FINAL REPORT TO THE PORT OF SAN DIEGO

CHEMICAL ANALYSIS OF THREATENED AND ENDANGERED SPECIES IN SAN DIEGO:

THE SAN DIEGO BAY TROPHIC TRANSFER PROJECT

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FINAL REPORT, JANUARY 31, 2011

Citation: Lewison et al. 2011. Chemical analysis of threatened and endangered species in San Diego

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EXECUTIVE SUMMARY

- The objective of this grant was to use isotope and element analysis to understand trophic structure, map isotopic variability (i.e. the isoscape) in San Diego Bay and to evaluate contaminant exposure and load in species of conservation concern in San Diego Bay, focusing specifically on East Pacific green turtle (EPGT) and California least terns (CLT). Led by Dr. Rebecca Lewison, the research team was composed of a SDSU faculty member (Dr. Lai), a senior NOAA scientist (Dr. Seminoff), a senior Scripps Institute scientist (Dr. Deheyn) and several SDSU graduate and undergraduate students.
- One key result from this project was the resolution of the diet composition of the endangered EPGT. This information is fundamental to effective protection of this species within San Diego Bay. Diet identification can also inform the identification of sources of contamination in this population. We applied two leading multisource stable isotope mixing models (Isosource and Stable Isotope Analysis in R, SIAR) to determine the main contributors to, and annual variation in, green turtle diet based on comparisons of isotope values of turtles and putative prey species.
- Isotope model outputs indicated that green turtles are omnivores, with mobile invertebrates having the greatest dietary input (62% with Isosource; 42% with SIAR) and seagrasses constituting the second most important diet item (16% with Isosource; 6% with SIAR). Green algae and sessile invertebrates were also identified as feasible prey species, although at reduced levels. Local seagrass pastures appear to be of high value to green turtles, serving both as a major food resource and by providing habitat for other green turtle prey.
- Based on significant inter-annual differences in the isotopic signal from discarded eggs across multiple CLT colonies, we found clear evidence of diet shifts in CLTs among years. These diet shifts may be linked to differences in prey species availability, spatial shifts in foraging areas or a combination of both factors. These shifts in food resources may be tied to observed variability in reproductive output.
- We had limited success in resolving CLT diet. Although we are able to differentiate isotopic signatures among prey items, limited information on the discrimination factor (also called fractionation factor), which determines how nutrients from the food sources are incorporated into the birds and their eggs, may explain why diet composition could not be resolved.
- Using isotope data from the most widely distributed species across the Bay *(Zostera marina*, *Gracilaria sp*. and *Ulva sp*.), we generated isoscapes for San Diego Bay, identifying locations of nitrogen enrichment in the South Bay. Nitrogen enrichment is likely the result of increased nutrient loading, likely anthropogenic in nature, in the Bay and is an indicator of degraded water quality. Nutrient inputs

in the Bay are probably driven by non-point sources (e.g., surface runoff, groundwater, atmospheric deposition and shoreline erosion).

- We focused contaminant analyses on two classes of compounds, metals and organics in a wide range of sample types. Some turtle blood was re-screened for organic compounds with more sensitive instruments because of low detection limits. For turtle blood, we also completed a more in-depth exploration of the metal analyses to identify the potential cellular pathway by which toxic compounds may be impacting this species.
- A range of different metals were detected in the samples we analyzed. In EPGT, silver, cadmium, copper, manganese, selenium, strontium, vanadium, and zinc were the most prevalent bioaccumulating metals**.** Strong spatial trends of copper and manganese drove spatial differentiation in EGPT food items, while a different suite of metals were found to influence accumulation patterns in sediment across regions within the Bay. These results indicate that metal levels in biota (all plants and invertebrates) and sediment are highly dissimilar. This suggests that toxicity reference values based on localized sediment testing are likely to be less accurate for risk assessments of higher organisms like EPGT.
- In the CLT forage fish sampled, cadmium, copper, manganese, lead, selenium and vanadium were the most prevalent metals detected although there were some spatial variation in levels. Cadmium was detected at greater concentrations in topsmelt at Imperial Pier compared to all other sites. Copper, manganese and selenium were all detected at higher concentrations in topsmelt in the central part of the Bay. The majority of contaminant levels detected in the forage fish species did not exceed identified risk levels identified for birds, although the accumulation patterns and levels of these compounds in CLTs is unknown. However, levels of selenium detected may exceed threat thresholds.
- We focused organic analyses on EPGT samples. There were a number of organic compounds that were commonly detected in the EPGT samples analyzed: γ benzene hexachloride (BHC) was present in all plasma samples, and p'p'- DDE and γ chlordane were frequently detected. Using a more sensitive instrument array, PCBs were found at the highest level in all the blood and plasma samples among all organic compounds tested. These more sensitive analyses highlight the clear presence of PCBs and PBDEs in the San Diego Bay food web.
- The chemical analyses conducted during this project provide a robust baseline for future study of nitrogen enrichment and contaminant levels in sediment and a wide range of species in San Diego Bay.

INTRODUCTION

San Diego Bay is a highly urbanized estuary that ranks as one of the most polluted coastal bodies of water in the United States (Long et al. 1996), but it also provides critical habitat for many sensitive species. Its shores are prime nesting ground for the Endangered California Least Tern (CLT) (*Sternula antillarum browni*), marshes and mudflats support thousands of shorebirds, and extensive eelgrass beds *(Zostera marina)* serve as nursery habitat for many fish species and key foraging grounds for the Endangered East Pacific green turtle (EPGT) *(Chelonia mydas)* (Zeeman 2004). Degradation of coastal habitats due to anthropogenic activities have been found to severely negatively affect species' health and success (Vitousek et al. 1997, Jackson et al. 2001b) and point and non-point pollution in the Bay from historical and contemporary sources has long been a standing issue of concern (USDoN 1999). San Diego Bay has experienced a long history of intense industrial and recreational use. Much of the Bay is impacted by industrial development, including numerous shipyards, two military bases, a major cruise ship terminal, and the South Bay Power Plant (SBPP), a once-through cooling power generating facility located in the extreme southern portion of this bay.

The widespread effects of pollution on sensitive wildlife and overall ecosystem health is a major issue of concern in San Diego Bay and similarly urbanized coastal ecosystems (Bryan and Langston 1992, USDoN 1999). To better understand how these pollutants enter and are transferred through the food web in the San Diego Bay, we compared isotopes, trace metal loads and contaminants in two of the sensitive species, EPGT and CLT, as well as a suite of forage species for both of these organisms throughout San Diego Bay. Here, we use isotopes to identify key food resources for EPGT and CLTs and also use these data to develop an isoscape for the Bay. Isoscapes provide data on resident organisms and environmental condition using their isotopic signatures. This project also directly analyzed bioaccumulation and spatial variability of contaminants in San Diego Bay food webs and in EPGT. This analytical approach provides fundamental information needed for

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more effective species management and more accurate risk assessments of habitats and higher-order species in the biodiverse, urbanized coastal environment of San Diego Bay.

