Indirect effects on seabirds in northern North Island

POP2017-06

Methodology report for project (Milestone 1)

NORTHERN NZ SEABIRD TRUST

30 November 2017

Prepared by: Chris Gaskin, Project Coordinator, Northern New Zealand Seabird Trust, with Prof. Andrew Jeffs, Dr. Brendon Dunphy, Dr. Emma Carroll (all University of Auckland), Dr. Nigel Adams (Unitec Institute of Technology), Dr. Megan Friesen (NNZST/Audubon Seattle) and Peter Frost (Science Support Service)

Cover: Fluttering shearwaters and kahawai school, Hauraki Gulf, October 2017. *Photo: Edin Whitehead*

This page: Buller's shearwaters, fluttering shearwaters and fairy prion over shoaling blue mackerel. *Photo: Edin Whitehead*

Description of Services

Objective 1

Identify the range of potential seabird prey species within fish work-ups.

- Characterise fish work-ups by identifying and estimating abundance of the suite of predator species and record observations of their feeding behaviour.
- Quantify the composition of the mesozooplankton community (i.e., 0.2→20 mm) associated with fish work-ups

Scope of work

Carry out boat trips into the Hauraki Gulf on at least six occasions spread out between October 2017 and March 2018 to sample fish work-ups, i.e., spring (Oct-Nov), early summer (Dec), late summer (Jan/Feb) and autumn (March-April). The sampling will identify the location and characterise fish work-ups, and collect samples using nets, underwater digital video, and observations to determine the make-up of marine organisms in the water column associated with schooling fish. Upon locating a fish work-up, the researchers will quantify, using visual counts or estimates of abundance, the species composition of avian and marine mammal predators. The respective feeding/non-feeding behaviour of each species will be recorded. Underwater digital video footage will be recorded and the identity of fish predators/prey will be identified where possible. Replicate vertical hauls from 30 m depth with a zooplankton net within the fish work-up will be used to identify the species of mesozooplankton associated with the work-up and provide an estimate of abundance. Expert taxonomic identification will be used for establishing a set of voucher specimens of commonly encountered zooplankton. A simple visual guide of key taxonomic diagnostic features will be developed to facilitate future identification of commonly encountered mesozooplankton species. Using the results of the study, we expect to identify where fish work-ups are commonly found in different seasons, and the associated avian fauna that is utilising these events. Comparisons of the composition of the suite of predators present in fish work-ups and the composition of the associated zooplankton community will assist in characterising different types of work-ups and indicate whether the species and abundance of zooplankton is responsible for generating differences in the predatory species that are attracted to the work-up.

Objective 2

Identify food fed to chicks of key surface feeding seabirds.

- Collect natural regurgitations, stomach flush samples and faecal samples from range of seabird species (gulls, terns, gannets, shearwaters and prions)

Scope of work

Opportunistic and targeted collection of diet samples from surface nesting seabirds as key priority and burrow nesting seabirds where trips coincide with chick rearing periods. Store whole or washed samples in alcohol or other storage mediums for later identification to family, genus or species. Samples collected from suitable northern offshore islands visited in 2017-18 and 2018-2019.

Objective 3

Compare prey availability in fish work-ups with the diet of the target seabird species.

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

Scope of work

Catalogue samples from fish shoals, surface waters away from shoals and seabird diets using expert voucher samples. Match the composition of these samples to determine how important fish schools are to these key seabird species.

Objective 4

- Collect baseline population data on surface nesting seabirds (Australasian gannet, red-billed gull and white-fronted tern).
- Collect population data on a sample of burrowing petrel species if part of collaborative projects (Buller's and fluttering shearwaters, fairy prion).

Scope of work

Carry out aerial population census of gannets, gulls and terns in Year 1 around northern New Zealand. Produce population reports.

Carry out ground-based population surveys on islands in Year 1 or 2 for shearwaters and prions. Produce population reports.

Figure 1. Two purse seiners hauling bait fish, off Taranga (Hen) Island – 8 Nov 2017. Photo: Chris Gaskin

Project coordination

Chris Gaskin (on behalf of the supplier Northern New Zealand Seabird Trust – hereafter NNZST)

Lead investigators/supervisors

Professor Andrew Jeffs (Institute of Marine Science, University of Auckland) – Objectives 1 and 3

Prof. Dr. Stefanie Ismar (GEOMAR/University of Auckland) – Objectives 1 & 3

Dr. Nigel Adams (Unitec Institute of Technology) – Objectives 2 & 3

Dr. Brendon Dunphy (School of Biological Sciences, University of Auckland) – Objectives 2 & 3

Dr. Emma Carroll (University of Auckland) – Objectives 2 & 3

Peter Frost (Science Support Service, Whanganui) – Objective 4

Dr. Megan Friesen (NNZST/Audubon Seattle) – Objective 4

James Ross (NNZST) – Objective 1

Personnel

Objective 1

Student TBA (University of Auckland) (collection and preserving samples; collation and identification

