

Grantee Organization	San Diego State University
Grant Title	Chemical analysis of threatened and endangered species in San Diego: San Diego Bay Trophic Transfer Project
Primary Investigator	Dr. Rebecca Lewison

PROGRESS REPORT SUMMARY

- The focus of this grant is to **use isotope and element analysis to understand trophic structure, map isotopic variability (isoscape) in San Diego Bay and evaluate contaminant exposure and load in species of conservation concern in San Diego Bay**, specifically focusing on East Pacific green turtle (EPGT) and California least terns (CLT). The research team currently includes two SDSU faculty (Lai & Lewison), a senior NOAA scientist (Seminoff), collaborative efforts of a senior Scripps Institute scientist (Deheyn), graduate students (Fournier, Komoroske (graduated) & Gaos) and a number of project interns who are current students or recent undergraduates from UCSD and SDSU.
- We have completed all isotope analyses. This includes analyses of forage fish species (anchovy and topsmelt), several species of algae, 2 types of sessile invertebrates, and 2 mobile invertebrates **Resolution of turtle trophic structure was extremely challenging and required additional sampling in the last time period, but we are now confident that we have identified the key diet items on which green sea turtles rely in San Diego Bay.**
- We have conducted contaminant analyses on the following samples: forage fish (anchovy and topsmelt), several species of algae, 2 types of sessile invertebrate, and 35 sea turtle blood and 63 scute samples. We also completed a second component to the metal analyses to identify the potential cellular pathway by which toxic compounds may be impacting the species of interest. Low detection limits of organic contaminants in the sea turtle blood samples required further analyses. This will be completed in Fall 2010. Our final report will be submitted by 1/31/2011.

ACTIVITIES SINCE APRIL 2010

Database construction

Since the completion of data collection, 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 begun work comparing 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.

Stable Isotopes

Over 200 hundred samples were run through isotope analysis. These samples include eelgrass and two other types of vegetation, invertebrates, fish, and EPGT blood and tissue as well as CLT egg shells. 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 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 CO₂ and N₂ 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. These data form the foundation for understanding annual diet composition of the focal species, EPGT and CLT.

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 in the water, sediment, habitat and forage species for both EPGT and CLT. For the fish sampled, muscle as well as gill and liver tissue were tested to pinpoint whether the source of the metals is being ingested by the fish or being absorbed from the water environment. All these analyses will establish concentration levels and point to metal sources across the sampled species.

Together with colleagues at CSU, Long Beach, we completed a second component to the trace metal analyses to identify the potential mechanism by which a toxic compound may be impacting the species of interest. 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 that the trace metal may be affecting.

EPGT blood plasma was also 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.

RESULTS TO DATE

Stable Isotopes

The examination of our isotope analyses results are in the final stages of interpretation and point to some interesting patterns. Although some sampled species have shown little variability among sites, there is some indication of significant differences in isotope signatures from Site 6 (labeled DBN in Fig 1). There was evidence of nitrogen enrichment in Site 6 from several different species groups. The terrestrial inputs of carbon can be observed in the forage fish samples. Samples from the main bay show increased carbon that could have been the result of carbon enrichment inside the bay compared with those in oceanic habitats. While the cause of

this difference is not clear, the inter-site differences point to an important application of stable isotope analyses – an ability to map the isoscape for San Diego Bay.

