

# NEW DIETARY FACTS FROM FAECES



NICK GALES

DNA analysis of the faeces of lactating Antarctic fur seals showed that they ate squid more frequently than conventional faecal analysis suggested, and that mackerel ice fish – a target of commercial fisheries – were also an important part of their diet.

**“Statistics show that of those who contract the habit of eating, very few survive.”**

*George Bernard Shaw*

All animals must eat to exist, and knowledge of a species’ diet is fundamental to understanding its biology and role in an ecosystem. In the marine environment, we need to better understand complex food webs in order to evaluate such things as the indirect impacts of fisheries and the effectiveness of marine protected areas. Since it is rarely possible to observe marine animals feeding, determining what they are eating is a challenge.

One way to get dietary information is to see what is in an animal’s stomach. For some marine species, such as fish that are caught by commercial fisheries, this type of analysis is feasible. However, it is not so easy (nor always ethically acceptable) to obtain stomach samples from top-level predators such as seabirds, seals and whales. Consequently, several alternative methods of dietary analysis have been developed.

Some methods rely on chemical signatures from prey that are incorporated into a predator’s tissue. By carrying out chemical analysis on small tissue samples collected from predators (such as blubber biopsies, whiskers and/or feathers) it is possible to get broad information about their diet.

In seals, more detailed dietary information is often obtained by sorting through their faeces to identify the origin of bones and other hard parts that survive digestion – and you thought studying marine mammals was glamorous! Unfortunately, fragile bones of some prey species do not survive digestion and some hard parts, such as large squid beaks, are regurgitated. This means conventional faecal analysis provides a biased view of what animals are actually eating. Another problem is that many animals, such as penguins and whales, leave almost no visually identifiable remains in their faeces because they digest their food so thoroughly. In response to these problems the Applied Marine Mammal Ecology group at the Australian Government Antarctic Division has been researching the potential for using DNA in animal faeces to study animal diet.

DNA is probably the most famous biological molecule. It is well known as a natural information store and has been studied in detail in humans through the Human Genome Project. The information in DNA, and the ability to recover DNA from trace



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Promising results from analysis of prey DNA in macaroni penguin samples indicate the need for invasive stomach flushing could be reduced in future penguin diet studies.

amounts of tissue, has made it indispensable in police forensic investigations. These same features give it the potential to be used to identify the food of animals – even after digestion.

We began our research in 2001 at a time when very little work had been done in the area and there were many unsolved technical difficulties involved in reliably isolating and identifying prey DNA from faeces. The challenges arise largely because DNA is broken into very small pieces during digestion and the prey DNA found in animal faeces is also mixed in with DNA from gut bacteria and parasites, and DNA from the gut cells of the predator. We looked at many laboratory techniques for separating out the DNA of prey species and eventually succeeded in isolating and identifying prey DNA in the faeces of a few different marine predators.

The next step was to determine if the prey DNA we isolated was representative of what an animal was eating. We decided to study animals in captivity, as the exact diet of the animals was known and their faeces could be collected and tested. Studies were undertaken on Steller sea lions housed at the Vancouver Aquarium in Canada (with colleagues from the University of British Columbia), and on fur seals at Sea World on the Gold Coast, Australia. In each case, the captive animals were fed a daily diet of several different prey species.

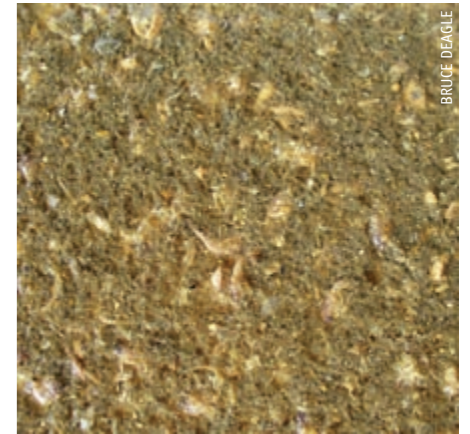
Back in Tasmania, the faecal samples were analysed in the laboratory using ‘group-specific PCR’ (a technique that allows detection of minuscule amounts of prey DNA). Both studies showed that prey DNA could be reliably recovered. In the Steller sea lion study, even prey that was eaten in small amounts (for example, squid fed as 5% of the diet) could be dependably detected. The proportions of prey DNA in the faeces were also roughly proportional to relative amounts of the different prey items consumed. The Sea World study compared DNA detection with recovery of prey hard parts. The results showed that even