Kerry Lukies (student, University of Auckland) – NNZST sub-contractor (collection and preserving samples for archiving)

Edin Whitehead (University of Auckland) – photography

Tom Trnski (Auckland Museum) (identification)

TBA – other experts required for identification of specimens

James Ross (NNZST) – skipper *Waimania* (vessel to be used in the Hauraki Gulf for sampling)

Jochen Zaeschmar - skipper *Manawanui* (vessel to be used north or the Hauraki Gulf)

Auckland Dolphin and Whale Safari (through a collaboration with NNZST) have undertaken to sponsor two students on board their vessel to collect plankton samples and record observations of work-ups, particularly of gannet, common dolphin and fish schools within the Inner Hauraki Gulf.

The University of Auckland research vessel *Hawere* may undertake additional sampling as other research projects allow.

Objective 2

Gannet sampling and analysis (also potentially red-billed gull and white-fronted tern analyses)

Dr Nigel Adams (Unitec Institute of Technology)

Prof. Dr. Stefanie Ismar (Helmholtz Zentrum für Ozeanforschung Kiel (GEOMAR) (Germany)/University of Auckland)

Unitec lab for preliminary tissue analysis

TBA – lab for faecal analysis
Collection of red-billed gull and white-fronted tern faecal samples
NNZST - sub-contractors and volunteers TBA

Buller's and fluttering shearwaters, and fairy prion sampling and analysis

Collection of regurgitations and faecal samples from all three species
NNZST - sub-contractors and volunteers TBA

Analysis of regurgitations and faecal samples (including stable isotopes provided separate funding is available)

Dr Brendon Dunphy (University of Auckland)
Dr Emma Carroll (University of Auckland)
TBA = lab for analysis of both regurgitations and faecal samples
Dr Sarah Bury (NIWA) – stable isotope analysis

Objective 3

Chris Gaskin (NNZST)
Dr Nigel Adams (Unitec Institute of Technology)
Student TBA (University of Auckland) under supervision Prof. Andrew Jeffs.

Objective 4

Gannet and red-billed gull aerial surveys

Peter Frost (survey coordination)
Olivia Hamilton (flight log and synchronising with photographers)
Neil Fitzgerald (first photographer)
Richard Robinson (second photographer – wide shots)
Chris Gaskin

Poor Knights surveys (Buller's shearwaters, fairy prions)

Dr Megan Friesen (planning only)
Chris Gaskin (overall coordination)
James Ross (team leader – December)
Graeme Loh (seabirds – Level 3 banding)
Sandra Anderson (habitat –Level 3 banding)
Karen Baird (team leader – February and April trips – Level 3 banding)
Cathy Mitchell (April trip – qualified vet, now retired, Level 3 banding)
Volunteers TBA

NW Chickens/Bream Islands surveys (fluttering shearwaters)

Chris Gaskin (overall coordination)
Pete Mitchell
Cathy Mitchell
James Ross (boat transfers) – alternative (Trevor Jackson – El Pescador Charters)
NNZST – volunteers as required.

Burgess Island, Mokohinau Group

Christy Wails (AUT/Northern Illinois University)

Other researchers on the island in 2018 TBA
Boat transfers - various

Reporting

Reporting deliverables and milestones are set out in the contract, as follows:

Objective 1: Produce an interim report of the fish shoal sampling activities by 30 June 2018, and a final report categorising the data samples collected within fish shoals and adjacent seas by 15 December 2018.

Objective 2: Produce an interim report of the field diet sampling activities undertaken by 30 June 2018, and a final report summarising all diet samples collected from each seabird species by 30 April 2019.

Objective 3: Produce an Excel database that catalogues all samples from fish shoals, surface waters away from shoals and seabird diets using expert voucher samples to identify specimens. Match the composition of these samples to determine how important fish schools are to these key seabird species. Produce a final report summarising the results of the indirects food availability and seabird diet project by 30 June 2019.

Objective 4: Produce an interim report by 30 June 2018 outlining seabird population studies carried out in Year 1 and preliminary results. Produce a final report on seabird population studies by 30 June 2019.

Milestones

1. Draft methodology report for project (15 Nov 2017)
2. Interim report on sampling from fish shoals (30 April 2018)
3. Interim report on sampling diets of seabirds (30 April 2018)
4. Interim report for seabird population assessments (20 June 2018)
5. Final report of the at-sea fish shoal sampling (15 December 2018)
6. Final report of the colony-based seabird diet sampling (30 April 2019)
7. Final report of the indirect effects project comparing fish shoals and seabird diet in northern New Zealand (20 June 2019)
8. Final report of the seabird colony and population assessments (20 June 2019)

Contract ends 30 June 2019

Wildlife Act Authorities and Permits

Wildlife Act Authority **38016-FAU** covers survey work on the Poor Knights, Bream, Northwest Chickens and Mokohinau Islands. Australasian gannet work on Mahuki and Horuhoru Islands is covered by a Variation of 38016-FAU. This Authority expires mid-2018. **45473**-FAU covers work at Tawharanui Regional Park/Open Sanctuary. The NNZST with collaborators will be applying for a new Authority in January 2018 once iwi consultation is completed. This will replace and extend current Authorities.