METHODS

Field data collection

Over the course of this project, comprehensive field data collection occurred and representative samples were taken from multiple trophic levels for both isotope and contaminant analysis. Sampling began in June 2008 at nine permanent sampling sites and one reference site outside the Bay (Figure 1) that reflect the stratified ecoregions from the State of the Bay report (2007). Sampling was repeated in the spring/summer and fall/winter for all sites to allow for seasonal comparisons. For these analyses, we evaluated habitat, prey species as well as the two target species to understand the impact of trophic structure and contaminants on threatened and endangered species in San Diego Bay**,** specifically focusing on EPGT and CLT.

Scientific name	***Common Name***
Zostera marina	Eelgrass
Gracillaria spp	-
Ulva spp.	-
Zoobotryon verticillatum	-
Navanax inermis	California aglaja
Bulla gouldiana	California bubble snail
Ascidian spp.	Sponge/Tunicates
Aplysia californica	Sea hare
Ptilosarcus spp.	Sea pen
Antherinops affinis	Topsmelt
Engraulis mordax	Calif. anchovy
Cymatogaster aggregata	Surfperch

Table 1. Species sampled across sampling stations

To sample potential contaminant sources for EPGTs and CLTs, we collected at least five water, sediment, and eelgrass samples via SCUBA or with a light-weight grab at each site. For isotope analysis, potential prey items for EPGTs were collected at the identified sampling locations across San Diego Bay. Tissue from putative prey species (hereafter referred to as habitat samples) were collected during SCUBA line-transects at areas of interest throughout the Bay, as well as opportunistically during field efforts. We collected entire organisms (i.e. whole body) for all but eelgrass, for which only the blades were gathered. These habitat samples were cleaned with distilled water and frozen at −10°C. We collected samples of (*Zostera marina)*, red and green algae, and numerous invertebrates including sponges, bryozoans, tunicates and mollusks (Table 1). Less common species (*Navanax* and *B. gouldiana*) were collected opportunistically, as these species have

variable spatial and temporal distributions. To resolve the key prey items in the CLT diet, we collected four species of fish prey from each sampling site with a surface purse seine net. These samples also were used to examine the potential heavy metal contaminant pathways for CLTs. Topsmelt (*Antherinops affinis*), California anchovy (*Engraulis mordax*), and surfperch (*Cymatogaster aggregata)* were among the species sampled and run through both trace metal and isotope analysis.

Database construction

All data have been organized into a comprehensive database that integrates the data collected from this project, related projects at SDSU, and data from the Southwest Fisheries Science Center. We have used this database to compare the results of our study to the findings from other investigations of contaminants in the Bay, such as those by SWFSC and the Department of Fish and Game.

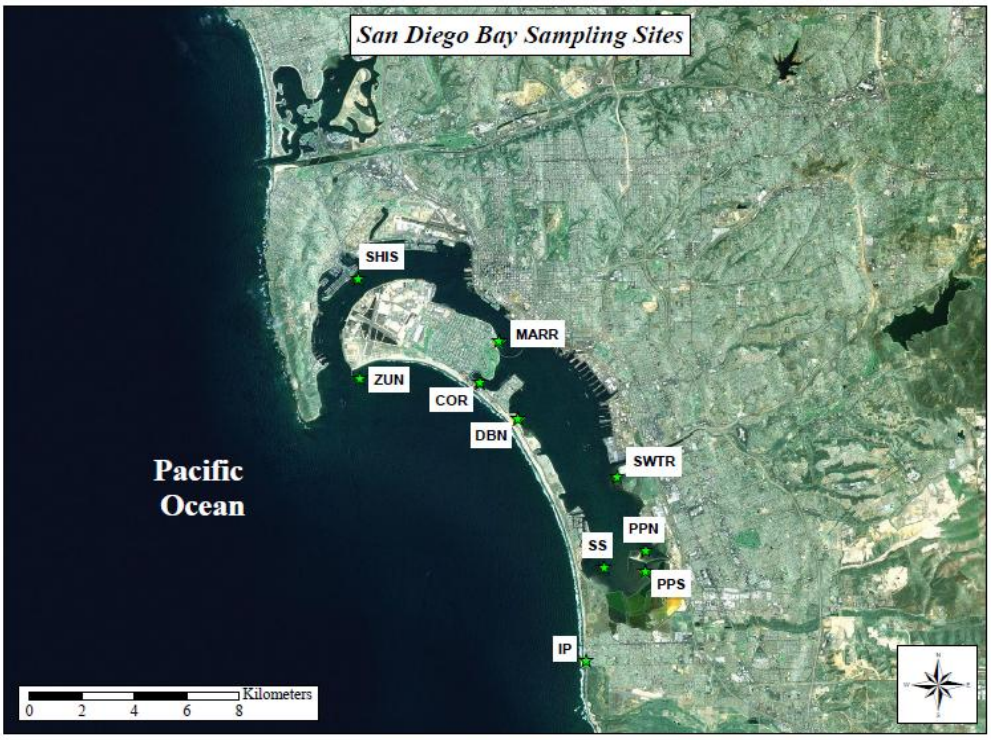

Figure 1. San Diego Bay Trophic Transfer Project Sampling Sites

Stable Isotopes

Over 500 samples were for analyzed for isotope composition. These samples include eelgrass and two other types of algae, invertebrates, fish, and EPGT blood and tissue as well as CLT egg shells. Prior to analysis, samples were thawed, weighed (wet weight), and dried at 60°C until sample weight remained constant (i.e. dry weight), then were homogenized into a fine powder using a mortar and pestle. Lipids were removed from skin samples and a portion of each habitat sample using a Soxhlet apparatus with a 1:1 solvent mixture of petroleum ether and ethyl ether for at least two 10-h cycles. Samples then were dried at 60ºC for 24 h to remove any residual solvent. For the EPGT samples, approximately 0.60 mg of diet and tissue samples were loaded into sterilized tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at the University of Florida, Gainesville USA. We used a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT, Bremen, Germany) to a Deltaplus gas isotope-ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Analysis of forage fish and CLT eggs was conducted at the San Diego State University Ecology Analytical Facility with a CarboErba NCS 2500 elemental analyzer to obtain relative concentrations of carbon and nitrogen. The resulting CO_2 and N_2 from combustion were then run through a Thermo Finnigan Delta Plus mass spectrometer to obtain isotopic ratios of each element. We also ran samples at the University of Florida Light Stable Isotope Mass Spec Laboratory because of equipment repair needs at SDSU.

Contaminants: Metals and Organic Compounds

We conducted trace metal analyses at Scripps Institution of Oceanography (University of California at San Diego), using nitric acid and hydrogen peroxide digestion followed by simultaneous quantification of 15 trace metals with an Inductively Coupled Plasma Optical Emission Spectrum (ICP-OES) spectrometer. These analyses were used to compare trace metal levels across samples. For the fish sampled, whole fish were tested to establish concentration levels and point to metal sources across the sampled species.

Together with colleagues as CSU, Long Beach, we completed a second component to the trace metal termed metal speciation analyses. Metal speciation is a process by which the specific form of an element can be determined and can be used to identify particular cellular pathways a trace metal may be affecting and helps identify the potential mechanism by which toxic compounds may be impacting turtles in the Bay.

EPGT blood plasma was analyzed for persistent organic pollutants (POPs) by Mississippi State Chemical Laboratory (Mississippi State, MS). We analyzed samples using these methods for a panel of 28 POPs including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), polybrominated diphenyl ethers (PBDEs), and other common pesticides. As many samples fell below detectable levels, blood and plasma from 22 individuals were run through testing with a new equipment array in the analytical laboratory of SDSUs School of Public Health's Division of Environmental Health using an Agilent GC/MS in Electron Capture Negative Ion (ECNI) mode, which is more sensitive equipment that has a higher probability of detection.

