ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
PO Box 25526
Juneau, AK 99802-5526

COOPERATIVE ENDANGERED SPECIES CONSERVATION FUND

GRANT AND SEGMENT NR: E-12

PROJECT NUMBER: 1

PROJECT TITLE: Chlorinated Fatty Acids in Northern Sea Otters in Alaska

PRINCIPAL INVESTIGATORS: Lorrie Rea (ADF&G)

COOPERATORS: John M. Kennish, University of Alaska-Anchorage; Angela Doroff, USFWS

PROJECT DURATION: 1 July 2007 – 30 June 2008

REPORT PERIOD: 1 July 2007 – 30 June 2008

I. PROBLEM OR NEED THAT PROMPTED THIS RESEARCH

Since 2002 the USFWS has recorded a marked increase in sea otter mortality in Kachemak Bay. These animals belong to the southcentral Alaska population stock which is located immediately adjacent to the ESA listed southwest Alaska Distinct Population Segment (DPS). The predominant cause of death has been a heart defect (specifically acute valvular endocarditis) and sepsis which is likely caused by a *Streptococcus bovis/equinus complex* infection. A small number of cases of this disease have also been documented within the listed population at Kodiak, the Alaska Peninsula, and the Aleutian archipelago, but the extent of this infection in the listed stock is not yet clear because the reporting and recovery of stranded otters is less prevalent in these more remote regions of Alaska. At present, the mechanism for the infection in sea otters is not understood. The mortality due to this vegetative heart lesion has been significant in Kachemak Bay and in August 2006, was declared as an Unusual Mortality Event (UME) under the Marine Mammal Protection Act. There is some concern that an underlying environmental stressor may have a compromising impact, making these individuals more susceptible to infection. We have investigated the presence of chlorinated fatty acids (CFA) in the cardiac and skeletal muscle tissue of stranded northern sea otters to determine if this population has been exposed to and has accumulated this contaminant, and determine if there is a higher occurrence of CFA contamination in otters which have died as a result of disease (e.g., acute valvular endocarditis) compared to those which have died as a result of trauma (e.g., boat strike). Since CFAs tend to accumulate in the cardiac muscle tissue of other Alaska wildlife species and clinical research on other mammalian species has shown negative effects of CFA contamination on membrane function we believe that high levels of this contaminant may act as an environmental stressor to compromise the health of otters.

II. REVIEW OF PRIOR RESEARCH AND STUDIES IN PROGRESS ON THE PROBLEM OR NEED

Chlorinated fatty acids are found in biota in areas where organochlorine chemicals are present (*e.g.*, PCB, DDT and derivatives, etc.) and may represent a significant load (e.g., Ewald 1999;

Milley *et al.* 1997). Chlorine moieties incorporated into the fatty acids of marine invertebrates may be passed along to higher trophic levels through the food chain. CFA have been detected in mussels and in marine sea ducks collected in the Aleutian Islands, both species that feed in the same nearshore habitat that is important for sea otter foraging. Given previous similarities in contaminant responses between sea otters and sea ducks in this region (i.e., P-450 responses), it is important to determine if sea otters have also experienced similar exposure to CFA in this environment. Surprisingly, the highest concentrations of CFA have been detected in the heart muscle of these ducks (Hoffman, *et. al.*, 2002). Recent analysis of skeletal and heart muscle tissue collected from Kenai River Chinook salmon found that CFA were concentrated in the phospholipids (important for membrane structure) of both muscle types at a level of 1-2 mg/g lipid (King et al. 2006). The physiological effects of CFA appear to be targeted at the cell membrane, causing small changes in membrane structure which lead to leakage of ATP (Ewald and Sundin 1998) potentially affecting the animal's energy budget and the production of gametes in males. These are physiological effects which would impact not only the individual but ultimately the population. Despite the demonstrated toxicity of CFA, organisms do not appear to differentiate or attempt to eliminate the compounds. Unlike traditional chlorinated pollutants, chlorinated fatty acids do not activate the detoxifying P-450 enzyme system (Håkansson *et al*. 1991). This suggests that CFA are not recognized by organisms as xenobiotics and therefore are not preferentially expelled from the body, giving them a greater possibility for bioaccumulation.

