DNA-based Methods for Studying the Diet of Marine Predators

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Declaration of originality

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institute, and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Bruce E. Deagle March 30th, 2006

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Thesis Abstract

Diets of large marine predators have been extensively studied to assess interactions with fisheries, monitor links between diet and reproductive success, and understand trophic interactions in marine ecosystems. Since marine species can rarely be observed foraging directly, most studies rely on the identification of prey remains in stomach contents or faeces to determine the prey items being consumed. While this approach has provided a wealth of information, it has several limitations resulting primarily from difficulties identifying digested prey and from biased recovery of remains due to differential digestion. My thesis explores the use of molecular genetic methods in dietary studies of large marine predators. DNA-based identification techniques have been used in several diet studies, but the methods and applications are still in the early stages of development. Through a number of studies, I investigated the ability to recover genetic data from various dietary samples using a range of genetic techniques.

A) *Genetic screening for prey in the gut contents from a giant squid* – I assessed the use of polymerase chain reaction (PCR)-based methods for isolation of prey DNA from an *Architeuthis* gut content sample. A taxonomically informative molecular marker was selected and a screening method developed using denaturing gradient gel electrophoresis. The methodology was used to identify prey from otherwise unidentifiable hard-part remains and the amorphous slurry component of the squid gut sample. The techniques developed here provided a framework for later chapters.

B) *Analysis of prey DNA in faeces of captive sea lions*

Part I: DNA detection, distribution and signal persistence – A feeding trial with captive Steller sea lions (*Eumetopias jubatus*) was carried out to investigate the use of genetic faecal analysis as a tool to study diet. I used group-specific PCR detection to determine: (i) the reliability of prey DNA recovery, (ii) the distribution of prey DNA within faeces and (iii) the persistence of the genetic signal after a prey item was removed from the diet. The proportions of prey DNA in several samples were also determined using a clone library approach to determine if DNA quantification could provide semi-quantitative diet composition data. Results show that the prey DNA could be reliably detected in sea lion faeces and the genetic signal could persist in samples up to 48 hours after ingestion. Proportions of prey DNA isolated from faeces were roughly proportional to the mass of the prey items consumed.

Part II: DNA quantification – Quantitative real-time PCR was used to further investigate if quantitative diet composition data could be obtained through quantification of the DNA present in faeces. I quantified the relative amounts of DNA in three fish species being fed to captive sea lions, then determined the amount of DNA recovered from these prey items in the sea lions' faeces. The results indicate that diet composition estimates based on the relative amounts of DNA in faeces can be biased due to the differential survival of DNA from different fish species; however, these biases may be less than those commonly observed in the conventional analysis of prey hard remains.

C) *Quantification of damage in DNA recovered from faecal samples* – I developed a general method to quantify the frequency of DNA damage present in specific gene regions. The technique was applied to assess the amount of DNA damage in predator and prey DNA recovered from sea lion faeces. The estimated frequency of DNA damage was always higher for the prey DNA than for the predator DNA within a faecal sample. The findings have implications for marker development and comparison of results obtained in future DNA-based diet studies.

D) *Studying seabird diet through genetic analysis of faeces* – I investigated the diet of macaroni penguins **(***Eudyptes chrysolophus***)** through conventional analysis of stomach contents and through the analysis of prey DNA extracted from faeces. Genetic data was obtained from faecal samples using PCR tests to determine the presence or absence of DNA from potential diet items and also using a clone library approach. Approximately half of the faecal samples tested positive for one or more of the prey groups targeted with PCR tests. Euphausiid DNA was most commonly detected in early stages of chick rearing and DNA from a myctophid fish was prevalent in faeces collected later; this trend mirrored the data obtained from the stomach contents. Analysis of prey sequences in "universal" clone libraries revealed a highly biased recovery of sequences from fish prey; this bias is most likely caused by the use of degenerate primers with a higher binding affinity for fish DNA template compared to DNA from other prey groups. Results obtained from the genetic and traditional approaches are compared, and potential future applications of the genetic techniques to studying seabird diet are discussed.

This series of studies has contributed significantly to our understanding of the strengths and the limitations of DNA-based diet analysis. The work identifies situations where genetic methods can be successfully applied to study the diet of marine predators and provides guidance for future studies in this emerging field.

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