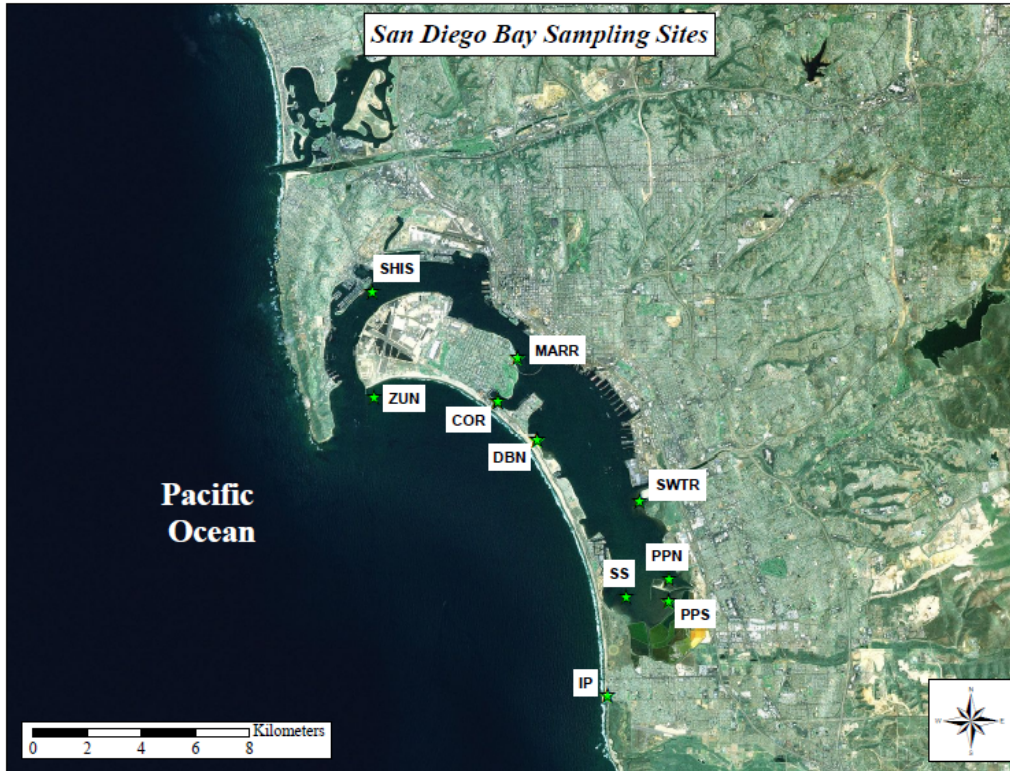


Figure 1. San Diego Bay Trophic Transfer Project Sampling Sites

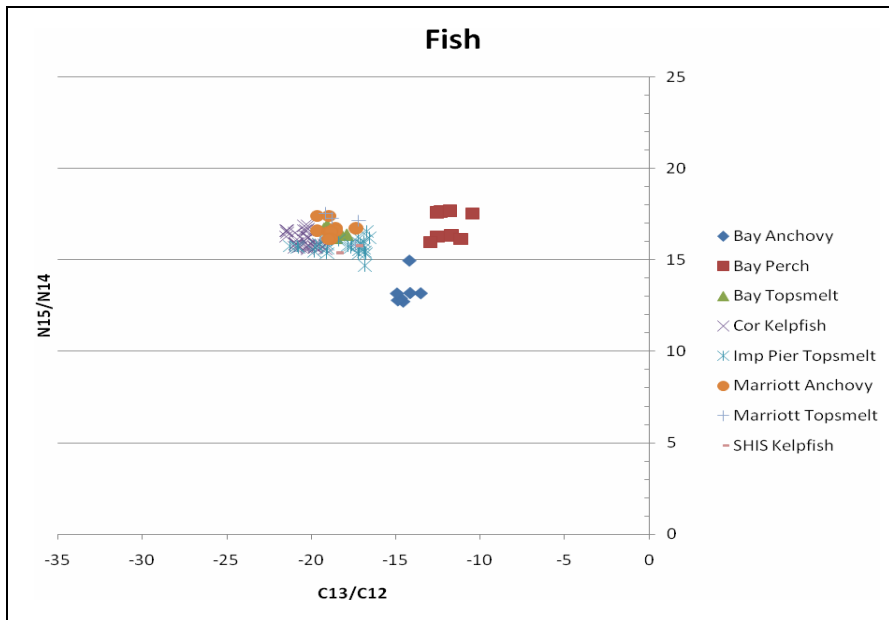


Figure 2. Isotope signatures for forage fish species across multiple bay and oceanic sites.

Results from a multisource isotope mixing model (Isosource and SIAR) for EPGTs revealed an omnivorous diet, with invertebrates constituting up to 65% (isosource) and 80% (SIAR) of the green turtle diet (Figure 3). We determine the relative importance of eel grass to the green turtles diet while also showing the highest level of invertebrate consumption yet reported. This in turn provides information that will allow for more successful monitoring in order to mitigate potential interactions between sea turtles, their habitat and anthropogenic influences in the San Diego Bay.

