

Prepared by: Chris Gaskin, Project Coordinator, Northern New Zealand Seabird Trust (for NNZST) and Nigel Adams (United Institute of Technology)



Cover: Buller's shearwater chick, Aorangi island, Poor Knights Islands. Photo: Edin Whitehead

Figure 1 (this page). Horuhoru Australasian gannet colony. Photo: Kylie Connell

Introduction

Dense fish schools create a phenomenon known as fish work-ups. These fish drive up prey items to the sea surface and observations suggest that this forms an important food source for a range of seabird species. There is currently poor knowledge of both the diet of surface-foraging seabirds and the prey items are being made available to seabirds from fish work-ups. This is currently limiting our understanding of the mechanisms through which changes in the distribution and/or abundance of fish work-ups may be driving seabird population changes (population status and annual breeding success).

Comparison of food availability from fish shoals with what food is fed to the target seabird species is required to establish how important those fish work-up species are in the diet of the seabirds.

This report outlines the opportunistic and targeted collection of diet samples from surface nesting and burrow nesting seabirds during chick rearing periods in 2017-2018. In the case of burrowing seabirds, samples were collected from two sites for two species (Buller's and fluttering shearwaters) and have been archived for later molecular analysis and potentially identification to family, genus or species. For Australasian gannets, we present preliminary results from an independent study conducted in 2017 and 2018. Timing of breeding for the remaining three species in this project (fairy prion, red-billed gull and white-fronted tern) meant only the investigation of suitable sites for collection of diet samples in 2018-2019 could be undertaken within the contract period.



Figure 2. Australasian gannet colony on Mahuki Island, Aotea Great Barrier Island Group. *Photo: Nigel Adams*

Australasian gannet Morus serrator

Prepared by Nigel Adams, Unitec Institute of Technology

Introduction

The research study outlined here was conducted jointly by Unitec Institute of Technology and Auckland Council. Due to the overlap of goals results are being made available to this project's (POP2017-06) reporting. The research was conducted under Wildlife Act Authority 38016-FAU (Variation).

We present the preliminary results of a study examining trophic relationships of the Australasian gannets utilizing the Hauraki Gulf. We utilized traditional and molecular approaches to describe diet of gannets and the diet of the prey targeted by gannets breeding in the Hauraki Gulf (Mahuki Island, Outer Gulf) and Horuhoru Rock (Inner Gulf) during January 2017 and January 2018. Based on samples obtained from 116 birds we have demonstrated substantial differences in the diet of birds at different locations within the gulf and across different years. Consistent with historical studies, gannet diet comprises a range of surface shoaling fish and squid including arrow squid Nototodarus gouldi, anchovy Engraulis australis, pilchard Sardinops sagax, saury Scomberesox saurus, redbait Emmelichthys nitidus and Jack mackerel Trachurus spp.. Diets of fish and squid prey recovered from gannets in January 2017 were dominated by the Euphausiid Nyctiphanes australis with all stomachs containing this taxon. A substantial range of shrimp and crab taxa were also recovered but their frequency of occurrence was substantially less. Changes in gannet diet between years may reflect changes or differences in the structure of the food web between years and sites and hence in energy flows between components of the food web. We have also demonstrated the utility of using molecular approaches to identify dietary items that would otherwise remain unidentified.

Methods

Australasian gannets are a native, protected but common seabird distributed among 28 colonies concentrated on the north-eastern coastline of New Zealand and with a particular concentration in the Hauraki Gulf. This project focussed on adults attending large chicks at Mahuki Island in the outer gulf and Horuhoru Rock in the inner gulf. Gannets were captured at their breeding colony immediately on arrival at nest sites to feed chicks. Capture involved the use of a long handled (3-4 m) modified shepherd's hook which was placed around the neck and then wedged under the lower mandible. Once restrained birds could be pulled towards the handler. Such birds frequently regurgitate food spontaneously on handling. These samples were collected by upending birds over a bucket during the handling procedure. Samples were then collected into plastic bags and preserved by chilling at the study colony and then freezing for later analysis. Once birds regurgitated they were held briefly in a plastic crate to collect faecal samples. In addition, faecal samples were collected opportunistically from around nests.