Health & Safety Plan

Northern New Zealand Bird Trust Health and Safety Plan supplied 6 November 2017. Refer DOC-3206772

Figure 3. Krill (euphausiids) swarm with kahawai school, NW Reef, Hauraki Gulf, 16 Oct 2017. *Photo: NNZST*

Proposed methods

Objective 1

Sampling will be conducted in the Hauraki Gulf on at least six occasions spread out between October 2017 and March 2018 when weather conditions permit. A sampling voyage is expected to last for an entire day, departing from Whangateau Harbour (or other), travelling to inner and outer Hauraki Gulf locations and then returning to start. A time-stamped GPS track of the vessel movements will be kept from each voyage along with regular records of sea state and weather conditions whilst underway. Whilst underway observers will continually scan the waters around the vessel using binoculars to spot any fish work-ups based on the presence of seabirds, marine mammals or disturbances to the sea surface by feeding fish. A record will be kept of the estimated visual range surveyed during the voyage. Upon sighting, any fish work-up will be visited by the vessel to allow closer observations and sampling.

Upon approaching a fish-work up the GPS position of the work-up will be recorded, as well as observations of the estimated size of the fish work-up and other characteristics, such as horizontal movement of the fish work-up, the extent of fish breaching the surface, and patchiness of the work-up. Observations will also be made of the species composition of avian and marine mammal predators and each species will be quantified with visual counts or estimates of abundance. The respective feeding/non-feeding behaviour of each species will be recorded. including the proportion of each species displaying the behaviour. Underwater digital video footage will be recorded using a Go-Pro camera on a floating support and the identity of fish predators/prey will be identified subsequently, where possible, from the recordings.

Three replicate vertical hauls from 30 m depth with a zooplankton net within the fish work-up will be used to identify the species of mesozooplankton associated with the work-up and provide an estimate of abundance. The three hauls will be taken some distance apart but still within the main body of the work-up location. The mesozooplankton net consists has a 750mm diameter opening with 250µm cod end. The net will be lowered to 30m depth and then hauled to the surface at a rate of 1 m min^{-1}, i.e. reaching the surface after 30 seconds. The contents of the cod-end will be washed into a 250µm sieve and then washed into a sample jar with a minimum amount of seawater and preserved with 90% ethanol and sealed. A paper label will be included with the sample which has the date, location and replicate number and sampling personnel written on it with pencil. Where a sample is too large for the sample jar it will be placed in the plastic measuring jug and the volume made up to a convenient quantity, then stirred vigorously, whereupon half of the sample will be tipped out of the jug. The remaining contents of the jug will be sieved and stored in the sample jar as outlined previously, and a note made on the paper label that the sample has been subsampled (halved).

Upon return to the laboratory the mesozooplankton samples will be sorted into different species and counts made of each species where possible. Expert identification of difficult taxonomic groups will be used for establishing a set of voucher specimens of commonly encountered zooplankton. A simple visual guide of key taxonomic diagnostic features will be developed to facilitate future identification of commonly encountered mesozooplankton species.

Using the results of the study, we expect to identify where fish work-ups are commonly found, and whether there is any association of any of the predatory, fish or mesozooplankton species with these locations. Associations between mesozooplankton and the characteristics of the work-ups, such as the species of predators present and their corresponding feeding behaviour, will also be examined. Given the non-parametric nature of the data, associations will need to be tested using non-parametric statistical methods such as contingency tables.

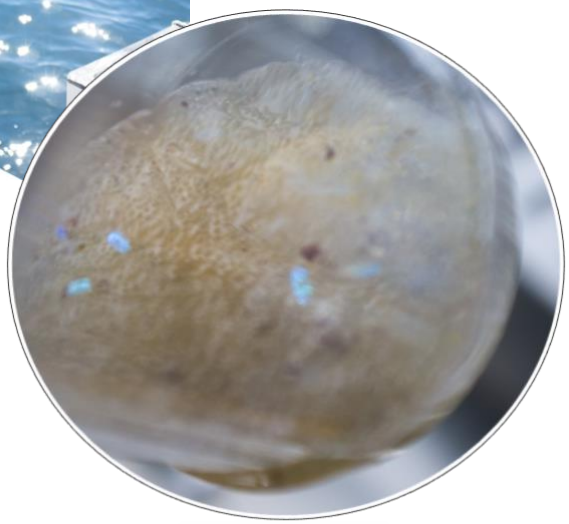
Supplementary

Field photography and underwater video

Throughout our sampling programme we will be recording what is observed at the surface - fish species, bird species, the nature and extent of the work ups, and conditions. Observations will be recorded for each sampling location, and collated together with topside photography and the use of a remote free-floating underwater camera rig to video the fish schools, their prey, as well as other activity and organisms – e.g. spawning, fish larvae.

