes or increased benthic grazing (Alpine and Cloern 1992; Kimmerer 2004). This study observed a decline in chlorophyll *a* potentially indicating that less phytoplankton was produced locally, and leading to a shift towards more δ^{13}C-depleted sources that are usually found upstream in the freshwater. The δ^{13}C-depleted sources could be freshwater phytoplankton or terrestrial C3 plants in the form of detritus from the watershed.

The rapid decline in chlorophyll *a* after 1986 corresponds to the time of the clam's, *P. amurensis*, introduction and establishment in the LSZ. This correlation, highlighted by Kimmerer (2002) and Winder and Jassby (2010), suggests that the clam filtration altered the base of the food web. One hypothesis is that the zooplankton are now more subsided by allochthonous primary production. The invasive clam began depleting phytoplankton populations and therefore the zooplankton that are present in the LSZ are either able to feed on smaller particles (such as the now abundant invasive cyclopoid copepod, *Limnoithona* spp), or shifted their diet to food sources subsidized from upstream (e.g. *E. affinis* and *P. forbesi*). Smaller zooplankton species (*Limnoithona* spp.) better adapted to feeding on smaller phytoplankton and microzooplankton became more abundant in the LSZ compared to larger herbivorous zooplankton species, *E. affinis* and *P. forbesi* (Winder and Jassby 2010). Kimmerer (2004) concluded that more trophic steps are less efficient and leads to a shift from large to small copepods available for fish, potentially explaining the recent declines in pelagic fish population in the San Francisco estuary. Similarly,

the introduction of zebra mussels to the Irish Lake, Lough Erne, reduced chlorophyll *a* values, and lead to an increase reliance on allochthonous food sources for *E. affinis* in the lake ecosystem (Maguire and Grey 2006).

Zooplankton $\delta^{13}C$ and $\delta^{15}N$ isotope compositions

Fewer phytoplankton impacts zooplankton diet. Nutrient levels can limit the growth of biomass (Kimmerer 2004). Wastewater discharge contributed to the change in nitrogen nutrient loads in the San Francisco estuary (Gilbert 2010). The mean $\delta^{13}C$ values were highest in diatom era and declined in flagellate era and cyanobacteria era for *E. affinis*, *P. forbesi*, and *S. doerrii*. The $\delta^{13}C$ and $\delta^{15}N$ values declined for *E. affinis*, *P. forbesi*, and *S. doerrii* from 1976 to 2012, indicating a shift in the origin and trophic level of food source. $\delta^{13}C$-depleted values from 1976 to 2012 corresponded to food originating from freshwater sources (Cloern 2002). Higher $\delta^{15}N$ values for *N. mercedis* indicate a shift towards feeding from higher trophic levels. One hypothesis explaining an increase in $\delta^{15}N$ values is because fewer phytoplankton may have caused a shift towards a slightly more carnivorous diet in *N. mercedis*, or an increased reliance on detritus, which tends to become $\delta^{15}N$-enriched as a result of bacterial degradation. With limited phytoplankton available as a food source, zooplankton are forced to rely on additional sources for nutrition.

Effect of clam invasion. The timing of changes in $\delta^{13}C$ and $\delta^{15}N$ values for *E. affinis*, *N. mercedis*, *P. forbesi*, and *S. doerrii* corresponds with the establishment of the invasive *P. amurensis*. The grazing effects of the invasive clam modified the environment by increasing predation on phytoplankton, which reduced the food available for zooplankton (Sommer et al. 2007; Kimmerer 2004). Studies found *E. affinis* population declined in 1987-1988 potentially caused by *P. amurensis* consuming copepod nauplii (Kimmerer et al. 1994). Maguire and Grey (2006) used stable isotope analysis to determine the change in zooplankton diet after the introduction of zebra mussels. Similar to Maguire and Grey (2006), the decrease in $\delta^{13}C$ represented a shift from a phytoplankton food source to allochthonous matter from terrestrial sources. After the clam invasion, $\delta^{15}N$ values decreased for *E. affinis* and *S. doerrii*, which corresponds to feeding at a lower trophic level. However, the $\delta^{15}N$ value for *N. mercedis* increased and is potentially feeding from a higher trophic level. Benthic grazing is a leading cause for reduced biomass values (Alpine

and Cloern 1992) and the sharp decline in chlorophyll *a* values after the clam invasion indicates that the clams reduced phytoplankton biomass and food availability for zooplankton species that were previously abundant.