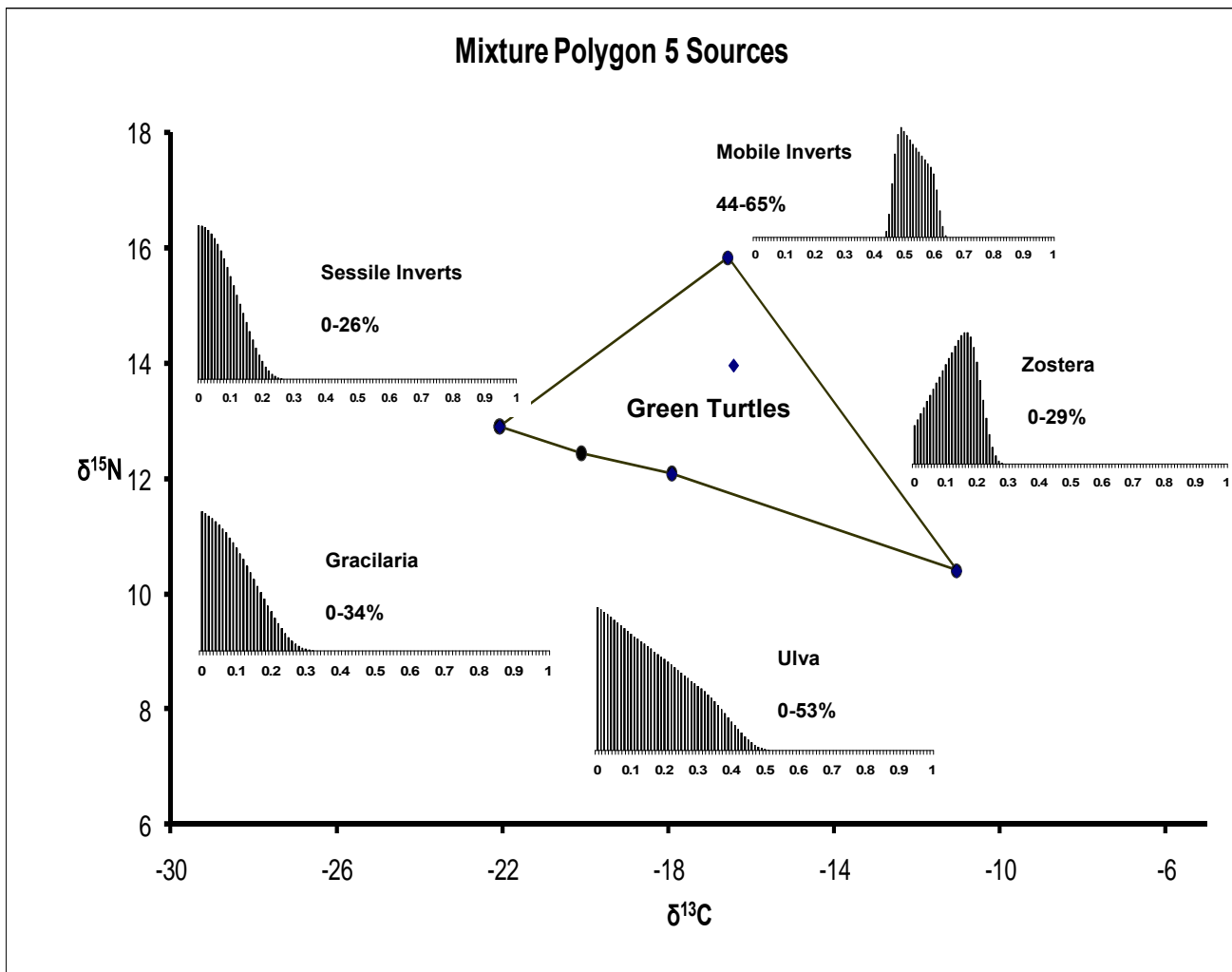


Figure 3. Isosource polygon with 5 aggregated groups. (Phillips et.al. 2005). Histograms next to each food item show distribution curves of the percent contribution to the turtle's diet.

Isotopic data on CLT eggshells and forage fish species were evaluated in the context of reproductive success to consider potential routes of trophic transfer. Although there are differences between the years in CLT clutch size, there was no relationship between annual variation in prey source, as measured by site averaged $\delta^{15}\text{N}$ values, and clutch size or hatching success. Measurements of $\delta^{13}\text{C}$ were not correlated to clutch size or hatching success. The particularly notable results is that, although not strongly related statistically, temporal patterns in concentration of chlorophyll were mirrored in nitrogen stable isotope measurements from CLT eggs.