Data analyses

$\delta^{13}C$ and $\delta^{15}N$ isotope values for all habitat and prey species were averaged by site. We then used these values to create an isoscape map of San Diego Bay for the most widely distributed species: *Zostera marina*, *Gracilaria* spp. and *Ulva* spp. Isoscapes were developed in GIS through kriging interpolation. The $\delta^{13}C$ and $\delta^{15}N$ values for green turtle tissues were compared among all years using ANOVA to gauge the consistency in isotopic values through time. To establish the probable dietary groups consumed and assimilated by green turtles in San Diego Bay, we used the isotope mixing model programs Isosource (Phillips et. al., 2003) and SIAR (Inger et al., 2010b). We used both programs to take advantage their respective strengths and to examine the variation in output values of two leading mixing models. Using Isosource, we created a mixing polygon that produced an intuitive graphical relationship among $\delta^{13}C$ and $\delta^{15}N$ of green turtle skin and potential diet items. With SIAR we generated a series of prey contribution distributions, which 11 integrated the variance of green

turtle and habitat isotope values, and represented the probability distributions for each potential group's feasible contribution to green turtle diet.

For CLTs, we used abandoned eggs from multiple colonies in and around San Diego Bay from 2003-2009. We specifically targeted the egg membrane as our sample tissue because this tissue represents most recent diet choices, i.e. approx. 2 weeks. We analyzed for $\delta^{15}N$ and $\delta^{13}C$ values after verifying there was no significant difference between $\delta^{15}N$ and $\delta^{13}C$ values of hatched and unhatched eggs. We used a general linear model with year and site as predictors to test for significant temporal or spatial variation in $\delta^{15}N$ levels. We also used SIAR to identify diet composition for CLTs based on values from egg membranes and the documented CLT prey items.

EPGT habitat and prey species sample replicates for metal analysis were averaged by sample, and we calculated means and medians for each sample type per sampling event. We calculated enrichment and bioaccumulation factors to evaluate patterns among sites and used paired t-tests to detect overall bioaccumulation patterns for each forage type. Subsequently, to examine regional patterns of accumulation within and between each forage type, we calculated bioconcentration factors (BCF) defined as:

$$\frac{metal\ concentration_{biota}}{metal\ concentration_{sediment}}$$

To distinguish spatial relationships, we employed main effects Analysis of Variance (ANOVA) models by forage type for each metal and deconstructed the variance to determine the percentage of variability explained by each predictor. We compared the Bayesian Information Criterion (BIC) between fine (i.e. site and season) and coarse (i.e. region and season) models to identify if spatial differences were dependant on local "hotspot" site metal levels, or exhibited larger scale regional patterns. Principal Components Analysis was used to describe overall correlation patterns for sediment and biota and to create multivariate metal factors. In EPGT

plasma samples tested for organic compounds, concentration values were averaged and the number of independent samples above level of detection for the instruments (LOD) was calculated.

Tissue concentrations in parts per million (ppm) of all metals tested for forage fish in the CLT food web were averaged by species, site and metal tested. Kruskal-Wallis one-way analysis of variance tests were used to determine if concentrations of arsenic, cadmium, copper, manganese, lead, selenium or vanadium differed between sites in topsmelt samples, the species with representative samples at the most sites. Metals that displayed significant differences in concentrations across sites were then utilized for kriging interpolation to determine if there were regional patterns of metal concentrations.

RESULTS

Stable Isotopes

The examination of our isotope data point to some interesting patterns, as can be seen in an isoscape map of $\delta^{15}N$ values for *Zostera marina*, *Gracilaria* spp. and *Ulva* spp. (Figure 2). Although some of the other sampled species showed little variability among sites, data from these species point to important geographic differences in isotope signatures, with higher nitrogen levels detected at several sites in the South Bay. However, the specific locations of high nitrogen hotspots were different among species.

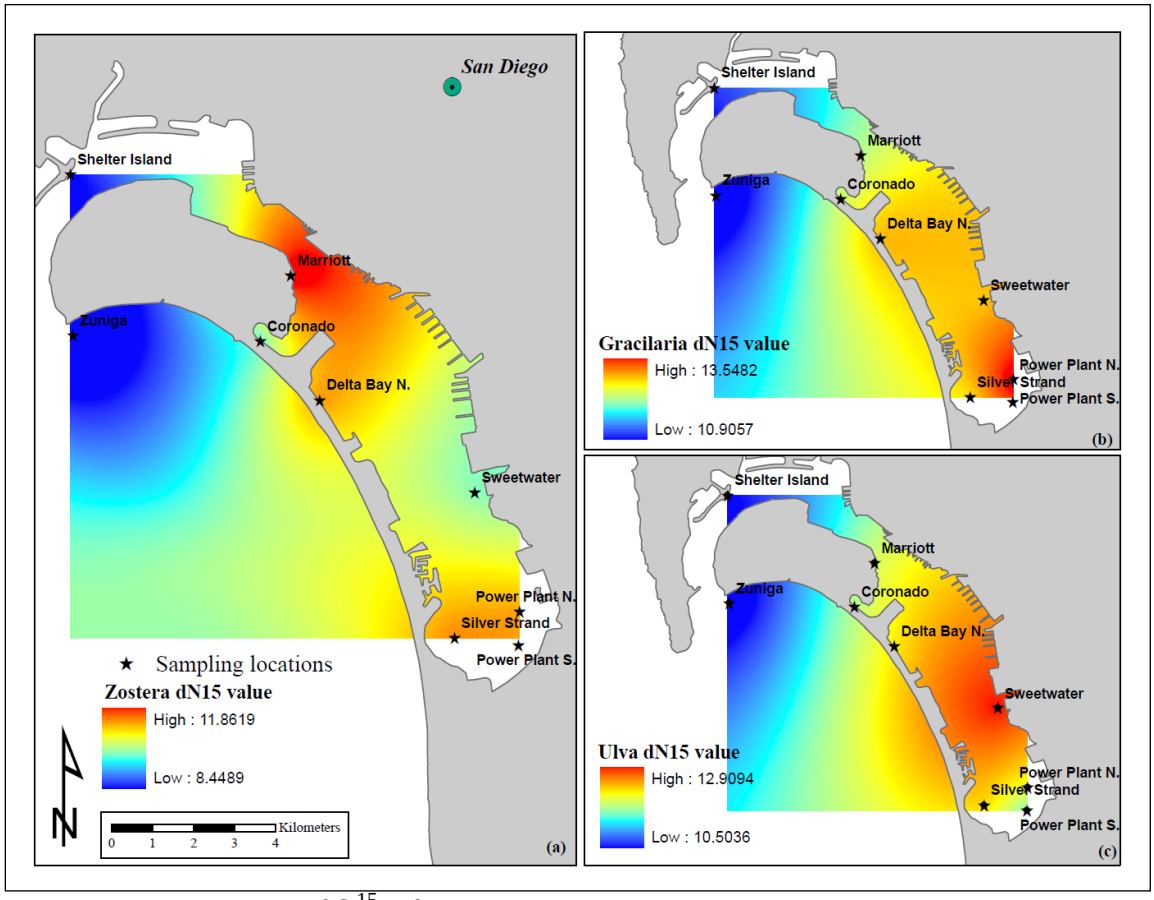

Figure 2. Bay isoscape of $\delta^{15}N$ for (a) *Zostera marina*; (b) *Gracilaria* spp.; and (c) *Ulva* spp.

