

## **TITLE: Determination of Plasma Amino Acid Concentrations in Wild and Captive Sea Otters.**

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### **HYPOTHESES AND OBJECTIVES**

The objective of this study is to determine the plasma amino acid profiles in sea otters in captivity and in wild populations in mainland California (CA); Prince William Sound, Alaska (AK); and Kodiak Island, AK. The study will establish the range of normal values for plasma amino acids in sea otters. Data will be evaluated for effect of known diet, indications of metabolic diseases or dietary amino acid deficiencies and differences among otter populations.

### **EXPERIMENTAL PLAN**

#### *Plasma sample collection and processing*

Plasma samples will be collected from approximately 80 sea otters. These will include 20 otters from the captive (permanent and rehabilitation) populations at Long Marine Lab (LML), University of CA, Santa Cruz and the Monterey Bay Aquarium (MBA) in Monterey, CA. All of these otters are anesthetized for routine physicals, and blood can be drawn at that time. Plasma samples from free-ranging otters will be obtained during captures in coordination with ongoing research efforts. Otters are anaesthetized when captured, and blood is drawn at that time. Samples can be collected from 20 free-ranging otters from mainland CA; 20 from Prince William Sound, AK; and 20 from Kodiak Island, AK.

A minimum of 2 ml of blood will be drawn from jugular or femoral veins into a heparinized syringe. The blood will be placed into a glass tube containing lithium heparin and centrifuged. The plasma will then be placed in 0.5 ml aliquots. One aliquot will be saved untreated, and to each remaining aliquot, 0.5 ml of 6% sulphosalicylic acid will be added to precipitate the plasma proteins. Samples will be shipped frozen to the laboratory at UC Davis and stored at -80° C until analyzed.

At the same time that blood samples are obtained from the captive, rehabilitation or wild otters, morphometric measurements will be taken. Whenever possible, known diet history and time of feeding relative to the blood draw will be recorded. Written support has been obtained from Dr. David Jessup, senior wildlife veterinarian associated with LML and mainland CA capture activities; Michelle Staedler of the Sea Otter Research and Conservation Program at MBA; and Jim Bodkin, research biologist responsible for capture activities in AK (attached). Samples will be collected under existing permits held by these individuals and organizations. Copies of these permits can be supplied upon request.

#### *Food sample collection and processing*

We are currently collaborating on a study to measure amino acid concentrations in otter prey species from mainland California. Data from that study will be used to calculate the amino acid composition of wild otter diets whenever data is available on the dietary preferences of the individual otter. However, there are some prey species in Alaska that are different from the mainland CA species. Also, some foods fed to captive otters have unknown amino acid composition. For these prey species and foods, we will collect 10 g of fresh tissue in plastic bags and freeze at -80°C until analysis. Samples will be shipped frozen on dry ice to the laboratory at UC Davis. Prior to analysis, samples will be thawed and homogenized using a Waring blender.

#### *Sample and data analysis*

Amino acid concentrations of plasma and food samples will be determined in the laboratory of Dr. Andrea Fascetti at the University of CA, Davis with an automated amino acid analyzer (Biochrom 30, Cambridge England) that incorporates cation-exchange high-pressure liquid chromatography,

separation and ninhydrin-reactive colorimetric detection. Reference intervals will be established for the twenty four most commonly evaluated plasma amino acids. Results will be evaluated with respect to otter population location. Among the captive otter population sample, dietary protein and amino acid intake will be calculated from the known current diet and compared to plasma amino acid levels. The same analysis will be performed when estimated diet history of wild otters is known. Significant differences will be considered as potential indications of dietary amino acid deficiencies and/or metabolic diseases.

## **SIGNIFICANCE TO OILED WILDLIFE HEALTH**

This project will help to fill one of the goals of the OWCN's Research and Technology Development Program: "To assess wildlife health... by compiling biomedical health parameters of commonly oiled wildlife species." We propose to analyze plasma amino acids from several wild and captive populations of sea otters to establish the normal range of amino acid concentrations, and potentially identify deviations from the normal. The measurement of plasma amino acid concentrations has proven to be a useful tool in human and veterinary medicine for diagnosing dietary inadequacies in addition to genetic, nutritional and metabolic diseases (Zicker and Rogers 1990). Population trends (Estes et al. 2003, Kreuder et al. 2003, Tinker 2004) suggest nutritional deficiencies or imbalances may be limiting the threatened sea otter populations of California and Alaska. The project proposed here will evaluate one facet of nutrition of sea otters by measuring the plasma amino acid concentrations of wild and captive otters.

Plasma amino acid analysis can be used to determine a deficiency of an essential amino acid in the diet of an animal (Broderick et al. 1974, Rogers and Morris 1979, Zicker and Rogers 1990). In addition, deviations from normal plasma amino acid concentrations are associated with several diseases. Low taurine concentration is associated with central retinal degradation in cats (Hayes 1975) and dilated cardiomyopathy in cats (Pion 1987) and dogs (Fascetti et al. 2003). Protein calorie malnutrition (PCM), also known as Kwashiorkor in children in third world countries, results in a general increase in nonessential amino acids and a decrease in essential amino acids in plasma (Badger and Tumbleson 1974, Holt 1963, Worthington et al. 1979). Severe PCM is associated with increased septicemia and mortality, probably due to decreased immune function (Stinnett 1983, Scragg 1978). Diseases of the liver and kidney can also cause alterations of plasma amino acids as a secondary effect of the disease process (Strombeck and Rogers 1978, Kopple 1978, Langer et al. 1988).

The proposed study will measure the plasma amino acid concentrations of several populations of wild sea otters with varying success and growth rates, all at risk of oil exposure. The database produced by this project will establish normal reference intervals for plasma amino acids in the sea otter, providing a foundation for a potential diagnostic tool in this species.

## **PROJECT DURATION: 1 year**

### **ESTIMATED BUDGET**

<u>Supplies</u> for sample collection (test tubes, needles, solutions)	\$ 500
<u>Travel</u> for sample collection (within CA)	\$ 600
<u>Other expenses</u>	
Plasma amino acid analysis (80 plasma samples at \$73.19/sample)	\$5,855
Food amino acid analysis (10 samples at \$83.75/sample)	\$ 838
Sample transport (frozen from CA and AK to UC Davis via overnight courier)	\$ 600
Statistical assistance (8 hours at \$50/hour)	\$ 400
Publication costs	\$ 500
<b>Grant Total Requested:</b>	<b>\$9,293</b>

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