Breeding gannets generally restrict foraging to coastal waters and, in contrast to a range of other more pelagic seabirds, the prey in the crop of generally near-shore feeding gannets may be recovered largely intact. Consequently, the stomachs of ingested fish and squid have been dissected and the contents analysed separately from the primary prey.

Initial sampling in 2017 involved collection from 53 gannets caught at two breeding colonies namely Mahuki Island (n = 40) in the outer Hauraki Gulf and Horuhoru rock (n = 12) in the inner gulf (see below). This sampling effort has been repeated for another 64 birds during the 2018 breeding season. A total of 43 birds were sampled at Mahuki and 21 at Horohoru. Before release at the nest site birds were weighed and measured and a sample of contour feathers were taken from the breast region. These feathers were used to sex gannets. Analysis for sex was completed by laboratory at Massey University using chromosomal analyses.

Fresh prey items in regurgitate samples collected in 2017 and 2018 were identified using appropriate guides (Paulin et al. 1989), confirmed, as appropriate, from hard part remains. During this identification procedure the stomachs of fish and squid were dissected and the contents removed and stored for molecular analysis. The primary diet of gannets was analysed as the total number of prey items of each taxon recovered across all samples (Numerical Abundance), number of times a particular taxon was recorded as present across all samples (Frequency of occurrence) and as the accumulated mass of each taxon recorded across all samples (Duffy & Jackson 1986).

Molecular analysis

Identification of prey based on recovery of DNA has been conducted on the stomach contents of intact fish and squid recovered from the gannet regurgitate and on faecal samples collected in 2017.

Prey identification from samples has involved the extraction, amplification and purification of remnant DNA from fish and squid (prey) tissue, stomach contents of fish and squid recovered from gannets and gannet faecal samples.

For more detail on methodology, refer to our earlier report (POP2017-06 Milestone 1)

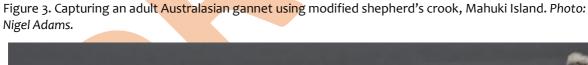
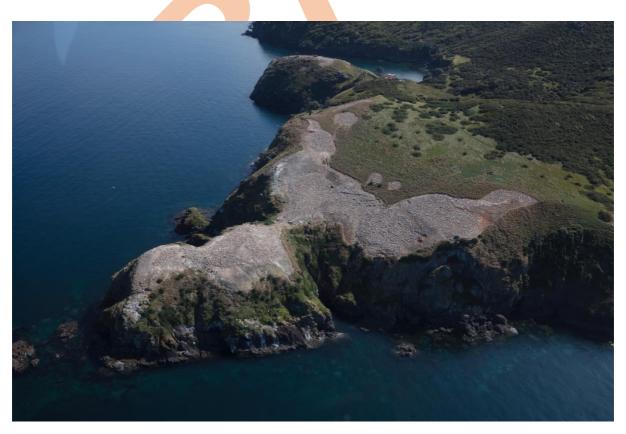




Figure 4. Australasian gannets nesting on the summit ridge of Horuhoru Rock, 27 November 2017, during aerial survey of northern North Island gannet colonies. *Photo: Neil Fitzgerald*



Figure 5. Australasian gannet colony on Mahuki Island, 27 November 2017, during aerial survey of northern North Island gannet colonies. *Photo: Richard Robinson*



Preliminary results

Samples include regurgitated stomach contents of gannets (fig. 6), faecal samples and the stomach contents of fish and squid recovered from the regurgitated contents. Morphological-based analyses of the primary prey from 2017 and 2018 breeding seasons is completed. Sequencing of molecular samples collected in 2017 has also been completed and preliminary taxonomic identifications obtained.

