

# Application of stable-isotope methods to monitoring Bald Eagle restoration and management on Santa Catalina Island, California

## *A Report to the Science Review Panel for the Northern Channel Islands Bald Eagle Feasibility Study*

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### **Background**

Stable isotope methods are being used increasingly in ecological studies and range from ecosystem-level scales such as the delineation of terrestrial or marine foodwebs to more local scales involving diets or movements of individuals (reviewed by Rundel et al. 1988, Lajtha and Michener 1994, Michener and Schell 1994, Hobson 1999, Kelly 2000). There has also been rapid progress in the application of stable isotope methods to better understand the behavior of contaminants since isotopic measurements may allow insight into dietary sources and trophic levels of individuals (reviewed by Kidd 1998). This report will examine the potential for using such isotopic methods to better understand ecological factors influencing contaminant load in Bald Eagles reintroduced to Catalina Island, California. Here, the stable isotopes of carbon and nitrogen will be considered as tools to monitor trophic position and possibly source of feeding by eagles associated with marine foodwebs off this region of southern California.

Carbon occurs in two stable forms as  $^{13}\text{C}$  and  $^{12}\text{C}$ . Carbon isotopic compositions (expressed in delta notation as  $\delta^{13}\text{C}$  and representing the relative abundance of the heavier to lighter isotope,  $^{13}\text{C}/^{12}\text{C}$ , compared with an arbitrary standard) of animals reflect those of the diet within about 1‰ (DeNiro and Epstein 1978, Peterson and Fry 1987). For

this reason, stable-carbon isotope analysis is an ideal tool for tracing origins of nutrients in foodwebs since carbon may enter the base of foodwebs with characteristic isotopic signatures due to a variety of biogeochemical processes and these change little throughout the foodweb. The main processes of interest here are those resulting in differences in  $\delta^{13}\text{C}$  values of terrestrial vs. marine foodwebs and  $\delta^{13}\text{C}$  values associated with inshore or benthic foodwebs and offshore or pelagic foodwebs.

Carbon in the atmosphere is depleted in  $^{13}\text{C}$  relative to dissolved carbonate in the oceans by about 7‰ (Craig 1953, Chisholm et al. 1982). This difference arises from the fact that dissolved inorganic carbon in the oceans is derived ultimately from the atmosphere. Effectively, heavier carbon dioxide enters into bicarbonate ion exchange at the ocean/atmosphere interface more readily than does lighter carbon dioxide. In general then, marine foodwebs tend to be enriched in  $^{13}\text{C}$  compared with terrestrial C-3 foodwebs and this difference has been used to trace the relative contributions of terrestrial vs marine protein in the diets of contemporary (e.g. Hobson 1986, 1990) and prehistoric consumers (e.g. Chisholm et al. 1982, Hobson and Collier 1984). Within marine systems, inshore or benthically linked foodwebs also tend to be more enriched in  $^{13}\text{C}$  than offshore or more pelagic foodwebs (reviewed by France 1995) and this effect has been used successfully to examine feeding locations of a variety of seabird species (Hobson et al. 1994).

There are two stable forms of nitrogen,  $^{14}\text{N}$  and  $^{15}\text{N}$ . In terrestrial systems, nitrogen enters foodwebs through symbiotic fixation or through direct conversion of atmospheric nitrogen within plants (reviewed by Nadelhoffer and Fry 1994). These processes typically lead to different isotopic signatures of plants adopting these strategies. In marine systems,

inorganic nitrogen occurs as molecular nitrogen, ammonia, nitrate, nitrite and nitrous oxide. Relatively little is known about the isotopic fractionation effects occurring between these nitrogen pools and phytoplankton (Owens 1987, but see Wada and Hattori 1976).

As was found for stable-carbon isotopes, the stable nitrogen isotopic composition of animals (expressed as  $\delta^{15}\text{N}$  values) is related ultimately to the isotopic compositions of their diets (DeNiro and Epstein 1981, Macko 1981, Minagawa and Wada 1984). Through processes associated primarily with differential excretion of  $^{14}\text{N}$ , animals incorporate dietary  $^{15}\text{N}$  into their tissues preferentially, an effect due to discrimination against the lighter isotope during protein amination and deamination (Gaebler et al. 1966, Macko et al. 1982, Minagawa and Wada 1984). A broad survey of field and laboratory data confirms that there is a significant linear relationship between  $\delta^{15}\text{N}$  values of an organism and its diet (DeNiro and Epstein 1981, Owens 1987). This phenomenon forms the basis of using  $\delta^{15}\text{N}$  measurements to infer trophic level. Because of this strong trophic linkage,  $\delta^{15}\text{N}$  measurements can be used as a powerful surrogate for trophic level in contaminant studies. The slope of the relationship between contaminant concentration and  $\delta^{15}\text{N}$  values of consumers in a foodweb in fact represents the bioaccumulation behavior of that contaminant in that foodweb (Jarman et al. 1996).

A fundamental advantage to using the stable isotope approach over more conventional means of dietary analysis (see Duffy and Jackson 1986) is the *time integrated* nature of the technique (Hobson and Clark 1992a,b). Because different tissues have different metabolic rates, the residency time of each element in a metabolically active tissue is directly related to its metabolic rate. Blood plasma has a relatively fast turnover rate (with

an elemental half-life of about 3 days) compared with the cellular fraction of blood representing a much longer period of dietary integration (i.e. a half life of approximately 30 days, Hobson and Clark 1993). Metabolically inactive tissues such as feathers represent diet of individuals during the growth period only, after which the dietary signal is effectively locked in. Thus, by examining several tissues, a dietary or trophic history of an individual can be assembled. The analysis of egg components can provide information on the diet or use of endogenous tissues by females during the period of egg formation. Different components of eggs can represent different periods of formation with the egg yolk representing the longest period and the shell and shell membrane the shortest (Hobson 1995, Sydeman et al. 1997).