Macro imagery

We will also be using the University of Auckland School of Biological Sciences technical unit to have macro images of plankton samples. For images of larger plankton stacking multiple images together ('photo-stacking') to get a wider depth of field resulting in higher quality images with everything in focus. Smaller samples will be done under a compound microscope camera rig.



Figures 4-6. Trial sampling October 2017, Kerry Lukies (UoA) and Chris Gaskin (NNZST). Photos: Tony Whitehead (top) and Edin Whitehead.

Figure 7 (below). Sample collected 1 December 2017 showing massed small spherical salps with some *Sappharina* copepods (their transparent bodies reflect blue light at certain angles of incidence designed to confuse predatory fish) Taken at time of collection. Photo: Edin Whitehead

Table 1. Fish school/prey types where Procellariiforms have been observed associating or feeding directly:

	Fish school/prey type	General description of activity	Species
1	Trevally *Pseudocaranyx dentex* (and mixed trevally, kahawai *Arripis trutta* & kingfish *Seriola lalandii*)	Tightly packed, very active dense schools, sometimes with several schools merging to form very large schools. Birds either forage in the wake of the schools, or in some cases feed ahead of and around the schools. Fish will erupt explosively if disturbed either from below (e.g. predatory fish) or from above (e.g. gannets flying low over a school). Shearwaters and prions have been filmed diving in the wake of school activity.	Buller's shearwater, fluttering shearwater, fairy prion, sooty shearwater, flesh-footed shearwater, short-tailed shearwater, white-faced storm-petrel, Cook's petrel (with red-billed gull, white-fronted tern and occasionally grey noddy at some locations)
2	Kahawai	Fast-moving schools, birds moving in 'leap-frogging' formations, shearwaters plunging and diving.	Fluttering shearwater (with white-fronted terns moving with them)
4	Blue (slimy) mackerel *Scomber australasicus*	Highly eruptive, mobile schools (see spread over large area accompanied by thousands of shearwaters and prions (one occasion northwest of Little Barrier Island, October 2017) – see figure 1. *https://voices.nationalgeographic.org/2017/10/30/a-krills-eye-video-of-new-zealand-seabirds/*	Buller's, fluttering and sooty (occasional) shearwaters, fairy prions; also northern giant petrel and white-capped albatross
3	Saury *Scomberesox saurus*	One instance of shearwaters and gannets diving on saury, catching fish close to the surface. Out beyond Mokohinau Islands, north of Great Barrier Island.	Flesh-footed shearwater, black petrel and sooty shearwater (with Australasian gannet)
5	Baitfish species (e.g. Jack mackerel *Trachurus novaezelandiae*, pilchard *Sardinops neopilchardus*, koheru *Decapterus koheru*)	Often tightly packed schools, sometimes forming spinning 'bait balls' below the surface. Birds plunging/diving and pursuing prey underwater. Dramatic.	Fluttering shearwater, flesh-footed shearwater, Buller's shearwater, white-faced storm-petrel, Cook's petrel (with Australasian gannet and cetaceans)
6	Skipjack tuna (*Katsuwonus pelamis*)	Fast moving spread-out schools with birds following.	Fluttering shearwater (with gulls and terns)

Contd.

6	Crustaceans (no visible fish schools)	Mainly euphausiids (*Nyctiphanes australis*) with birds actively feeding from the surface, often well- spread, occasionally across several sq. kms.	Buller's shearwater, fluttering shearwater, fairy prion, common diving petrel, white-faced storm-petrel, sooty shearwater.

Figure 8. Gannets diving on baitfish, common dolphins and flesh-footed shearwaters also feeding. Photo: Derek Tearne.

Figure 9. Not all work-ups are in open sea. Gannets diving over fish school, close inshore at Bushy Point, Bream Head. Later in day thousands of fluttering shearwaters and hundreds of gannets were active in same area, 1 December 2017. Photo: Edin Whitehead.

Objective 2

The range of methods used to describe the diet of seabirds and thereby explore their trophic dynamics include identification of intact prey brought back in the bill (e.g. terns and puffins), sampling and visual identification of partly digested stomach contents and recovery and identification of species-specific and digestive resistant hard parts recovered from stomach contents, regurgitated pellets or faeces. More recently molecular techniques have been used to identify prey from DNA recovered from gut contents or faecal samples.

Australasian gannet

We will use a combination of approaches to describe the trophic web upon which Australasian gannets (*Morus serrator*) depend.

Sampling will be focussed adults attending larger chicks. Sixty gannets will be caught at their breeding colony (Mahuki or Horuhoru) immediately on arrival at nest sites to feed chicks. Capture involves the use of a long handled (3-4 m) modified shepherd's hook which is placed around the neck. Such birds frequently regurgitate food spontaneously on handling. These samples will be collected into plastic bags and preserved for later analysis. Once birds have regurgitated they will be held briefly in a plastic crate to collect faecal samples. In addition, fresh faecal samples will be collected opportunistically from around nests. Before release feather samples will be plucked for genetic analysis to determine the sex of sampled adults to explore whether there is any intra specific trophic differentiation between male and female bird.