Regurgitation samples

The mean mass of regurgitate recovered from gannets was 232 ± 110.1 g (mean \pm standard deviation) The maximum regurgitated mass recovered was 507 g around 25 % of body mass. Individual regurgitate samples were most commonly homogenous with 68.6 % containing only one species, 25.4 % two species and 5.9 % containing 3 species.





Composition

Consistent with findings from historical studies, the diet of gannets is dominated by shoaling fish and squid. Most of these are known to exploit the surface waters of the Gulf. Important species identified during the study period include arrow squid Nototodarus gouldi, anchovy Engraulis australis, pilchard Sardinops sagax, saury Scomberesox saurus, redbait Emmelichthys nitidus and Jack mackerel Trachurus spp..

Frequency of Occurrence

In 2017 arrow squid was the most frequently recovered prey item from gannets followed by anchovy, jack mackerel and saury. This was particularly so at Mahuki Island where 27 of 40 regurgitate samples contained squid. At Horuhoru squid and pilchard occurred in a similar number of stomachs. Arrow squid was recovered substantially less frequently from regurgitate samples in 2018 being replaced by Jack mackerel, saury, pilchard and red bait. The more frequent recovery of saury and the frequent occurrence of red bait at Mahuki in 2018 was also notable.

Frequency Abundance

In terms of frequency abundance, diet composition gives added emphasis to small prey taxa. Accordingly, the proportion of the diet comprising the generally small anchovy was substantially higher. This was evident at Mahuki Island in 2017 and at Horuhoru in 2018. Anchovy caught by gannets at Horuhoru were particularly small in 2018.

Discussion

There were substantial differences between years and between sites. Notable in 2017 was the importance of arrow squid. This was replaced by saury, redbait and anchovy in 2018. Further analysis of this data will allow characterization of the size distribution of prey taken. The differences in diets between the two breeding sites suggests spatial separation of foraging of gannets and differences in the structure of the food web between the generally shallow water of the inner gulf and deeper water of the outer gulf. Sea surface temperatures in 2018 were substantially higher than 2017. Differences between the diets of gannets between these two years may reflect the differences in prey species abundance and availability related to these differences in oceanographic conditions.

Details of the diets of these fish and squid prey await complete analysis of the molecular data however there are clear indications of the importance of the local krill species (*Nyctiphanes australis*) and the pelagic life stage of a number of crab and shrimp species. Assessment of the utility of using gannet faecal samples to give an indication of the occurrence of particular species in the diet also awaits full analysis however we were able to detect a range of both primary and secondary prey items. Preliminary analysis has indicted that we can indeed detect both primary and secondary prey in faecal samples which in the case of primary prey were consistent with identifications for morphological analyses.

The approach adopted here lends support for the expansion to these approaches to other seabirds within the Hauraki Gulf – including other species in this study i.e. Buller's and fluttering shearwater, fairy prion, red-billed gull and white-fronted tern.

Buller's shearwater Puffinus bulleri

Introduction

The work outlined here was undertaken during the Buller's shearwater survey on Aorangi and Tawhiti Rahi Islands, Poor Knights Islands 24March to 3 April 2018 (Northern NZ Seabird Trust with funding from Birds NZ Research Fund). A trial was conducted, as members of the previous 2016-2017 season survey team had reported a determined reluctance of Buller's shearwater chicks to regurgitate during handling (M. Friesen, C. Mitchell pers. comm.). Methods were discussed with G. Taylor (Marine Species and Threats, DOC) prior to the trial. The research was conducted under Wildlife Act Authority 38016-FAU.

Methods

Regurgitation samples were collected from 8 chicks on Aorangi Island, Poor Knights Islands in March 2018. Two were collected opportunistically during handling, the other six using the 'flushing' technique. A crop tube was used with saltwater fed from a syringe in 20ml increments. If the bird did not regurgitate, then the process was repeated up to 100ml. In all six cases small amounts of oil were regurgitated and collected.