All EPGT prey items sampled had varying isotopic signatures compared to each other with the exception of the two types of algae whose nitrogen signature similarities can be attributed to their similar composition and life histories (Figure 3). Our two mobile invertebrates revealed an interesting correlation as they not only had the highest nitrogen value of all our prey items (15.83 ± 1.04) but also a nitrogen value that by simple observation has a similar signature to that of the green turtles nitrogen value. Furthermore, the mobile invertebrates produced carbon isotopic signature (-16.56 ± 1.21) very similar to our turtle carbon signature (-16.03 ± 1.52). When these data were incorporated into the multisource isotope mixing model (Isosource and SIAR) for EPGTs, they revealed an omnivorous diet, with invertebrates constituting up to 65% (isosource) and

80% (SIAR) of the green turtle diet (Figure 4). We determined the relative importance of eelgrass to the green turtle's diet while also showing the highest level of invertebrate consumption yet reported.

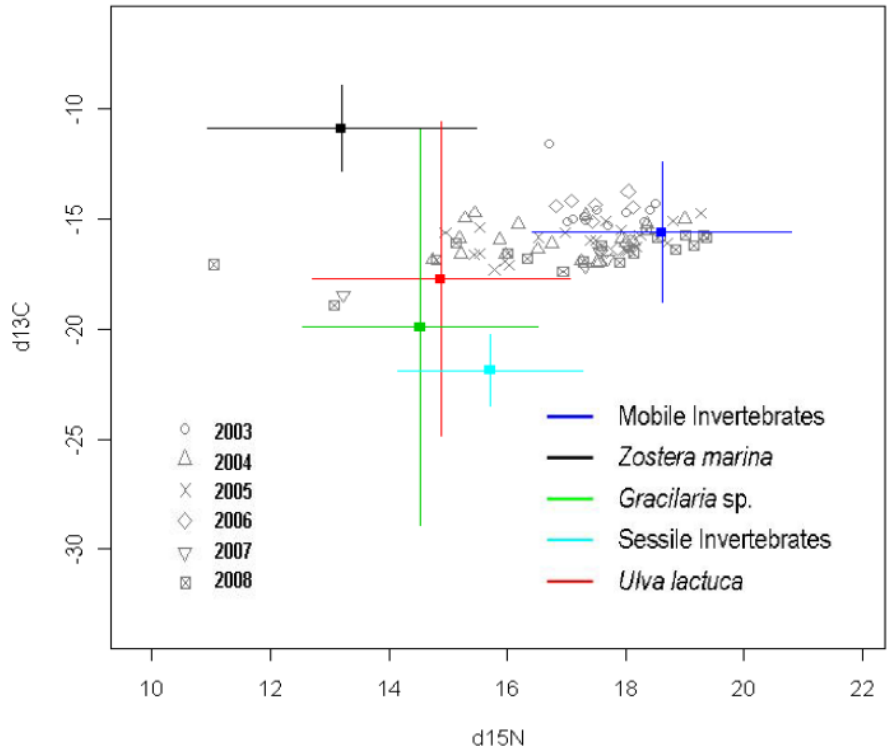

Figure 3. Isotopic signatures for EPGT prey items sampled between 2003 and 2008.

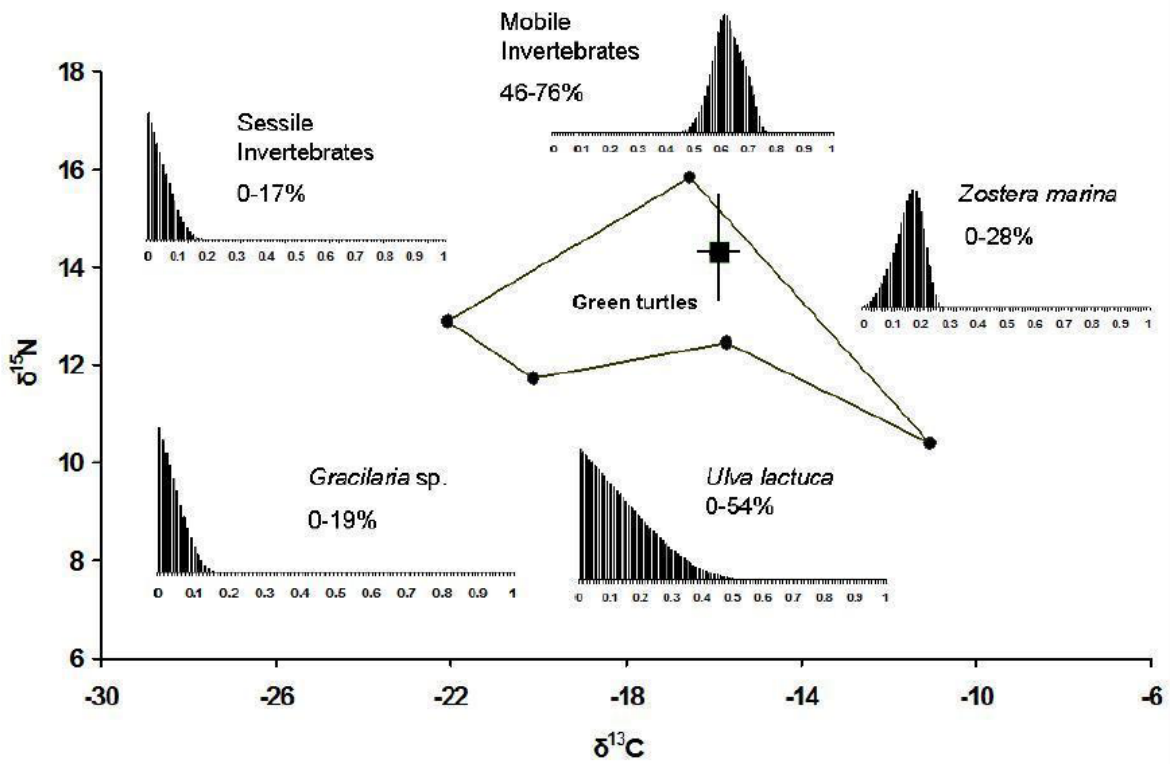

Figure 4. Isosource polygon with 5 aggregated groups. (Phillips et.al. 2005). Histograms next to each food item show distribution curves of the percent contribution to the turtle's diet.

For CLT egg membranes samples, student's t-test showed that there were no significant differences in average $\delta^{15}N$ measurements between the hatched (14.697 ‰) and unhatched (14.592 ‰) membranes ($t = 1.001$, $p = 0.323$) or average $\delta^{13}C$ values ($t = 1.600$, $p = 0.118$) between hatch (-18.370‰) and unhatched (-18.216‰) membranes. We did find clear evidence of significant inter-annual differences in $\delta^{15}N$ (Figure 5), with year as the most influential predictor variable ($r^2 = 30.4$, $F_{df,5} = 20.68$, < 0.001, BIC = 597.3).

