An investigation into the possible relationship between killer whale (*Orcinus orca*) predation and the continuing decline of the Steller sea lion (*Eumetopias jubatus*) population

Graham A.J. Worthy, University of Central Florida, Orlando FL
Markus Horning, University of Alaska, Fairbanks, AK
Marilyn E. Dahlheim, National Marine Mammal Lab, NMFS, Seattle, WA

In 1997, the western population of the Steller sea lion (*Eumetopias jubatus*) was classified as "endangered" and the eastern population as "threatened" under the Endangered Species Act. A number of reasons have been proposed as possible causes of this decline, but most have been eliminated from consideration. The leading hypothesis that is still being considered being a potential change in food availability (e.g., Castellini 1993, Merrick and Calkins 1996), however, despite numerous studies attempting to link the continuing decline in the size of the Steller sea lion population with nutritional stress, there has yet to be a definitive linkage made. An alternate cause for the continuing decline in the sea lion population has recently been proposed. While predation by transient killer whales (*Orcinus orca*) is not likely to have caused the Steller sea lion decline, it may now be a contributing factor.

Discerning the feeding habits of cetaceans can be difficult, however, there are several indirect methods of study available. In recent years a number of investigations have used stable isotopes of carbon and nitrogen to determine trophic relationships of a variety of birds and mammals (*e.g.,* Schell *et al.* 1989, Hobson and Montevecchi 1991, Kurle and Worthy 2001, Kurle and Worthy In press). Isotope ratios are ultimately determined by the food that has been incorporated into the animal over the past several weeks or months, and can give an overall idea of the average diet. With multiple types of food generally available, isotope ratios can indicate, but cannot prove, that a certain type of food was used; they can however, sometimes prove when a food has not been eaten and assimilated.

Isotope data derived from a pilot study on killer whales in Prince William Sound (PWS), AK suggested that some transient killer whales had been feeding at a higher trophic level than resident whales (Worthy and Abend 1998). This could imply that they had been feeding on marine mammals, but based only on isotopic data the possibility that they may have been feeding on piscivorous fish cannot be excluded. These stable isotope data do suggest that some transient whales were feeding at higher trophic levels than other transients or residents, however, it was considered unlikely that any whales were feeding solely on marine mammals (Worthy and Abend 1998).

Fatty acid signature analysis has emerged recently as a method that potentially has the resolution to determine individual prey species (Iverson *et al.* 1997). Marine food webs contain many long chain fatty acids (LCFA) that are specific to individual prey items, and are generally incorporated into marine mammal blubber with minimal modification (Iverson 1993). By examining the blubber layer, it is possible to use LCFA as indicators of possible prey items (Iverson 1993, Samuel and Worthy 1999). Ultimately, this approach will require knowledge of the fatty acid signatures of all likely prey species, but it has the potential to answer the question of whether a particular whale had been feeding on a specific species.

Objectives:
The objectives of this research project were to analyze killer whale blubber and skin samples for stable isotope and fatty acid signatures; to assign trophic status to known transient and resident killer whales that have been sampled from California to the Aleutian Islands using nitrogen isotope ratios; and to assess fatty acid signatures of Steller sea lions to attempt to interpret killer whale fatty acid signatures.

Progress:
Thus far we have made progress towards achieving objective 1, but have not gone further due to continuing delays in receiving funds.

Preliminary analysis to determine if fatty acid composition changes across blubber depth in the killer whale have been completed. Blubber samples from six individuals were subdivided into six equal vertical parts (layers), from skin to muscle. The outermost layer was the region located immediately adjacent to the skin, while the innermost layer was located just above the muscle. Lipid was extracted from each sub-sample using a 2:1 chloroform/methanol solution (Folch et al.1957; Iverson et al. 1997), and fatty acids subsequently esterified through a combination of 0.5N sulfuric acid in methanol and dichloromethane (Hilditch and Williams 1964). The resultant fatty acid methyl esters (FAMEs) were stored in hexane, and then analyzed using a Perkin-Elmer gas-liquid chromatograph linked to Turbochrome 6.1.1 software for chromatogram editing. FAMEs were converted to percent amounts for each layer, and fatty acid composition then compared among layers.

An increase in long-chain fatty acids (*i.e.,* 18 carbon length or greater) was observed across the blubber, with the largest percentage found in the innermost layers and least amount detected within the outermost layers. Medium chain fatty acids (12 to 17 carbons in length) were also found to decrease from the outermost to innermost layer. Classification and Regression Tree (CART) statistical analyses were able to consistently separate the outermost two layers from the innermost two layers. The two middle sub-samples tended to classify more closely with the outermost layer, but were not as readily distinguishable when compared to the outer and inner sub-samples. The outermost blubber, therefore, has a very different fatty acid composition relative to the innermost layers. It is likely that the middle layers act as a transitional zone, and may vary depending on the health, season, age, and/or reproductive status of the animal at the time of sampling.

In the next few months, analysis of the remaining killer whale and Steller sea lion samples will be completed. Ultimately, interpretation of feeding habits will require knowledge of the fatty acid signatures of all likely prey species, but this approach has the potential to answer the question of whether a particular whale had been feeding on a specific species. FASA has shown very promising results in initial applications to other species and while it has yet to be fully validated in controlled studies with cetaceans, it has a great deal of potential for resolving the question of feeding habits in killer whales. This first phase of inquiry will need to be followed by additional sampling of both killer whales and their prey to develop a more complete picture of feeding habits and the potential ramification of those habits for Steller sea lions.

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