Figure 7. Flushing seawater through a crop tube, Aorangi Island March 2018. Photo: Edin Whitehead

Figure 8. Buller's shearwater chick, March 2018. Photo: Edin Whitehead

Molecular analysis

Regurgitation and faecal samples collected have been sent to Unitec to see whether DNA can be extracted. The following will be undertaken: Spectrophometry for estimating nucleotide concentration; PCR using chordata primers; PCR using malacostra primers; Gel elctrophroesis, and a short report. Additional Sanger sequencing will also be undertaken by Unitec.

Results

Table 1. Samples collected for Buller's shearwater, Aorangi and Tawhiti Rahi Islands, Poor Knights.

Date	Chick/	Band #	Sample	Island	Preserv.	Comments
	adult		type			
24/02/18	Chick		Regurg	Aorangi	90% Eth	Originally collected in plastic bag - emptied into jar with ethanol
3/04/18	Chick		Faecal	Tawhiti Rahi	90% Eth	Feather collected; sourced from ground in entrance to burrow
31/03/18	Adult		Faecal	Tawhiti Rahi Tawhiti Rahi	90% Eth	ground in character to bullow
2/04/18	Adult		Faecal		90% Eth	
1/04/18	Adult		Faecal	Tawhiti Rahi	90% Eth	
27/03/18	Chick		Faecal	Aorangi	90% Eth	Feather collected
27/03/18	egg		Egg	Aorangi	none	
25/03/18	Chick		Regurg	Aorangi	90% Eth	Small amount of oil regurgitate when handling
25/03/18	Chick		Regurg	Aorangi	90% Eth	Small amount of oil regurgitate when handling
25/03/18	Chick	H42312	Regurg	Aorangi	90% Eth	Feather collected; crop sample using salt water
25/03/18	Bone		Fish bone	Aorangi	none	Outside burrow
26/03/18	Fish		Fish	Aorangi	90% Eth	Found near burrow on main track, could have been dropped by something other than Buller's shearwater?
26/03/18	Chick		Regurg	Aorangi	90% Eth	Feathers collected; regurgitate when handling
28/03/18	Chick	H42345	Regurg	Aorangi	90% Eth	Feathers collected; regurgitate using crop flushing with saltwater; in 2 small tubes
28/03/18	Chick	H-42344	Regurg	Aorangi	90% Eth	Regurgitate using crop flushing with saltwater
28/03/18	Chick	H-42343	Regurg	Aorangi	90% Eth	Regurgitate using crop flushing with saltwater
28/03/18	Chick	H-42347	Faecal	Aorangi	90% Eth	

Fluttering shearwater Puffinus gavia

One regurgitation sample has been collected from a fluttering shearwater. This was collected opportunistically during a banding session at Tawharanui on 17 September 2017 (figs 9 & 10). The regurgitate was collected from the bird during handling when the bird was being measured. On preliminary inspection, it was found to be either totally, or mostly, euphausiids, species to be identified. Specimens from the sample were photographed under microscope at School of Biological Sciences, University of Auckland (fig 10).

Three sites have been identified for future work with fluttering shearwater – i.e. to collect regurgitates and faecal samples for this project (POP2017-06) and, potentially, for tracking and physiological studies across three years through funding from the Gulf Innovation Fund Together (G.I.F.T.). The sites are: Burgess Island, Otata Island (The Noises) and Taranga/Hen Island.

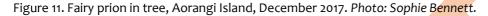
Figure 9. Fluttering shearwater captured during a banding session at Tawharanui Open Sanctuary, September 2017. Photos: Edin Whitehead.



Fairy Prion

No samples have been collected from this species to date.