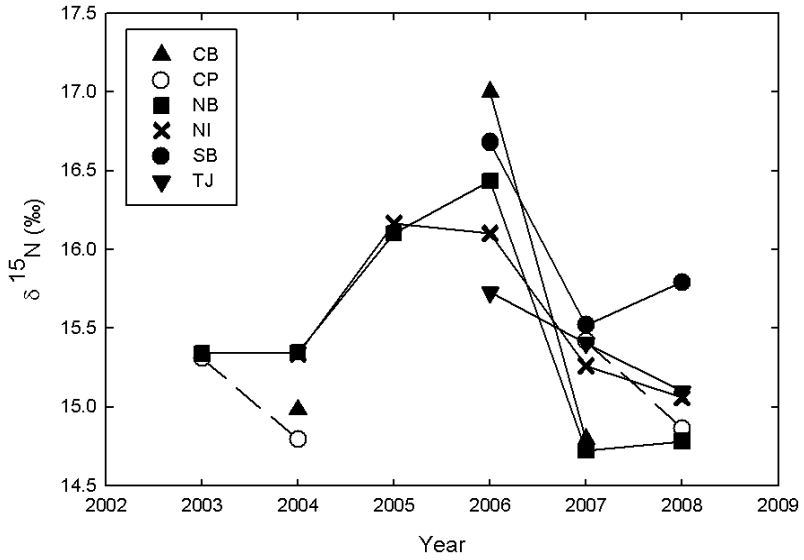

Figure 5. $\delta^{15}N$ measurements from abandoned CLT eggs from 2003-2008 at six sites in and around San Diego Bay. CB= Central Bay, CP= Camp Pendleton, NB= Naval Amphib. Base, NI=North Island, SB=South bay, TJ= Tijuana River.

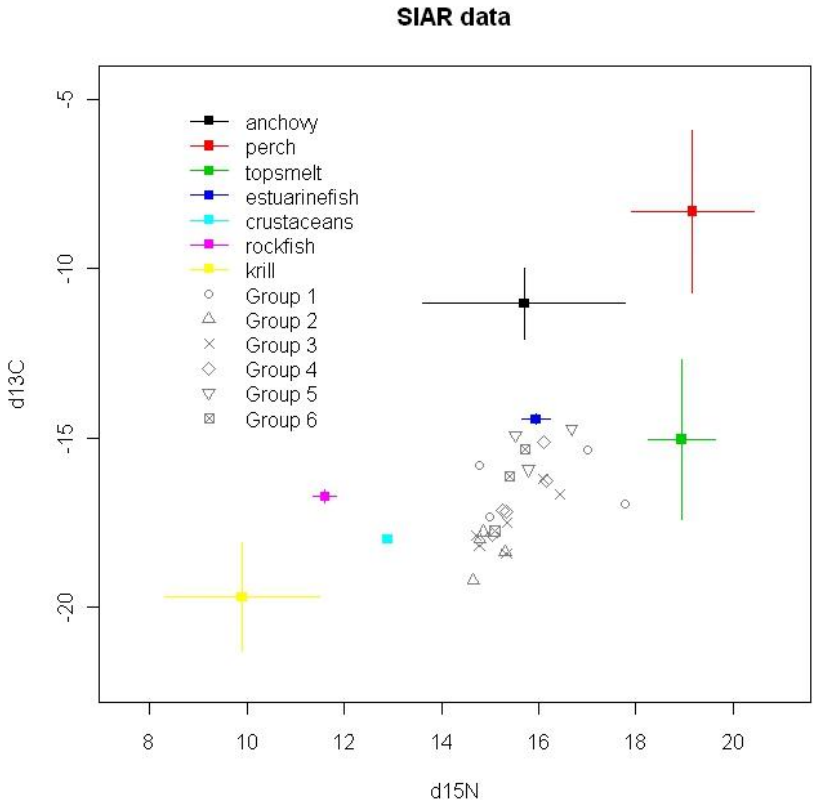

Figure 6. Isotopic signatures for CLT and their prey items.

Using the egg membrane data and all known prey CLT prey items, we were unable to definitively identify the species that contributed to the CLT diet. As seen in Figure 6, the bird values (shown as Group 1-6) are not closely linked to the food items we analyzed. This lack of resolution may be due to limited data on how prey nutrients are integrated into CLT tissue (termed the discrimination factor). It also may point to a missing prey item, although no other prey item has been documented for this species to date.

Metal Contaminants

Bioaccumulation patterns varied spatially and among samples representing the EPGT food web, with silver, cadmium, copper, manganese, selenium, strontium, vanadium, and zinc being the strongest bioaccumulating metals (Figure 7). Strong spatial trends of copper and manganese drove spatial differentiation in EGPT food items, while a different suite of metals were found to influence accumulation patterns in sediment across regions within the Bay. These results indicate that metal levels in biota and sediment are highly dissimilar. This suggests that toxicity reference values based on localized sediment and invertebrate testing ex-situ are likely to be less accurate for risk assessments of higher organisms like EPGT. Beyond looking at site specific differences, we also considered whether there were accumulation patterns among the different regions of the Bay. Regional bioaccumulation patterns varied among trace metals. Certain metals exhibited BCF differences between forage types, but were generally consistent across regions. In contrast, other metals showed little BCF variation between forage type and Bay regions, while some were influenced by a combination of both factors.