Carbon values also varied between years, as measurements maintained set differences between some nesting colonies.

Metal Contaminants

Bioaccumulation patterns varied spatially and among biota types in the EPGT food web, with silver, cadmium, copper, manganese, selenium, strontium, vanadium, and zinc being the strongest bioaccumulating metals (Figure 4). Strong spatial trends of copper and manganese drove spatial differentiation in biota, while principal components analysis (PCA) identified a different suite of metals which dictated trends 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.

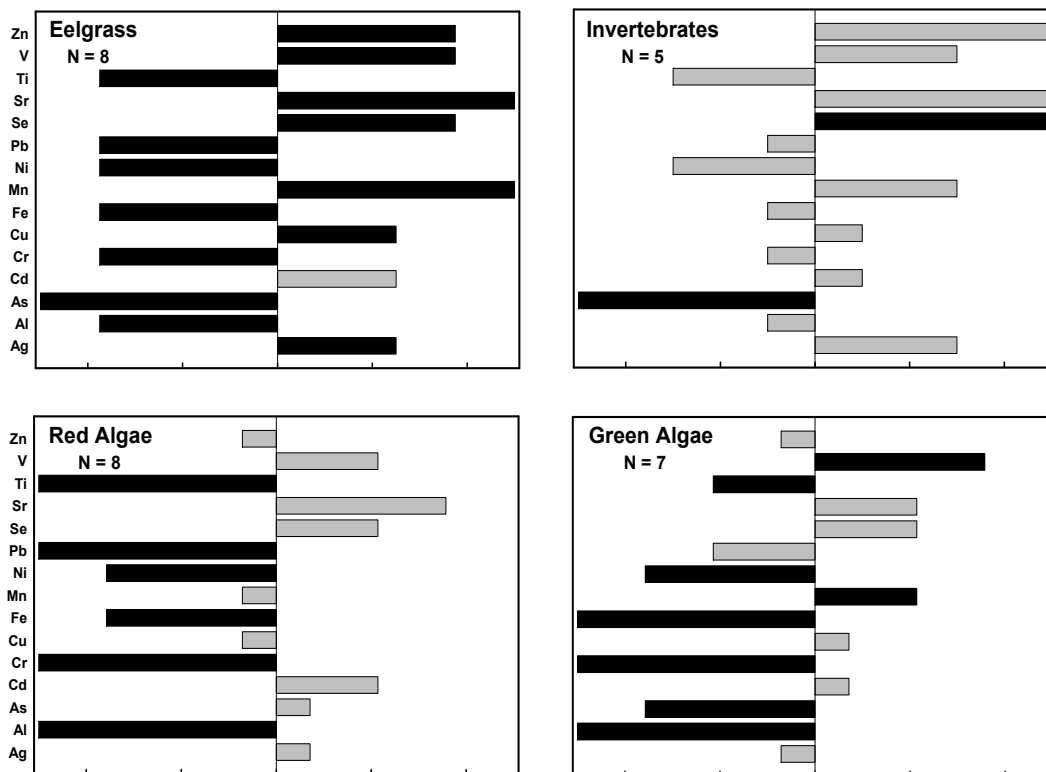


Figure 4. Percentage of sites exhibiting bioaccumulation in eelgrass, invertebrates, red algae, green algae averaged across seasons. X-axes indicate to the right: 100% biota: where the samples at all sites had higher concentrations

than that of corresponding sediment; X-axes indicate to the left 100% sediment: no bioaccumulation – metal levels in sediment had higher concentrations than samples. Metals with significant relationships ($\alpha=0.05$) from 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 indicating that most of the metal binding species probably represent native metalloenzymes and other metal-binding proteins. Results from this work also showed it is probable that co-eluting elements are indicative of competitive binding of multiple metals to a common ligand. In the case of non-essential metals, such as Cd, it may represent a site of molecular toxicity binding to a Zn and Mn requiring protein(s).

Metal concentrations in the fish sampled for analysis of the CLT food web showed both spatial and seasonal variation that differed by metal and fish species analyzed. Initial review revealed that the maximum concentrations of most metals tested fell below avian risk-based dietary screening levels. Cadmium and manganese fell at borderline levels while concentrations of vanadium and selenium were higher than screening levels. Selenium in particular, has been associated with negative effects to bird fecundity. Most interestingly, when compared with a previous seabird study conducted in the Salt Works region of the bay (Zeeman et al. 2008), our study produced results that were slightly different for tissue concentrations of metal for iron, nickel and strontium and very different for arsenic, cadmium, manganese, lead and vanadium.