Limitations and Future Directions

In this study, different researchers collected samples over the time span of this project. However, both the Fish and Wildlife Service and Kimmerer Lab processed the samples by the same procedure. Samples were collected on a monthly basis, although there are some gaps in the dataset over the 34-year time scale. Some of the oldest samples from the monitoring program have been lost or dried out and unable to be used in this study. Careful preservation and curating practices should be encouraged to ensure future methods that would allow for new scientific studies. Using stable isotope analysis as a method to detect long-term changes in estuarine food web needs further investigation. This research is part of a larger, ongoing project comparing different sites in the San Francisco estuary. This site in particular is low salinity and will be compared to freshwater, and moderate to high salinity sites for a greater understanding of the dynamic estuary.

This research provides evidence of one possible cause for changes in the food web. When investigating the impacts of invasive species using stable isotope analysis, samples must include collections before the invasion (Maguire and Grey 2006). Stable isotopes are one method, however there are other methods suitable to detect a change in the food web. An isotope mass spectrometer is not the only method to measure isotope values. Lasers are used to detect isotope differences in gas molecules using infrared absorption bands (Fry 2006). A laser can produce thousands of isotope numbers each day, compared to 50 to 500 values produced by a mass spectrometer (Fry 2006).

Isotopes can be altered by factors other than nutrient loads and organic mater cycling. One possible factor affecting the nitrogen isotope composition is a shift in the nitrogen baseline signature where the $\delta^{15}N$ values increased linearly for each trophic level. In addition, an increase in $\delta^{15}N$ values can indicate a higher load of anthropogenic nitrogen sources, possibly from wastewater treatment discharge (Riera et al. 2000). The San Francisco estuary has been physically modified by humans, including diking and converting 95% of estuary's wetlands, introducing

exotic species, reducing or eliminating stocks of fish and invertebrates, increasing loading of contaminants from hydraulic mining, and disposing and accumulating agricultural and urban waste (Nichols et al. 1986). There are many factors that contribute to changes in the estuary's food web, which causes difficulty to find one definite cause for the change. For example, fertilizer input into the San Francisco estuary affect food webs by altering phytoplankton species composition (Gilbert 2010). However, this study detected a decrease in $\delta^{15}N$ values in the LSZ, but research from freshwater stations provides evidence of wastewater treatment discharge effects.

Broader Implications

This research is a part of a larger ongoing project comparing different salinity zones in the San Francisco estuary. Additional studies in other estuaries in North America and around the world can possibly support the findings in this study. Studies using stable isotope analysis could support or contradict this method as sufficient quantitative evidence. Stable isotope analysis can be used as evidence to support programs to restore and clean the bay. Costanzo et al. (2001) reported high ^{15}N nutrient releases from sewage treatment facilities, which stimulated progressive efforts to restore water quality. Additional long-term research on food webs would strengthen this study so that this study could be used in reference to other estuaries. Previous research and this study provide evidence that stable isotope analysis can be used to detect changes in a food web.

CONCLUSION

In this research, stable isotope analysis suggests that zooplankton diet shifted in the San Francisco estuary. Evidence shows a significant change in carbon and nitrogen isotope signatures after the invasive clam introduction. Stable isotope analysis is one method used to detect long-term changes in food webs. The San Francisco estuary zooplankton species can be used as bioindicators to assess water quality.

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APPENDIX A: Temperature and Salinity Values

Table A1. Values of environmental conditions. Temperature and salinity mean and standard deviation (SD) yearly from 1975 to 2012.

Year	Temperature Mean	Temperature SD	Salinity Mean	Salinity SD
1975	17.000	4.896	0.521	0.517
1976	19.525	5.250	5.590	1.510
1977	19.550	5.274	10.486	1.312
1978	19.895	5.646	2.065	1.917
1979	18.816	5.718	2.766	2.381
1980	18.528	4.303	1.438	1.431
1981	19.526	5.579	3.988	2.953
1982	18.361	5.795	0.259	0.339
1983	16.667	4.997	0.098	0.041
1984	19.333	6.413	1.181	0.876
1985	18.583	4.506	4.380	2.059
1986	17.700	4.891	2.419	2.232
1987	19.550	5.173	6.318	2.772
1988	19.700	4.137	7.083	2.447
1989	18.694	5.380	4.522	3.031
1990	19.583	5.375	6.552	2.310
1991	17.579	5.855	6.884	2.949
1992	21.025	5.139	6.580	3.204
1993	18.231	4.785	2.254	3.180
1994	17.633	5.156	5.751	2.348
1995	17.583	4.907	0.547	1.153
1996	16.467	4.064	1.851	2.645
1997	16.918	4.264	2.790	2.770
1998	15.318	5.274	1.846	3.194
1999	14.769	5.368	2.246	2.699
2000	16.395	4.446	3.853	3.559
2001	16.595	4.820	5.970	3.981
2002	16.438	4.109	4.271	3.310
2003	16.700	4.478	2.829	2.973
2004	16.693	4.311	3.653	3.118
2005	16.489	4.395	2.397	2.713
2006	16.650	4.866	2.227	2.748
2007	15.942	4.500	4.200	2.538
2008	16.508	4.676	4.641	3.020
2009	16.317	4.697	4.324	2.917
2010	16.008	4.110	3.230	3.257
2011	15.642	4.668	1.799	2.327
2012	14.533	4.661	3.113	2.806