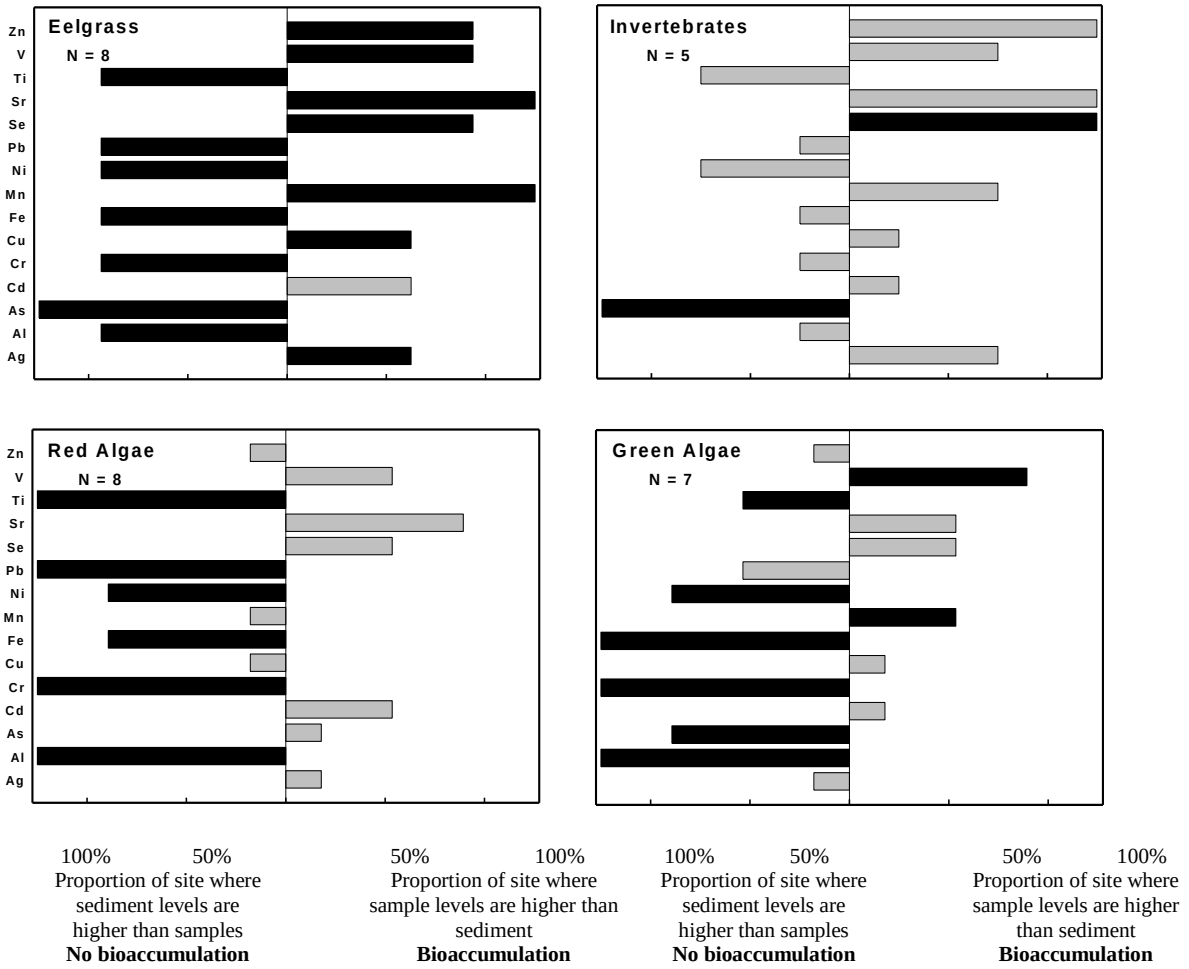

Figure 7. Percentage of sites exhibiting bioaccumulation in eelgrass, invertebrates, red algae, green algae relative to sediment. Values are averaged across seasons. Metals are listed on the Y axis. Bars to the right of the central X axis line indicate the proportion of sites at which metals were higher in biota samples than sediment. Bars to the left of the central X-axes indicate the proportion of sites at which sediment values were higher than biota, indicating no accumulation. Metals with significant relationships (α=0.05, paired t-tests) are indicated by black bar coloration.

The metal speciation work on EPGT plasma detected evidence of numerous metals and the coincident presence of distinct absorption peaks. These absorption peaks suggest that most of the metal binding species probably represent native metalloenzymes and other metal-binding proteins. This evidence of coincident absorption peaks points to co-eluting elements, i.e. elements that have similar profiles. This is indicative of competitive binding of multiple metals to a common ligand. In the case of non-essential metals, such as cadmium, the likelihood of competitive binding may represent a pathway of molecular toxicity, whereby non-essential metals at high levels, such as cadmium or lead are more likely to bind with cellular proteins.

Metal concentrations in the fish sampled showed both spatial and seasonal variation that differed by metal and fish species analyzed. Kruskal-Wallis tests of tissue concentration of cadmium, copper, manganese, lead, selenium and vanadium by site in topsmelt all showed significant (α=0.05) variation by site (Figure 8). Through kriging interpolation, regional patterns of some metal concentrations were detected for cadmium, copper, manganese and selenium (Figure 9). Cadmium was detected at greater concentrations in topsmelt at Imperial Pier compared to all other sites. In comparison, copper, manganese and selenium were all detected at higher concentrations in topsmelt in the central part of the bay based on samples at the Coronado and Delta Bay North sites.

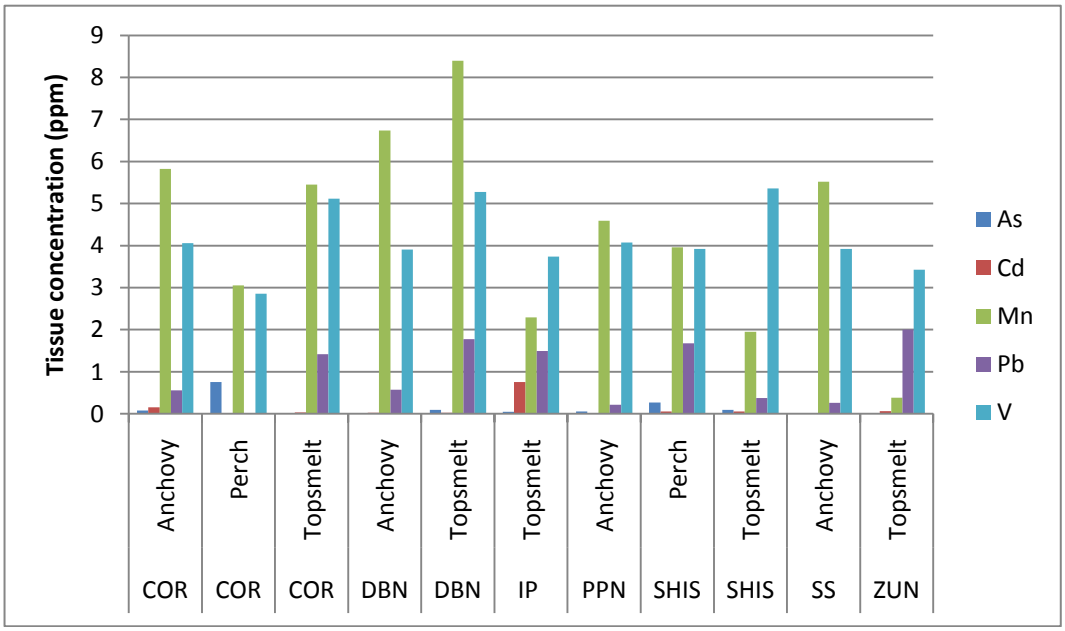

Figure 8. Tissue concentrations of select metals show differentiation by site and species.