III. APPROACHES USED AND FINDINGS RELATED TO THE OBJECTIVES AND TO PROBLEM OR NEED

OBJECTIVE 1: Determine the presence and abundance of chlorinated fatty acids in the cardiac and skeletal muscle of stranded northern sea otters.

Cardiac and skeletal muscle tissue samples (n=48, approx. 2 grams each) were collected by the USFWS personnel using standard necropsy protocols and be delivered to the UAA ASET laboratory for analysis. Chlorinated fatty acids were quantified using laboratory techniques recently published by King et al. 2006. Each muscle tissue sample was separated into three lipid fractions (triacylglycerols, TAG; fatty acids, FA; phospholipids, PL) using solid phase extraction. CFA concentration was analyzed in a total of 144 lipid fractions for this pilot study.

There was a trend for higher concentrations of total CFA in the lipids extracted from heart tissue compared to skeletal tissue extracts (Figure 1); however, this was not statistically significant due to high variance in the concentration of total CFA in heart tissue in particular. The highest concentrations of CFA were found in the structural lipid fractions (phospholipids, PL) and the lowest concentrations in the storage lipids (triacylglycerols, TG). Dicloromyristic acid ($C_{14}Cl_2$) concentrations were either below limit of detection levels or were not encountered at all in sea otter tissues. The concentrations of dichloropalmitic ($C_{16}Cl_2$) and dichloroarachidic acids ($C_{20}Cl_2$) were around 1 mg/g lipid for both muscle tissue types. The concentrations of dichlorostearic acid ($C_{18}Cl_2$) were higher in heart muscle (10 ± 14 mg/g lipid) than in skeletal muscle (4 ± 5 mg/g lipid), but a high level of variability was found in both tissues, possibly due to small sample size.

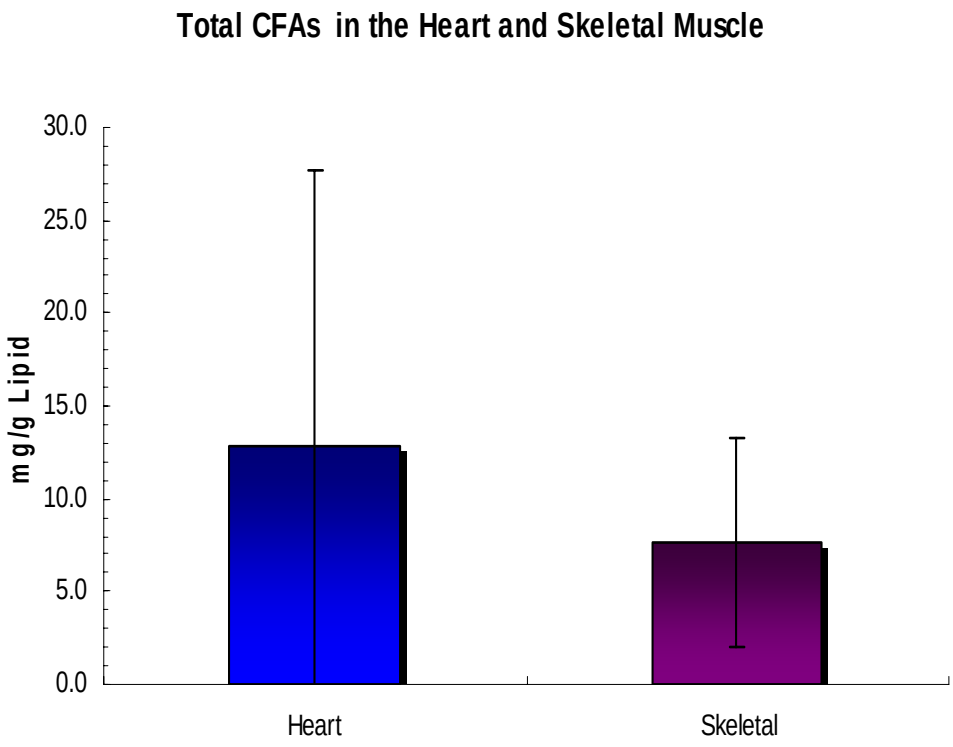

Figure 1. Comparison of total mean chlorinated fatty acid (CFA) concentrations (mg / g lipid) in northern sea otter heart and skeletal tissue.