Prey identification

Prey studies have indicated that gannets consume a relatively small range of fish and cephalopods. Fresh, intact prey items in food samples will be identified using appropriate guides and where appropriate confirmed by genetic analysis of tissue samples. Australasian gannets have a relatively short foraging range accordingly the prey in the stomach of gannets maybe largely intact providing the opportunity to analyse prey using traditional methods but also to dissect the stomach contents of ingested prey and analysed these separately. During this analysis stomachs of intact fish and squid will be dissected and the contents analysed using molecular techniques.

Molecular analysis

Prey identification from samples will involve 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. This DNA will then be sequenced using Sanger sequencing or Next Generation Sequencing as appropriate by a suitable laboratory and identified into taxonomic units using appropriate software (see below).

Prey muscle tissue

DNA extractions will be carried out using the Qiagen DNeasy® Blood & tissue Kit, as per the manufacturer's specifications and stored at -80°C.

A 710bp section of the mitochondrial gene cytochrome oxidase (COI) region will be amplified using the primers LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G) and HC02198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA). PCR products which produce a single band of approximately 650bp by this method will sequenced (Sanger sequencing) by Massey Genome Service, on a ABI3730 DNA Analyzer. The resulting sequences will be viewed and edited using Geneious 10.0 software. Sequences will then be queried in GenBank using the BLASTn search algorithm. Query matches of <2% dissimilarity will be taken to be a species level match.

Prey stomach contents and faecal samples

DNA from prey stomach contents and faecal samples will be extracted using the Qiagen DNeasy® Blood & tissue Kit and the Qiagen QIAamp® DNA Stool kit respectively following the manufacturer's specifications.

DNA extracted will be used in two additional PCR amplifications. One targeting a 155bp section of the 16S gene using the primers Chord_16S_F_TagA (5'- ATG CGA GAA GAC CCT RTG GAG CT) and Chord_16S_R_Short (5'- CCT NGG TCG CCC CAA C), and a second targeting a 200bp section using Mala_16S1F (5'-TGA CGA TAA GAC CCT) and Mala_16S2R (5'- CGC TGT TAT CCC TAA AGT AAC T). PCR products which produced a band of the expected 150-200bp size will selected for next generation sequencing.

Samples will be further purified and prepared for MiSeq illumina sequencing (Next Generation sequencing). This was formerly done by New Zealand Genomics Ltd, the lab for new work TBA. Appropriate software will be used to assemble sequences into molecular operational taxonomic units (MOTUs) at various levels of base pair differences. Basic bioinformatics analysis (quality control, chimera filtering, molecular identifier deconvolution, BLASTing and MOTU clustering) will be conducted in collaboration with the lab TBA. Briefly, the VSEARCH software will be used to quickly identify similar sequences. A conservative 97% identity threshold will be used in the first instance for the clustering of MOTUs. If the results are not satisfactory, the most probable species identity threshold for each primer pair may be calculated using the localMinima function in the R package SPIDER.

Red-billed gull and white-fronted tern

Similar methods to the above will be adopted for red-billed gulls and white-fronted terns in 2018-2019. The 2017-2018 season will allow the team to locate accessible colonies for procuring samples; also to trial working within these colonies with minimal disturbance and for sample collection.

Buller's shearwater, fluttering shearwater and fairy prions

Pending additional funding, UOA collaborators will undertake DNA-based analyses of Procellariiform (shearwater and prion) diet. This will involve two key aims. The first is to establish a reference database of DNA barcodes of potential prey items. This will use the samples collected under Objective 1 and will comprise multiple DNA barcodes (e.g., COI, 18S, 16S), depending on the taxa under study and resolution sought (e.g., order or species level).

The second objective is to identify prey DNA in faeces via targeted amplification of DNA barcodes and comparison with the reference database previously constructed. To minimise contamination risk, DNA will be extracted in a laminar flow UV hood and negative controls will be used throughout the DNA extraction and amplification steps. A targeted DNA barcoding approach will be used, where primers specific for DNA barcodes (e.g., 18S and COI) of key prey groups (e.g., copepods, krill, fish) will be used to preferentially amplify prey DNA. PCR products will be multiplexed and barcoded to link putative prey back to the individual bird sampled, and sequenced on an Illumina sequencing platform. The resulting DNA sequence data will be compared against the reference database to identify putative prey species.

Objective 3

Comparison of prey availability in fish work-ups with the diet of the target seabird species.

The aim of this objective is to establish how important those fish work-up species are in the diet of the seabirds. Aside from the collection of Australasian gannet samples and resulting analysis undertaken by Nigel Adams and his collaborators, samples from Objective 1, and the five other species collected under Objective 2, will be archived initially awaiting analysis. These will be catalogued by sample type, date and location in the first instance. For regurgitations/faecal samples, species will also be identified.