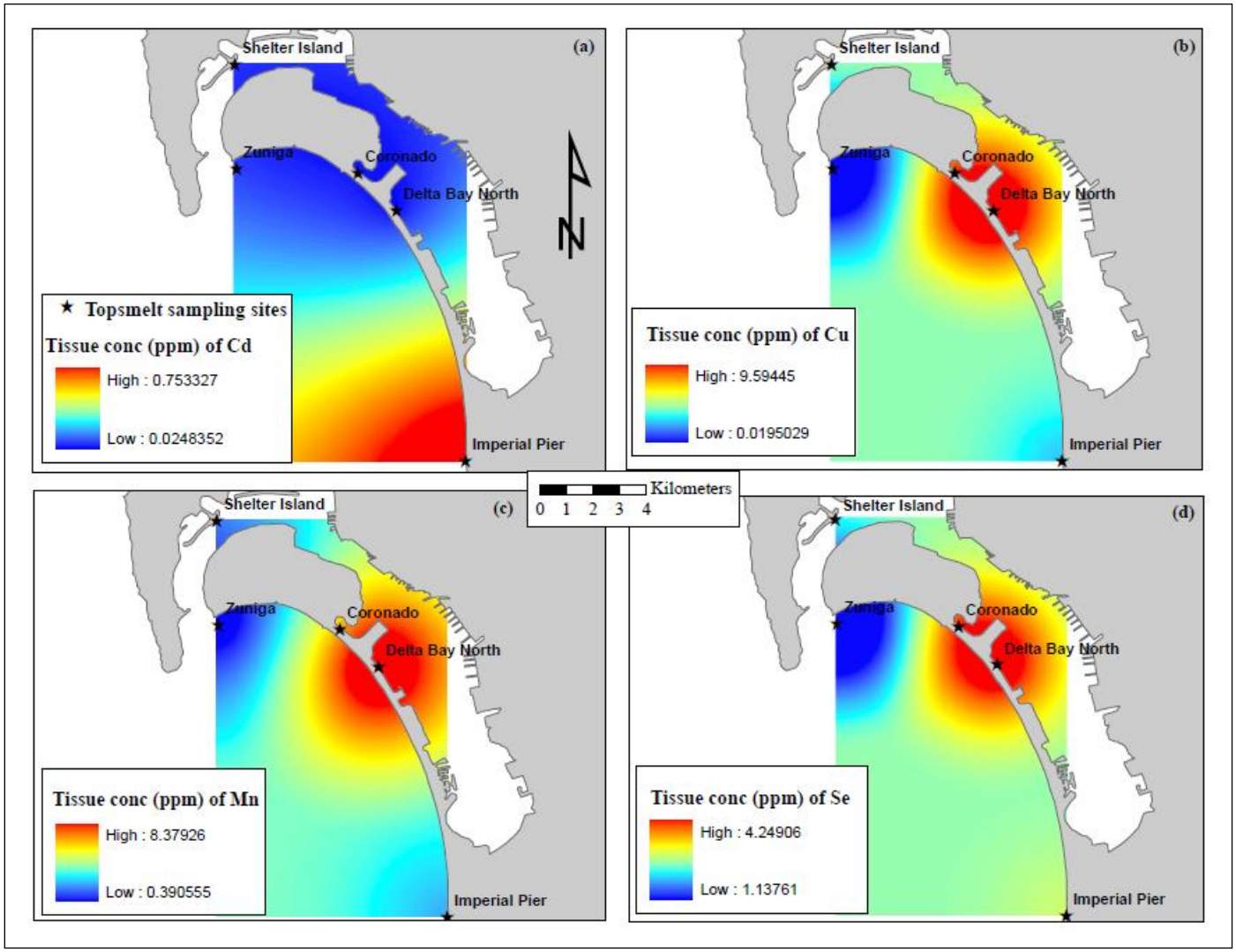

Figure 9. Geographic patterns of topsmelt tissue metal concentrations in ppm for (a) Cadmium; (b) Copper; (c) Manganese; (d) Selenium, based on kriging interpolation.

Organic compounds

There were a number of organic compounds that were commonly detected in the EPGT samples analyzed. γ benzene hexachloride (BHC) was present in all plasma samples, and p'p'- DDE and γ chlordane were frequently detected. Several other chemicals were detected in only a few individuals, including four congeners of polybrominated diphenylethers (PBDEs) detected in two individuals (Table 2). When blood and plasma were run through SDSU's new equipment array to validate results and establish values for samples that

had been below the limit of detection for the equipment (Table 3), PCBs were found at the highest level in all the blood and plasma samples among all POPs tested. These more sensitive analyses highlight the clear presence of PCBs and PBDEs in the San Diego Bay food web.

		Blood Plasma			
Contaminant	*N> LOD*	*Mean*	*SE*		*Range*
γ BHC	20	0.915	±	0.092	0.460 - 2.45
Heptachlor epoxide	1	0.516	±	n/a	< LOD - 0.516
α Chlordane	1	0.620	±	n/a	< LOD - 0.620
γ Chlordane	12	0.790	±	0.051	< LOD - 1.16
p'p'-DDE	14	0.965	±	0.078	< LOD - 1.56
PBDE #47	2	0.565	±	n/a	< LOD - 0.760
PBDE #99	2	0.480	±	n/a	< LOD - 0.730
PBDE #153	1	0.220	±	n/a	< LOD - 0.220
PBDE #154	1	0.230	±	n/a	< LOD - 0.230
Moisture (%)	20	92.5	±	0.425	86.3 - 94.6
Lipid (%)	20	0.462	±	0.135	0.126 - 2.77

Table 2. Organic compounds concentration values in EPGT (mean ± SE) rounded to three significant digits ($ng \cdot g^{-1}$ wet weight). N represents number of independent samples above level of detection for the instruments (LOD).

Sample (Turtle)	Collection date	blood wt. (g)	Chlordanes	p,p'-DDE	PCBs	PBDE
X105	1/8/2009	4.13	0.017	0.000	0.897	0.171
X110	3/25/2009	4.53	0.030	0.054	1.723	0.058
	3/25/2009	5.5	0.044	0.000	2.240	0.596
X143	12/17/2007	6.71	0.091	0.045	2.965	0.009
	12/17/2007	6.71	0.111	0.072	4.058	0.144
	2/27/2008	4.35	0.039	0.025	1.231	0.039
	2/27/2008	5.34	0.190	0.056	5.388	0.071
	3/27/2008	2.73	0.064	0.000	2.134	0.000
	4/3/2008	2.5	0.156	0.103	4.217	0.224
	4/3/2008	2.63	0.144	0.060	3.598	0.042
	4/3/2008	4.9	0.192	0.054	4.731	0.075
X161	1/30/2008	3.46	0.076	0.042	1.952	0.035
X169	12/17/2007	5.25	0.025	0.037	0.875	0.032
LB315	2/26/2009	3.65	0.016	0.135	1.908	0.063
	2/26/2009	5	0.028	0.091	1.336	0.081
LB319	2/15/2008	3.98	0.017	0.088	0.920	0.057
LB325	4/25/2008	4.05	0.015	0.054	0.527	0.159
	12/17/2007	5.93	0.011	0.141	0.521	0.174
	12/17/2007	5.94	0.018	0.120	0.678	0.252
LB326	3/27/2008	2.36	0.030	0.000	2.727	0.052
LB332	12/18/2008	3.48	0.010	0.000	0.569	0.073
LB342	2/15/2008	3.8	0.161	0.096	2.837	0.132
LB362	1/8/2009	5.16	0.011	0.095	0.574	0.105
	2/26/2008	3.32	0.028	0.096	0.967	0.105
76R	2/26/2009	4.3	0.006	0.132	0.773	0.050
	2/26/2009	3.42	0.014	0.000	0.773	0.064
	3/25/2009	3.99	0.018	0.130	0.800	0.107
126277750A	12/17/2007	5.48	0.006	0.000	0.082	0.029
132129225A	12/18/2008	4.56	0.012	0.095	0.472	0.157
132211311A	12/18/2008	2.64	0.019	0.051	0.459	0.124
26618298	3/12/2008	3.31	0.061	0.073	3.758	0.561
*O266182298	3/27/2008	3.83	0.067	0.039	4.118	0.466
126479146A	3/12/2008	6.64	0.014	0.055	0.262	0.028
126331466A	3/12/2008	4.1	0.005	0.054	0.120	0.083
HJ529	12/18/2008	4.45	0.036	0.073	2.971	0.166
Pappy	2/27/2008	2.43	0.040	0.141	1.083	0.702

Table 3. Results of more sensitive testing for organic compounds in EPGT conducted at SDSU. Concentration values (mean $\pm$ SE) rounded to three significant digits ($ng \bullet g^{-1}$ wet weight). Chlordanes represents sum of α- and γ- chlordanes and *trans*- and *cis*-nonachlors. *p,p'*-DDE is a main metabolite of DDT. PCBs represents sum of 35 PCB congeners. PBDEs represents sum of PBDE-47, 99, 100, 154, and 153. * indicates plasma.