OBJECTIVE 2: Determine if there is a pattern of association between muscle CFA contaminant levels and cause of death in sea otters.

Some of the variability in the concentration of total CFA in northern sea otter muscle tissues can be explained by the location in which the sea otter was sampled post-mortem and presumably was foraging prior to death (Figure 2). Small sample sizes currently prohibit detailed analysis of the relationship between total CFA concentration and ultimate cause of death, however, there was no obvious elevation in CFA concentration in animals diagnosed post-mortem with acute valvular endocarditis compared to those determined to be killed by trauma (Figure 3). A more detailed analysis will be completed once all contaminant and pathology laboratory findings are available for these animals.

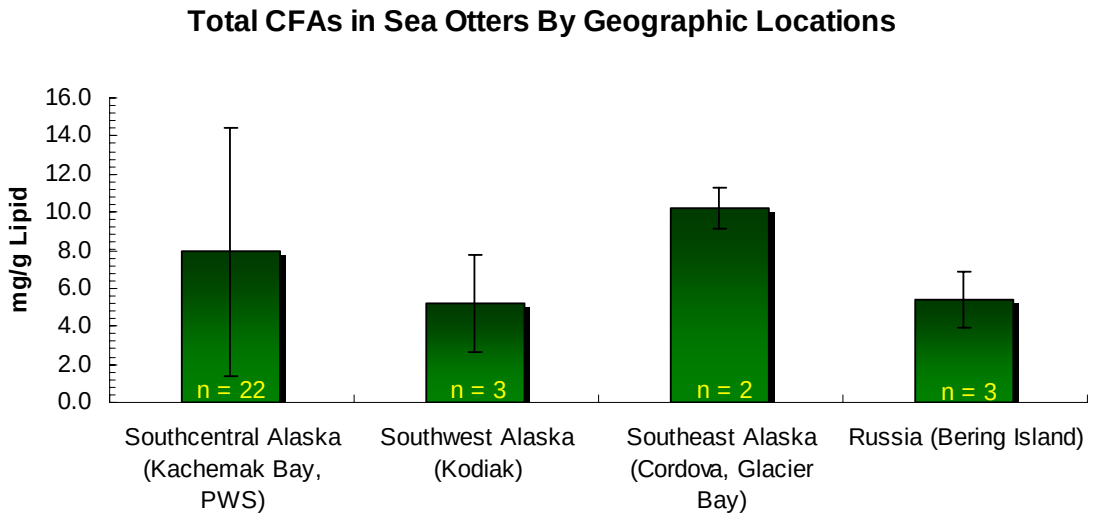

Figure 2. Comparison of mean total chlorinated fatty acid concentration (mg/g lipid) of northern sea otter skeletal tissues between four regions of collection (southcentral Alaska, southwest Alaska, southeast Alaska and Russia).