The samples collected in Objective 1 will require a student starting at the University of Auckland in 2018 before identification and analysis can begin. Samples collected in Objective 2 will be archived until Dr. Emma Carroll (currently at University of St Andrews, UK) returns to Auckland and the University of Auckland in 2018 and can make a start on this project. The results from both those objectives will feed into meeting Objective 3.

The final report, comparing the availability of food species in fish schools and how these are represented in different seabird diets, will be result of collaboration between Chris Gaskin, Nigel Adams, Emma Carroll and the authors of Objective 1, with Brendon Dunphy and Stefanie Ismar in advisory roles. The methods to be used for preparing will be worked out by the authors once results are completed and prior to work start.

Figure 10. Red-billed gulls and trevally school, November 2016. Photo: Richard Robinson.

Objective 4

Gannet and red-billed gull aerial surveys

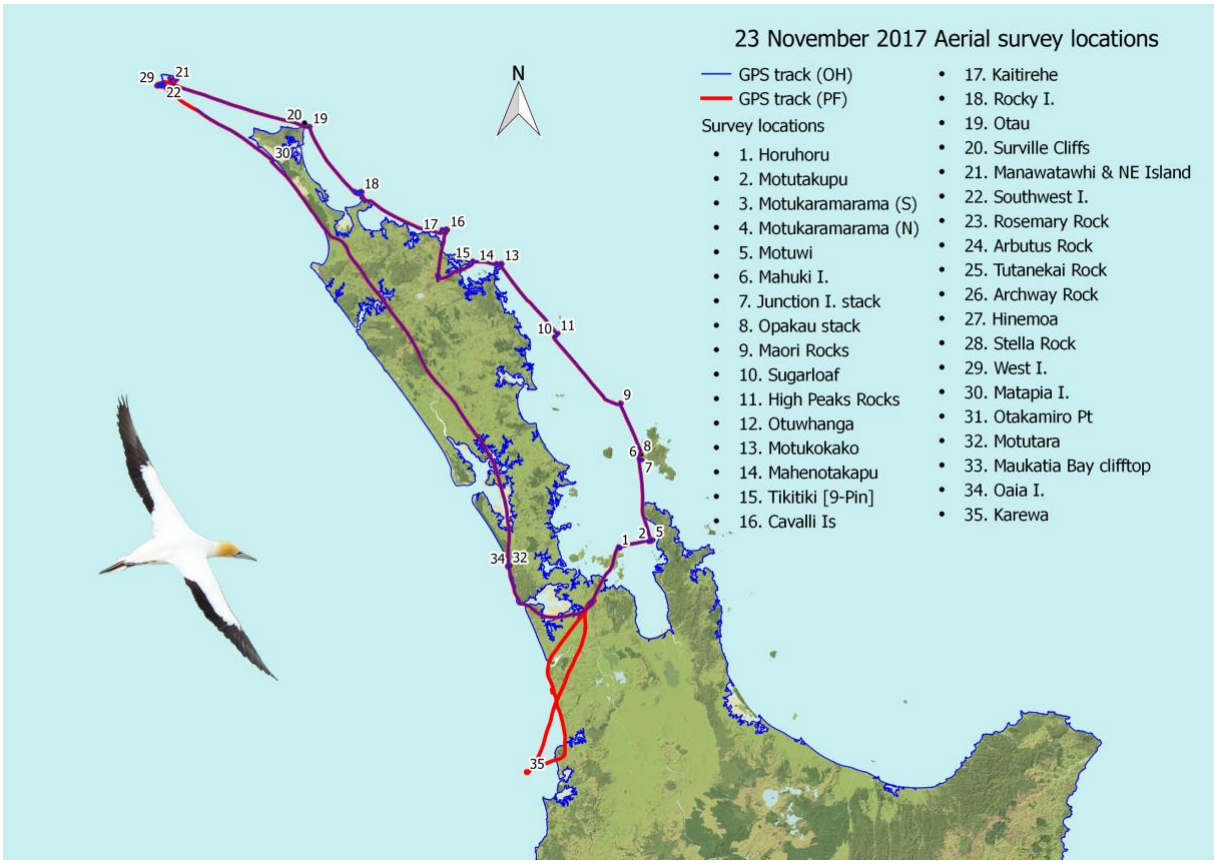
Planned approach to surveying surface-nesting seabirds

Given the general inaccessibility of many of the islands, the approach to be used centres on counting nesting birds from aerial photographs of the colonies taken around the time of peak egg laying or soon after. Aerial survey is generally the quickest, most cost-efficient and least disruptive means of locating and surveying colonies of these species, and they enable the composition, size and breeding status of the birds present at a site at a specific time of year to be determined.