CONCLUSIONS

Stable Isotopes

In light of the highly urbanized nature of San Diego Bay, the elevated $\delta^{15}N$ of green turtle skin and habitat values depicted in the isoscape mapping suggest that this system is experience nitrogen enrichment, particularly in the southern portion of the bay. Indeed commercial shipyards, naval shipyards and storm drain runoffs have been documented to contain high levels of pollutants for this system (Fairey et al., 1998), and presuming these point sources of pollution are linked with sewage runoff, this could lead to an enrichment of ^{15}N in affected habitats. These suspected sources can be compared with the results of our isoscape mapping of nitrogen enrichment in eelgrass and algae species to inform potential management options for these sources.

Despite the spatial variation in ^{15}N, temporally, values appear to have remained stable. Kwak and Zedler (1997) profiled isotopic signatures of numerous marine species in the San Diego watershed, including most of the putative EPGT prey species included in this study, and in these instances, the 20 values reported therein were highly similar to our results, an encouraging similarity considering the decade between the two studies. With respect to $\delta^{13}C$, the results of Kwak and Zedler (1997) also indicate low isotopic variability. This consistency supports the temporal stability in isotope signatures of EPGT individuals over the past eight years.

This research effort yielded some surprising results regarding EPGT diet in San Diego Bay. While Hatase (2006) used SIA to show that green turtle in oceanic environments also consume an omnivorous diet, ours is the first study using SIA to show high levels of omnivory in a coastal neritic habitat. In addition to highlighting the importance of specific prey groups, our results underscore the need for eelgrass conservation in San Diego Bay, particularly in light of the nitrogen loading in this system. Seagrass beds in coastal waters provide habitat and shelter for invertebrates and fish including variety of marine snails (Orth, 1984; Kharlamenko et al., 2001), and it is likely that conservation of this habitat type would have broader value for many different species, including green turtles, in San Diego Bay.

Metal Contaminants

We detected several metals that are anthropogenically enriched in sediments of San Diego Bay eelgrass ecosystems, a finding that supports results from previous studies that attribute contamination to both historical and contemporary sources (Katz and Kaplan 1981, MacDonald 1994, Fairey et al. 1998, USDoN 1999). However, presence of anthropogenically enriched sediments did not uniformly correspond to bioaccumulation of trace metals in local biota, perhaps due to complex processes of bioavailability and physiological functions. Eelgrass was the strongest accumulator of metals across sites, likely because eelgrass accumulates metals via roots and blades, reflecting trace metals in the water column as well as in sediment (Coelho et al. 2009). Red and green algae exhibited weaker accumulation trends, which may be related to their lack of root systems. Soft-bodied invertebrates displayed the fewest accumulation trends although this may be the result of small sample sizes due to their patchy distributions. Given the differences in metal sources among sampled species, specific diet choice and foraging sites may be driving factors of metal exposure and bioaccumulation for EPGT. Thus, while sediment toxicity reference values are very useful for species in which bioaccumulation and toxicity are well documented and understood, they may not be representative or indicative of metal risks for higher order organisms that feed on multiple trophic levels, such as EPGT and CLT.

A review of metal concentrations in the CLT forage fish sampled revealed that the maximum concentrations of most metals tested fell below established risk levels for avian species (references in Zeeman 2004) with a few exceptions. However, maximum concentrations of lead, cadmium, selenium, vanadium and zinc exceeded levels associated with adverse effects in some bird species. Selenium in particular, has been associated with negative effects to bird fecundity (Beyer et al. 1996). Most interestingly, when compared with a previous seabird study conducted in the Salt Works region of the Bay (Zeeman et al. 2008), results from our study differed somewhat from tissue concentrations of iron, nickel and strontium and were very different for

arsenic, cadmium, manganese, lead and vanadium. The differences observed in these values may be explained by the variability we detected per site and Bay region and likely point towards more localized sources of these elements in the San Diego Bay ecosystem. Similar to what was observed in the EPGT food web, bioaccumulation in the CLT food sources may be location-dependent and may also be influenced by shifts in prey availability. We expect that for many species at higher trophic levels in the Bay food web, bioaccumulation is driven by both spatial and species forage preferences. However, because metal accumulation was not studied directly in CLTs, this assertion is untested. Direct testing of CLT tissue is necessary to confirm that metals are accumulating in this species of conservation concern.

Organic Compounds

The presence of POPs serves as a clear signal of anthropogenic contamination because they are derived exclusively from manufactured man-made chemicals, while trace metals occur naturally but are toxic above certain thresholds (Bryan 1984). These pollutants can exert lethal and sublethal toxic effects in wildlife, including alteration of neurological and immune function, growth, and reproduction (Beyer et al. 1996). Compared to existing literature (Keller et al. 2004; Carlson 2006; Hermanussen et al. 2008; Swarthout et al. 2010; van de Merwe et al. 2010a,b), San Diego turtles had higher mean levels of chlordanes and p'p' DDE relative to all previous studies examined except for Kemp's Ridley's on the US Southeastern coast and one study of loggerheads in North Carolina (only the latter study was higher than San Diego for p'p' DDE). San Diego PBDEs were also higher than all other studies while PCBs fell within the range of values found in previous studies. The majority of these pollutants have already been identified as contaminants of concern for wildlife in San Diego Bay (Fairey et al. 1998), with DDT and possibly PBDEs linked to seabird reproductive failures (Zeeman et al. 2004). Many compounds detected in San Diego turtles have been banned in the United States for several decades, but remain as legacy pollutants in Bay sediments (Fairey et al. 1998; Deheyn and

Latz 2006). Of particular concern are PDBEs because they are still used prevalently in the U.S. as flame-retardants, despite a growing body of evidence that they have toxic and bioaccumulative effects (Hites 2004). Within this context, our results highlight the need for future monitoring of both contemporary and legacy pollutants in San Diego Bay wildlife.

The chemical analyses conducted during this project provide a robust baseline for future study of nitrogen enrichment and contaminant levels in sediment and a wide range of species in San Diego Bay. The isotope data was also a powerful technique to identify diet contributions and can be used to identify annual diet shifts. For EPGT, the data collected on this project provides the most accurate diet study for this species, to date. For CLTs, observed shifts in diet or foraging location may explain some of the variability in annual reproductive output. The contaminant analyses point to a level of impairment in many locations and for many species that exceeds established risk levels. However, testing to directly measure these compounds in CLTs and other at-risk seabird populations is needed to confirm the contaminant accumulation patterns observed in forage fish species.

One emerging message from this work is the need to account for spatial variability in isotope and contaminant analyses. We found clear differences in accumulation levels among sediment, plant species, invertebrates and higher-order animals. The spatial variability we detected points to differential risks of pollution and enrichment across the regions of the Bay. The difference in accumulation levels among samples highlights the potential limitations of contaminant risk assessments that are based on sediment or a single plant or invertebrate species at a single location. The dissimilarity among potential food items (prey species) and the long-lived species that consume them, such as the EPGT, points to the need for direct measurement of potential contamination risks in species of conservation concern.

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