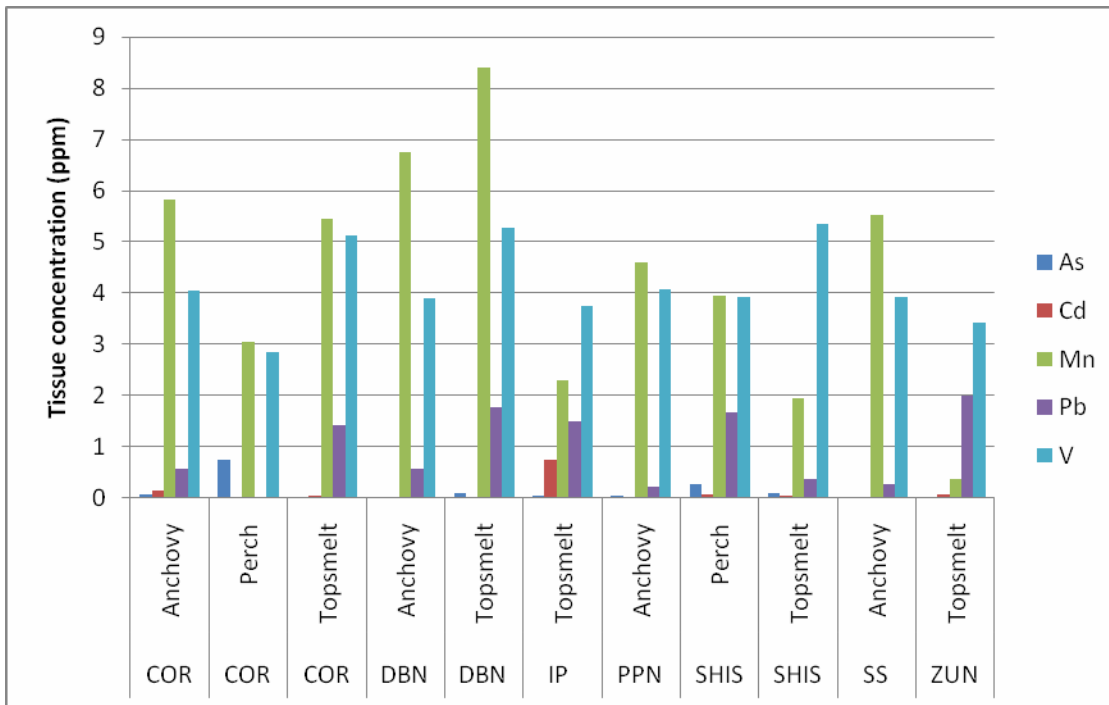


Figure 5. Tissue concentrations of select metals show differentiation by site and species.

Organic compounds

A number of POPs of concern, most notably total PCBs, were not detected in any individuals. However, 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.

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 1. Organic compounds detected in EPGT blood plasma

FINAL STAGE OF RESEARCH ACTIVITY

In the last several months of the project we will be focusing on:

Validating results: Although the analyses to date have helped focus attention on the organic compounds exposure in San Diego Bay, resolving the analyses on organic compounds in sea turtle samples using a more sensitive equipment array is needed. SDSUs School of Public Health’s Division of Environmental Health has a new equipment array that we will be using for these validations.

Data analyses: We are conducting statistical analyses to determine where differences may exist in trace metal concentrations and accumulations and making comparisons with previous data from other studies and water quality reports to determine potential inputs into the bay. A GIS-based examination of the data will also better help us understand where these hot spots of contamination are, whether they are spatially clustered and what nearby sources may exist. Furthermore, analysis is underway to compare results of metal concentrations in fish tissue to habitat and food sources for the species sampled to determine the patterns of bioaccumulation in the CLT food web.

Data interpretation: A comprehensive analysis of all components of the study is also underway to better understand the pathways of transference of contaminants based on diet, habitat use and trophic status. Work is also being conducted to better determine implications of our results from the perspective of which contaminants are of greatest concern for their potential toxicity and lethal or sublethal effects to species such as

EPGTs and CLTs. We are examining our results in the context of both human health risk based screening levels and dietary screening levels for fish or avian species.