The aim is to determine the number of breeding pairs of these species at the survey sites, to provide a baseline against which the results from future surveys can be assessed. Carried out at regular intervals across a range of sites, and provided both the survey and assessment methods are comparable through time, such surveys help determine both site-specific and region-wide trends in a species' status.

Aerial photographs of Australasian gannet and red-billed gull colonies on selected islands in the north-east of the North Island will be taken from a Gippsland GA8 Airvan. Fourteen gannet colonies have been identified between the southern Hauraki Gulf and the Three Kings Islands, with a further five colonies along the west coast between Muriwai and Karewa Island. being included for comparative purposes.

Figure 11. Australasian gannet and red-billed gull aerial survey locations, 23 November 2017. Map: Peter Frost and Olivia Hamilton



Seventeen sites with known or suspected breeding colonies of red-billed gull are also due to be surveyed, and a further 18 sites will be searched in fly-overs. White-fronted terns are known to breed in association with red-billed gulls at a number of these sites, and will be photographed as well. Any additional tern colonies seen during fly-overs of other islands will also be photographed, where possible. In all cases, the focus will be on documenting large colonies (>50 pairs).

At each colony being censused, three complementary sets of photographs will be taken:

(i) wide-angle images (35-50 mm focal length) of the area in general, showing key features that can be used to locate and orientate the fields of view of closer-focused photographs;

(ii) medium-scale images (85-135 mm focal length) showing nesting and other birds clearly enough that they can be identified and counted; and

(iii) close-up photographs (200-300 mm focal length) of groups of individual birds in sufficient detail that their status can be accurately determined (e.g. incubating adult, chick, adult on nest but not incubating, non-breeder).

Because medium-scale photographs usually cover only some of a colony, they will be taken so that they overlap to cover the whole area. By finding and marking common points in adjacent images a mosaic of discrete adjoining zones covering the whole colony will be created, each containing a unique subset of the birds present. Suitable control points include conspicuous or distinctive rocks or fissures, well-demarcated bare or eroded areas, notable patches of vegetation, or any other prominent feature. Panoramas, in which adjacent images are stitched together digitally, will generally not be used because of periodic omission and sometimes duplication of individuals in the merged zone, a consequence of photographs being taken from a moving platform.

The birds present in each zone will then be counted. To reduce the risk of either double counting or overlooking individuals, the areas are gridded. Each grid is then search systematically in turn, identifying and marking off individual birds with different coloured marks depending on their apparent status (e.g. incubating eggs, brooding chicks, accompanying a nesting bird, standing around). Once all birds in zone have been marked, the marks in each category are then tallied. The total tally should equate to the actual number of birds present in the colony at the time it was photographed.

Unlike the medium-scale photographs, close-up photographs do not have to cover the whole colony, but they should comprise reasonably random subsets within it, including central, peripheral and outlying areas. These serve the dual purpose of checking on the completeness of the count in the larger mosaics and for establishing the status of individual birds more accurately.

Individuals will be categorised as follows:

1. birds incubating, brooding or feeding small chicks (active breeders);
2. chicks alone (indicating an active nest but with the parents absent, presumably away foraging);
3. individuals accompanying an incubating or brooding adult;
4. birds occupying empty nests (no eggs or chicks—such birds may be failed breeders or ones that have yet to lay in the present season); and
5. birds not obviously associated with a nest and are presumed to be non-breeders (including loafers or future breeders prospecting for possible nest sites or mates).

In effect, close-up photographs serve as quasi-random samples from which the mean proportion of birds falling into each of the above classes can calculated, along with a measure of uncertainty

in the estimate. The emphasis is on determining the incidence of Apparently Occupied Sites (i.e. those with either an incubating or brooding parent in attendance, or one or more chicks in a nest in the absence of the parents: classes 1 and 2 above). When applied to number of birds counted overall, and if all birds in a colony have been accounted for, this will yield an estimate of the number of actively breeding pairs, an index of population size.

Figures 11. High Peak Rocks, Poor Knights Islands. Figure 12. Detail from colony in Motukawao Group. Photos: Richard Robinson during gannet and red-billed gull aerial survey 23 November 2017 (Objective 4 in this project)

Buller's shearwater survey

The objective of this study is to investigate Buller's shearwater populations and breeding biology at the Poor Knights Islands. This will be achieved by using controlled site burrow checks, acoustic surveys and population models. In 2016-2017 the NNZST completed the survey of Tawhiti Rahi. The NNZST has funding to undertake the survey of Aorangi in 2017-2018. Three trips are required for each island.

- Conduct burrow counts in November
- Assess occupancy rates in February
- Follow up trip in April to measure chick development and assess fledgling numbers. In 2018 parties will travel to both islands and compare occupancy and chick development between the permanent plots on each island.