fish with robust hard parts were more likely to be identified by their DNA in faeces, than by their bony remains.

The next step was to field test the technique’s capacity to address ecological questions. A perfect opportunity arose with the AGAD’s Heard Island Predator-Prey Investigation and Ecosystem Study (*Australian Antarctic Magazine* 7:6-7). This food-web study involved determining the diet of some key predators using conventional methods (identifying remains of prey in fur seals’ faecal samples and in the stomach contents of macaroni penguins), and trialling the new genetic approach.

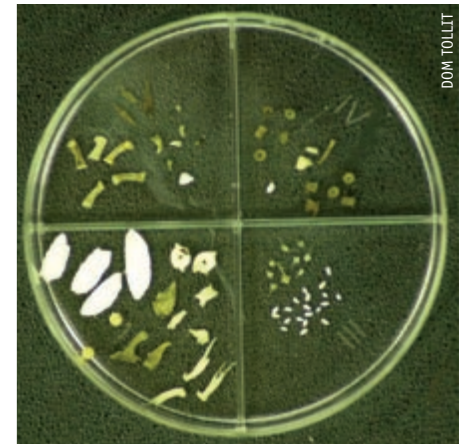
Tests for prey DNA provided a new perspective on the diet of female Antarctic fur seals that were nursing pups. DNA analysis showed that they consumed a more diverse fish diet, and that squid was eaten more frequently than conventional faecal analysis indicated. A major component of the diet was mackerel icefish, which is also a target species for commercial fisheries in the region. The potential for competition between lactating Antarctic fur seals on Heard Island and these fisheries is currently being assessed.

For the macaroni penguins, analysis of samples collected through stomach flushing showed that the diet changed from primarily krill in the early part of chick rearing, to primarily fish at later stages. The species of krill being eaten also changed during the course of the study. Both of these dietary trends were mirrored in the data obtained through DNA testing of penguin faeces. This comforting congruence indicates that non-invasive faecal analysis could reduce the need for stomach flushing in future penguin diet studies. The penguin DNA faecal analysis also allowed for improved taxonomic identification of some prey species. For example, two species of krill, difficult to distinguish in digested stomach samples, were easily identified by genetic differences.



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Species in dietary samples can be very difficult to identify. This photo shows the digested remains of krill and fish recovered from a penguin stomach.



DOM TOLLETT

Example of fish bones recovered from sea lion faeces (from top right, clockwise – herring, sandlance, pollock and salmon). Diet estimation based on recovery of bones can be biased since fish with fragile bones are poorly represented and some soft-bodied prey are not represented at all.

The studies have shown that DNA-based methods are useful for gleaned valuable dietary information from predators’ faeces. However, several hurdles remain before the technique can be routinely applied. For example, we are currently working on more effective ways of removing the large amounts of predator DNA from faeces.

In addition, DNA-based faecal analysis does not provide some information obtainable with traditional diet analysis, such as the size of prey species being eaten. However, the technique has obvious uses: in seals and sea lions it will allow detection of fish with fragile bones and soft bodied prey, such as squid; and in penguins it provides a new, less intrusive method for studying diet.

In the future the methodology may be semi-automated, allowing analysis of large numbers of samples collected in population level surveys. While the technique will not overcome all the complications of dietary analysis, it is another tool that will bring ecologists closer to understanding the important question of who’s eating whom in the ocean.

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