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APPENDIX B: Isotope Values by Nutrient Era

Table B1. Nutrient era isotope values. $\delta^{13}C$ and $\delta^{15}N$ values during each nutrient era. Minimum (Min), 1st quartile (1st Qu.), median, mean, 3rd quartile (3rd Qu.), and maximum (Max) values recorded for five species for diatom, flagellate, and cyanobacteria era.

Species	$\delta^{13}C$ Diatom Era						$\delta^{13}C$ Flagellate Era						$\delta^{13}C$ Cyanobacteria Era					
	Min	1st Qu.	Median	Mean	3rd Qu.	Max	Min	1st Qu.	Median	Mean	3rd Qu.	Max	Min	1st Qu.	Median	Mean	3rd Qu.	Max
A. sinensis	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	-31.35	-31.08	-30.80	-30.80	-30.53	-30.26	-30.84	-29.64	-28.81	-28.99	-28.54	-25.89
E. affinis	-28.92	-26.89	-26.65	-26.55	-25.75	-24.85	-33.87	-32.85	-31.84	-31.84	-30.82	-29.80	-33.87	-30.66	-29.46	-29.84	-29.05	-27.05
N. mercedis	-28.88	-28.48	-27.47	-26.72	-24.96	-23.84	-29.17	-28.95	-27.15	-27.74	-26.93	-26.12	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
P. forbesi	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	-31.40	-30.30	-29.51	-29.74	-29.25	-28.32	-33.53	-31.09	-29.91	-30.26	-29.42	-28.01
S. doerrii	-29.57	-28.67	-28.46	-28.33	-27.70	-27.30	-28.94	-28.94	-28.94	-28.94	-28.94	-28.94	-35.54	-31.03	-29.42	-30.11	-28.83	-27.53

Species	$\delta^{15}N$ Diatom Era						$\delta^{15}N$ Flagellate Era						$\delta^{15}N$ Cyanobacteria Era					
	Min	1st Qu.	Median	Mean	3rd Qu.	Max	Min	1st Qu.	Median	Mean	3rd Qu.	Max	Min	1st Qu.	Median	Mean	3rd Qu.	Max
A. sinensis	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	14.29	14.33	14.36	14.36	14.40	14.44	10.15	12.34	13.02	13.12	13.91	17.14
E. affinis	10.25	11.51	11.95	12.05	12.73	14.35	9.98	10.07	10.16	10.16	10.26	10.35	6.91	8.86	10.34	10.32	11.58	13.35
N. mercedis	10.61	11.16	11.51	12.07	11.80	16.44	12.48	13.55	14.25	14.40	15.27	16.46	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
P. forbesi	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	13.36	13.46	13.70	13.66	13.82	13.99	7.61	10.67	11.50	11.65	12.61	16.17
S. doerrii	9.09	12.10	13.24	12.51	13.76	13.80	12.67	12.67	12.67	12.67	12.67	12.67	7.76	10.04	11.00	11.06	12.23	13.23

APPENDIX C: Isotope Values by Date Collected

Table C1. Complete $\delta^{13}C$ and $\delta^{15}N$ data. Carbon and nitrogen isotope values for four species at each collection date.