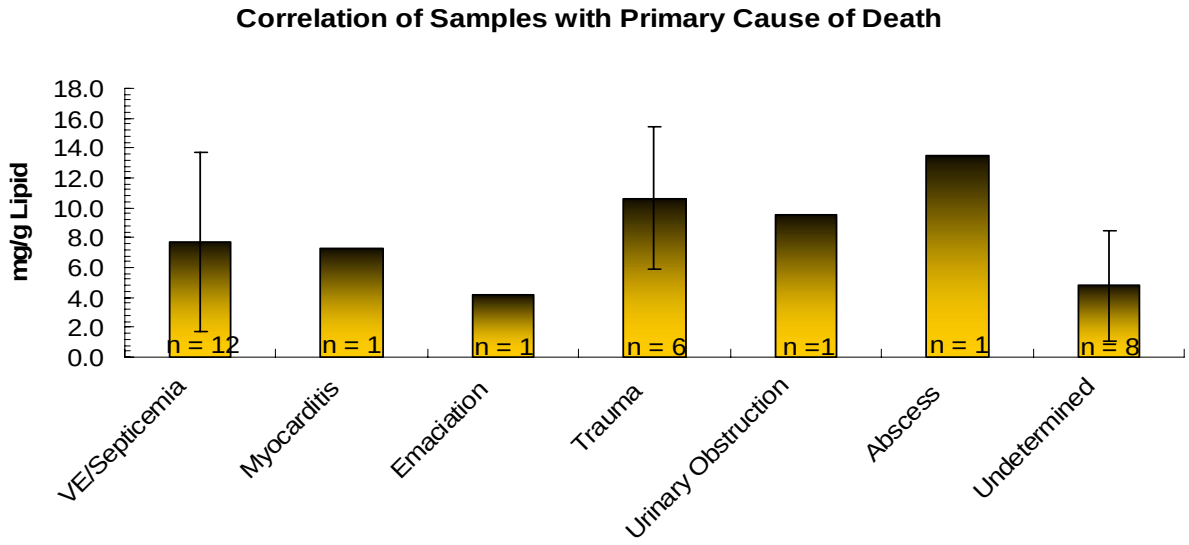

Figure 3. Comparison of mean total chlorinated fatty acid concentration (mg/g lipid) of skeletal tissues between northern sea otters with various causes of death.

OBJECTIVE 3: Determine need for continued study based on the results of pilot project.

This study indicates that CFA concentrations in the sea otter heart muscles are an order of a magnitude higher than the concentrations found in the heart muscle of Chinook salmon (King *et al.* 2006), and 4 fold higher than total CFA concentrations found in Steller sea lion heart tissue (unpublished data; Smith, Kennish, Rea et al.). These high concentrations accumulated in sea otter heart tissue (and to a lesser degree in skeletal tissue), along with preliminary indications of regional differences in exposure of otters to CFA's suggest that additional laboratory analysis is warranted in this species. Additional heart tissue samples should be analyzed to increase our ability to test for a correlation between CFA exposure and identified cause of death. Based on the results of this pilot project future analyses could be streamlined by concentrating on analysis of only the phospholipids fraction extracted from muscle tissue, since the highest concentration of CFA are found in this lipid fraction in northern sea otters.

Persistant organic pollutants such as PCBs and DDTs tend to accumulate in neutral storage lipids (Kawai *et al*. 1988) whereas CFAs tend to accumulate in polar lipids. Mu. H. (1996) suggests that the polar nature of these structural lipids, as well as the role of PLs in the structural formation of membranes slows down the turnover rate of CFAs. This causes CFAs to accumulate over time. The phospholipids in the heart and skeletal muscles of northern sea otters in this study contained the highest average concentrations of CFAs per gram lipid. This accumulation of CFAs in the heart tissues may alter the normal physiological functions of the heart by interfering with efficient membrane function.

IV. MANAGEMENT IMPLICATIONS

This study provides the first report of the presence and concentration of these compounds in northern sea otters using a unique set of archived tissues collected during necropsy of otters over the past 5 years. This data will compliment additional contaminant and pathology investigations conducted on these necropsy animals by the USFWS and their collaborators during the past 2 years. It is currently unclear if CFA concentrations are significantly linked to the prevalence of acute valvular endocarditis found in southcentral Alaska sea otters during the UME (possibly due to low sample size). We believe that further analysis of tissues collected during necropsy of northern sea otters is warranted beyond this pilot study and this information and recommendation will be provided to the UME consultant team, the Recovery Team, and the management agency (USFWS) so that they may judge this along with other contaminant and pathology results whether there is need to prioritize research on environmental contaminants as a potential cause of the decline of sea otter populations and potentially tailor future management actions to mitigate this risk in the environment.

V. SUMMARY OF WORK COMPLETED ON JOBS <u>FOR LAST SEGMENT PERIOD ONLY</u> (July 1, 2007 – June 30, 2008)

JOB/ACTIVITY 1A: Laboratory assay of chlorinated fatty acid concentrations in 120 muscle samples collected by USFWS during necropsy of stranded sea otters. This laboratory analysis will be performed by an ADFG college intern working in the UAA ASET laboratory under Dr. Kennish's supervision.

The majority of Federal aid funds were focused on the laboratory analysis of CFA. All analysis costs (approximately $9,000) were expended on the analysis of CFA homologs in 144 lipid fractions of sea otter tissues (24 more than the originally proposed 120 analyses). Approximately 90% of the College Intern personnel costs were directed at laboratory analyses.

JOB/ACTIVITY 1B: Data analysis and synthesis into reports of contaminant level findings to ADFG, the UME consultant team, the Sea Otter Recovery Team and appropriate USFWS sections. These results of research will also be included in a UAA senior thesis.