During the Buller's shearwater Poor Knights survey (2016-2018) areas where fairy prions have been found nesting have been recorded. A selection of these will be used for future work with this species – i.e. to collect regurgitates and faecal samples for this project (POP2017-06) and for tracking and physiological studies across three years through funding from the Gulf Innovation Fund Together (G.I.F.T.) initiative (Foundation North). A number of fairy prions were captured on Aorangi during nightly recapture sessions to check for bands on birds (i.e. Buller's shearwaters and fairy prions) banded by G. Taylor and others in 2012 and 2013.



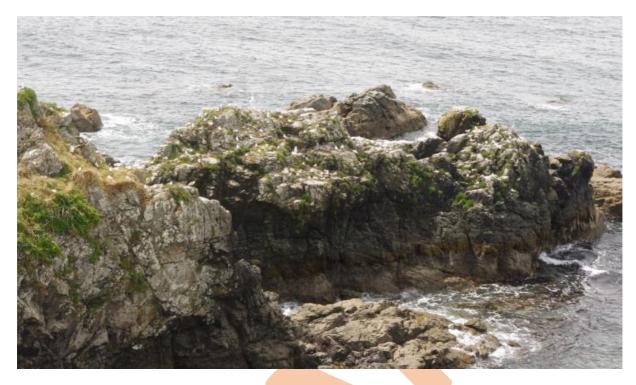


Red-billed gull

No samples have been collected from this species to date.

Three sites have been identified for future work with red-billed gulls – i.e. to collect regurgitates and faecal samples for this project (POP2017-06) and, potentially, for tracking and physiological studies across three years through funding from the Gulf Innovation Fund Together (G.I.F.T.) initiative (Foundation North). The sites are: Tawharanui Open Sanctuary, Tiritiri Matangi Island, Hawere (Goat Island) and Mokohinau Islands. The Tawharanui site was visited in November 2017 to assess access to the colony and how best to approach nests/chicks.

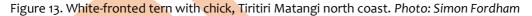




White-fronted tern

No samples have been collected from this species to date.

Two sites have been identified for future work with white-fronted terns – i.e. to collect regurgitates and faecal samples for this project (POP2017-06). The sites are: Tiritiri Matangi Island and Otata (The Noises).





Recommendations for follow-up work in 2018-2019

Our first priority is to complete the molecular analyses for the Australasian gannet and Buller's shearwater samples; the former through independent work by Adams et al. The latter relates to DNA and Sanger sequencing in order to determine that the amounts collected from reluctant 'regurgitators' (i.e. Buller's shearwater chicks) will be enough to yield results.

In terms of next season's work, we are recommending the following:

- 1. Instruction from Massey University with respect to the 'flushing' technique for Dr. Cathy Mitchell who will be the lead for Buller's shearwater and potentially other species.
- 2. Fluttering shearwater collection of regurgitations and faecal samples from three sites: Burgess Island, Taranga/Hen Island and Otata; visits from September.
- 3. Red-billed gull collection of regurgitations and faecal samples from three sites: Tawharanui, Hawere/Goat island; Tiritiri Matangi Island.
- 4. White-fronted tern collection of regurgitations and faecal samples from two sites: Tiritiri Matangi Island, Otata (The Noises); visits in October, November, December.
- 5. Fairy prion collection of regurgitations and faecal samples from Aorangi, Poor Knights Islands; visits in October and December.
- 6. Buller's shearwater collection of regurgitations and faecal samples from Aorangi Island; visits in December, February and April.
- 7. Australasian gannet collection of regurgitations and faecal samples will be conducted in conjunction with the tracking studies to be conducted under a G.I.F.T. initiative-funded project, from three sites; Mahuki Island, Horuhoru Rock and Motukawao Islands; visits in December and January.
- 8. Molecular analysis through Unitec for DNA and Sanger sequencing; then Genomics Auckland University of Auckland if sequencing extraction is successful.

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Figure 14. Red-billed gull at Burgess island, Mokohinau Group. Photo: Abe Borker