E. affinis			*N. mercedis*			*P. forbesi*			*S. doerrii*		
Date	$\delta^{13}C$	$\delta^{15}N$	Date	$\delta^{13}C$	$\delta^{15}N$	Date	$\delta^{13}C$	$\delta^{15}N$	Date	$\delta^{13}C$	$\delta^{15}N$
1976-05-06	-26.75	11.95	1978-05-18	-28.83	10.61	1990-08-28	-29.490	13.840	1977-08-09	-28.28	13.80
1976-05-21	-25.75	11.65	1978-05-31	-28.88	11.26	1992-04-24	-31.400	13.390	1977-08-24	-27.30	9.09
1978-06-14	-27.54	12.91	1978-08-24	-23.84	11.57	1992-05-11	-30.560	13.670	1980-04-05	-29.57	11.89
1978-06-28	-26.85	14.35	1979-05-21	-28.13	16.44	1992-05-25	-29.530	13.360	1980-06-10	-28.64	13.77
1978-08-10	-25.14	12.21	1980-08-06	-24.89	12.04	1992-08-04	-28.320	13.740	1980-06-23	-28.68	13.74
1978-08-24	-24.85	12.47	1980-08-20	-25.02	11.51	1992-08-21	-29.170	13.990	1980-07-22	-27.51	12.75
1978-09-11	-26.60	13.02	1981-06-15	-27.47	11.07	1996-05-14	-31.960	10.980	1983-08-03	-28.94	12.67
1978-09-25	-26.89	13.33	1983-08-03	-28.93	12.48	1997-07-14	-29.880	13.810	1996-05-14	-32.66	12.04
1979-05-21	-27.96	10.25	1990-04-19	-27.02	14.25	1998-05-20	-32.790	10.090	1998-07-17	-32.69	13.05
1979-07-05	-25.62	11.51	1990-05-02	-28.97	13.04	1998-06-18	-33.530	10.180	1999-05-13	-30.46	10.94
1979-07-18	-26.70	11.20	1990-08-28	-26.84	15.07	1998-07-17	-32.040	11.950	2001-05-18	-28.83	13.20
1979-08-30	-26.11	11.81	1991-05-22	-27.15	15.47	1998-08-13	-31.250	16.170	2001-06-15	-31.67	12.82
1980-06-10	-27.51	12.73	1992-05-25	-29.17	14.06	2000-08-09	-29.480	12.940	2002-04-09	-29.36	9.03
1981-04-30	-28.92	10.35	1992-08-04	-26.12	16.46	2000-09-08	-29.590	14.500	2002-05-07	-27.53	13.23
1981-06-15	-26.65	12.02				2001-05-18	-29.060	12.430	2003-05-12	-29.42	10.04
1981-06-29	-25.49	11.49				2001-06-15	-30.655	13.375	2004-04-15	-31.03	7.76
1981-07-10	-26.06	11.54				2001-07-17	-30.490	15.060	2004-05-13	-28.45	11.67
1998-04-22	-33.87	9.98				2002-07-18	-29.040	14.920	2005-04-20	-32.05	8.52
1999-05-13	-29.80	10.35				2003-09-05	-30.180	12.740	2005-05-20	-29.40	10.59
2001-04-18	-29.26	11.86				2004-05-13	-28.420	10.810	2005-06-17	-30.67	11.00
2001-05-18	-28.97	12.70				2004-06-10	-29.290	11.690	2006-04-24	-35.54	9.50
2002-04-09	-29.30	8.77				2004-07-09	-29.330	13.450	2006-06-23	-32.14	10.87
2002-05-07	-27.18	13.35				2004-08-25	-29.640	12.150	2007-04-12	-28.00	8.87
2003-05-12	-29.96	10.09				2005-04-20	-32.110	7.880	2007-05-10	-30.21	9.24
2004-04-15	-31.12	6.91				2005-05-20	-30.350	9.800	2008-04-14	-29.93	10.51
2005-04-20	-32.01	7.77				2005-06-17	-31.700	9.980	2008-05-12	-29.38	11.99
2005-05-20	-29.46	10.56				2005-07-15	-30.450	11.750	2008-06-11	-29.17	12.58
2006-05-23	-32.11	8.86				2005-09-27	-30.570	11.020	2009-05-18	-29.45	10.83
2007-04-12	-27.05	10.12				2006-06-23	-32.670	9.850	2009-06-17	-29.14	11.86
2007-05-10	-30.66	8.56				2006-07-19	-31.040	10.840	2010-05-07	-28.25	12.23
2008-04-14	-29.67	11.00				2006-08-17	-32.250	7.610	2010-06-04	-28.67	11.43
2008-05-12	-29.12	11.58				2006-09-15	-29.570	11.260	2010-07-06	-28.77	12.66
2009-05-18	-29.05	10.34				2007-05-10	-30.660	8.540			
2010-05-07	-28.73	12.59				2007-06-11	-30.220	10.020			
						2007-07-20	-28.410	11.000			
						2007-08-21	-29.220	12.770			
						2007-09-19	-29.570	10.590			
						2008-06-11	-29.460	12.270			
						2009-06-17	-30.230	11.320			
						2009-07-15	-29.940	12.570			
						2010-05-07	-29.470	10.780			
						2010-06-04	-28.940	10.700			
						2010-07-06	-29.450	11.790			
						2010-08-04	-28.010	12.360			
						2010-09-17	-28.650	10.700			