Approximately 40% of ADFG researcher personnel costs were directed to report preparation and interagency coordination of research. The College Intern involved in this project submitted her thesis research paper on a different contaminant analysis conducted on sea otter tissue supported through a separate grant funding through the UAA undergraduate research office. This student will be involved, at no cost to this grant, in the final preparation of this research for publication during Fall 2008, which will be her final semester of undergraduate tenure.

JOB/ACTIVITY 2A: Incorporate CFA concentration data with other data collected during necropsy of stranded sea otters, including final assessment of cause of death, to determine if there is an association between disease and contaminant exposure using an epidemiological approach. This will contribute directly to studies underway at USFWS.

The remaining 10% of College Intern personnel costs were directed at data analysis to determine significant relationships between CFA concentrations and region of collection and cause of death of the stranded sea otter. Approximately 50% of ADFG researcher personnel costs were directed to this data analysis and student mentoring. Final, more in depth analyses will be completed by the PI (at no cost to this grant) once full contaminant and pathology laboratory results are available from collaborating studies.

JOB/ACTIVITY 3A: Determine the need for CFA assays on additional samples based on level of accumulation of CFA in sea otter muscle tissue above detection limits and based on a power analysis using data on variability of exposure seen in this pilot study.

The remaining 10% of ADFG researcher personnel costs were spent on data analysis to inform decisions about the extension of this pilot project to further investigate CFA contamination in northern sea otters.

VI. PUBLICATIONS

Smith, R.T., A.M. Doroff, V.A. Gill, L.D. Rea and J.M. Kennish. 2008. Chlorinated Fatty Acids (CFAs) in Alaskan Sea Otters. Poster presentation at the Alaska Marine Science Symposium, Anchorage, AK, January 20-23, 2008.

Chlorinated Fatty Acids (CFAs) in Alaskan Sea Otters

Revathy T. Smith[a,b], Angela M. Doroff[c], Verena A. Gill[c], Lorrie D. Rea[d], and John M. Kennish[a,b]

University of Alaska Anchorage:
[a]Applied Science, Engineering, and Technology Laboratory;
[b]Department of Chemistry, Alaska 99508,
[c]U.S. Fish and Wildlife Service; Marine Mammals Management, Alaska 99503, and
[d]Alaska Department of Fish and Game, Wildlife Conservation, Steller Sea Lion Research Program, Alaska 99775.

Recently, Kachemak Bay sea otters have been declining at an unusual rate (USFWS, 2006). A significant percentage of necropsies conducted on these animals revealed heart damage (valvular endocarditis) caused by bacterial infection (*Streptococcus bovis/equinus;* USFWS, 2006). The goal of this study was to measure the concentrations of different CFA homologues in Alaskan sea otter cardiac and skeletal tissues. Jones et al. (1983) discovered rats developed heart lesions when fed brominated corn oil with the dibromostearic acid ($C_{18}Br_2$) homologue being the highest concentration in heart muscles. The impact of halogenated fatty acids like CFAs in the heart will be thoroughly investigated. The possible adverse effects of CFAs bound to membrane lipids may cause membrane disturbances such as improper regulation of membrane fluidity and permeability problems. The concentrations of CFAs in triglycerides, free fatty acids and phospholipids were obtained. Preliminary data revealed alarming levels of CFAs within sea otter heart and skeletal muscles. The total concentrations of CFAs per gram lipid were much higher in heart muscle (13 ± 15 mg/g lipid; n=18) when compared to skeletal muscle (8 ± 6 mg/g lipid; n=30). Most of the CFA homologues were incorporated into the phospholipids. Dicloromyristic acid ($C_{14}Cl_2$) concentrations were either below limit of detection levels or were not encountered at all in sea otter tissues. The concentrations of dichloropalmitic ($C_{16}Cl_2$) and dichloroarachidic acids ($C_{20}Cl_2$) were around 1 mg/g lipid for both tissue types. The concentrations of dichlorostearic acid ($C_{18}Cl_2$) were significantly higher in heart muscle (10 ± 14 mg/g lipid) than in skeletal muscles (4 ± 5 mg/g lipid). The lowest concentrations of CFAs were found to be in storage lipids. Free fatty acids contained the second highest concentrations of CFAs in both tissue types.