#### **Potential application to the Catalina Island study**

There is considerable potential for using stable isotope monitoring techniques as part of the Bald Eagle restoration and management program on Santa Catalina Island because the demonstrated broad trophic range of individuals in this population results in differential exposure to and bioaccumulation of OC contaminants. Stable-isotope analyses can allow trophic positions of individuals to be established for several time scales or periods of dietary integrations (depending on tissues used) and so allow this fundamental component of individual ecology to be factored in to aspects of survival, reproductive success and contaminant load.

The measurement of stable-nitrogen isotope abundance in various tissues of Bald Eagles will provide insight into their trophic positions and how these change through time. Stable-carbon isotope analyses will additionally provide insight into location of feeding pertaining to inshore vs. offshore sources of prey in the marine environment and

also possible terrestrial sources of protein. The type of information gleaned using the isotope technique will depend in large measure on the tissues available and how these are stratified by time, location, and age class. Because sampling tissues represents a major challenge in this study and analyses will be largely restricted to archived material as well as fairly opportunistic sampling, the following deals with the likely types of materials that can come available and their possible use from an isotope perspective.

i) *Blood and feathers*

Blood can be separated into plasma and cellular fractions for assaying short- and long-term dietary integrations, respectively (Hobson and Clark 1993). Feathers provide isotopic information for the period of feather growth. Depending on the molt pattern of Bald Eagles, feather samples could potentially be used to reconstruct diet over a period approaching the total duration of molt. Because only a small amount of material is required for analysis (of the order of a few milligrams dry weight), it would be relatively easy to sample a sequence of flight feathers without hampering the flight of an individual. For these reasons, the sampling of blood and feathers of all captured birds is desirable. The details of flight and body feather molt for birds at this southern latitude should also be established to assist in the design of feather sampling.

ii) *Eggs*

Eggs provide a rich source of material for isotopic analysis and can be separated into yolk lipid, yolk protein, whole yolk, albumin, egg shell membranes, and the organic and inorganic fractions of the egg shell (Hobson 1995). However, only the yolk material will

likely provide dietary information representing a significant period of several weeks (i.e. the period of yolk formation and possibly inputs from endogenous stores). If whole eggs are available for analysis, the egg yolk, or whole egg homogenate is preferable. More short-term dietary information can be gleaned from remains of eggshells in nests (i.e. egg shell and membranes).

### iii) *Foodweb samples*

In order to use the stable isotope values measured in eagle tissues to place birds into a trophic model for the local marine foodweb, it will ultimately be useful to obtain stable isotope analyses of marine food items that span several trophic levels and also represent inshore/benthic and offshore/pelagic organisms. This approach will ultimately allow the trophic level of eagles to be estimated based on known trophic linkages within this foodweb (e.g. Hobson and Welch 1992, Hobson 1993). However, such a trophic model is not necessary in the short-term in order to use the stable isotope approach to gain insight into the foraging ecology of individuals. For example, eagles with higher  $\delta^{15}\text{N}$  values can be assumed to be of higher trophic level and any isotopic analysis of tissues also with organochlorine or other contaminant concentrations can readily be interpreted using  $\delta^{15}\text{N}$  values as a surrogate for trophic level.

### **Recommendations**

- 1) Measure  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in archived egg yolk or homogenates for which OC contaminant values are available. Plot  $\delta^{15}\text{N}$  values vs. logarithm of contaminant concentration. This will allow us to test the hypothesis that individuals feeding at

higher trophic levels (i.e. higher  $\delta^{15}\text{N}$  values) are more susceptible to contaminant accumulation. A biplot of  $\delta^{15}\text{N}$  vs.  $\delta^{13}\text{C}$  values will also allow the interpretation of whether more contaminated individuals tend to cluster according to trophic level and location of prey. Eagle age, if available, may be an important covariate in this sort of analysis. Feather sampling should follow from information on molt sequence in order to maximize the period of dietary integration.

- 2) Opportunistically obtain blood and feather samples from all captured individuals. Again, the routine isotopic measurement of these tissues for wild caught individuals will allow the comparison of contaminant load with individual feeding history.
- 3) Opportunistically obtain tissues from nonviable eggs. This approach follows on from the first recommendation with the additional possibility of examining short-term diet using egg shells and membranes.
- 4) Recover feather and tissue samples (muscle and bone) from any carcasses. This will allow the creation of individual dietary history. Of particular value in the case of a carcass is the use of bones for the isotopic analysis of bone collagen. Bone collagen has an extremely low metabolic rate and so can provide an integration of diet over the lifetime of the individual.
- 5) Obtain a suite of foodweb samples for the creation of a foodweb isotope model.
- 6) Tissues other than feathers should be stored frozen. Feathers can be stored in individual paper envelopes. Stable isotope samples are not compromised by enzymatic breakdown but bacterial growth needs to be avoided. Thus, samples can go several hours without freezing. If samples are to be stored for long periods without freezing, soak them in 70% ethanol and remove the ethanol again before freezing for

longer term storage. Prior to stable isotope analysis, samples should be subsampled and freeze dried (preferable) or oven dried. Most labs will require samples to be weighed out into small tin cups prior to submission. For sample analysis, only a few milligrams dry weight are required.

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