Our survey methods will include a comprehensive application of passive bioacoustic techniques to build soundscapes across difficult terrain with many seabird species (Borker et al. 2014). he results will be obtained by carrying out a thorough occupancy and breeding population count on a subset of burrows, using passive acoustic devices at these known sites and at other sites deployed throughout the islands. Establishing a network of spaced devices will reduce the extent of working in heavily burrowed areas. Acoustic activity will be analysed in comparison to the known population of a subset of plots (as in Borker et al. 2014) and we also will use a predictive model (method as in Rayner et al. 2007) on Aorangi and Tawhiti Rahi including information about vegetation (de Lange & Cameron 1999).

This model will use a two-fold method to create the first thorough population estimate of Buller's shearwaters

1. plots at various elevations and vegetation types will address the density of burrows across landscapes and altitude,
2. transects will identify the occupancy rate of burrows.

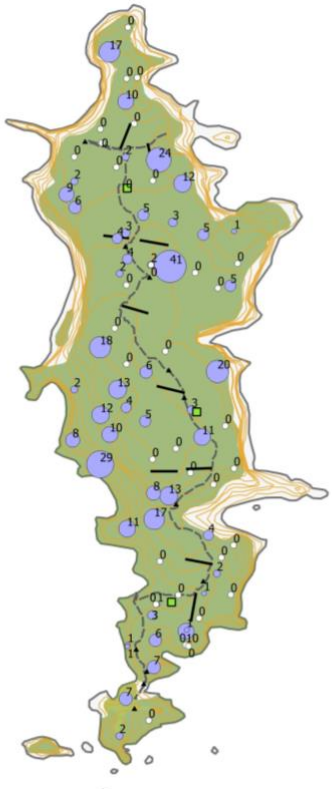


Figure 13. Tawhiti Rahi showing permanent plots (green squares), 100m transects (black lines), random plots (mauve of white circles) (numbers denote burrows) and recorder (black dots) locations.

Figure 14-15 (next page). Transect line and marking Buller's shearwater burrows on Tawhiti Rahi, December 2016. Photos: Steffi Ismar

Fairy prion survey

The Buller's shearwater surveys on the Poor Knights Islands (2016-2017, 2017-2018) together with previous acoustic surveys (Gaskin 2010-2012, Aorangi only) and spotlighting around the Poor knights during searches for NZ storm petrel (Gaskin 2006, 2007) have identified both areas on all islands where significant numbers of fairy prions are breeding, and, where they are absent (ie. areas dominated by Buller's shearwaters or completely free of all breeding Procellariiforms).

A number of these sites are in difficult areas – e.g. the big rock-filled gully on the south-western side of the island, east of the track leading up to the plateau; steeper western slopes and around Tatua Peak on Aorangi, and on Aorangaia and the easternmost stack of High Peak Rocks.

Surveys for fairy prions will adopt a modified approach to that for Buller's shearwaters and will be conducted in the 2018-2019 season. Collection of regurgitations (both opportunistic and through manipulation) and faecal samples will be conducted at the same time (Objective 2)

Fluttering shearwater surveys

This species breeds on many islands in the Hauraki Gulf and is seen at sea throughout the year, often in very large congregations. One of the mysteries, given the numbers seen at sea, and major gap in our knowledge of breeding species in the wider Hauraki Gulf, is the location of large colonies. During the survey of Tawhiti Rahi in 2016-2017 there was no evidence of the large population recorded by McCallum (1981) of 'many thousands of pairs making nest preparations'.

Population estimates will be made for three sites, one known and well-studied site (Mokohinau Islands) (Berg et al 2016) and two lesser known sites (North-west Chickens and Bream Islands) which potentially Harbour significant numbers of fluttering shearwaters. Survey protocol will follow that for the Poor Knights Islands (above) although considerable care will be required to mitigate against burrow damage in the densely burrowed and fragile areas on these islands

Surveys of Bream Islands will be undertaken in the 2018-2019 season. Collection of regurgitations (both opportunistic and through manipulation) and faecal samples will be conducted at the same time (Objective 2)

Complementary work

Visual imagery

The value of high quality images, both still and video should not be underestimated, and while hard won, their role in advocacy, supporting reporting (including building a catalogue of images of fish school types and seabird associations), and in presentations has been widely appreciated. To this end the NNZST will employ photographers for both topside and underwater photography/videoing and will explore multiple applications for the resulting work through a variety of media.

Investigate environmental gradients operating within the Hauraki Gulf using seabirds

Many animals sacrifice body condition to ensure the survival of their young. Seabirds however, will readily starve or abandon chicks if environmental stresses mount up; thus, providing a useful 'barometer' of ocean health. To better understand this, we will implement a world-first integrative project meshing together foraging behavior (GPS tracks), quantitative modelling (HMC models) and physiology (stress hormones). This exciting approach allows us to quantify the responses of seabirds living along an environmental stress gradient and identify tipping points which may lead to chick stress/abandonment. Such information is vital for predicting how vulnerable seabird populations will respond to future threats. Prey species can be frozen to provide intra- and inter-annual analyses of diet quality i.e. lipid and energy content.

Microbiomes

If funding is acquired, and samples are collected in the appropriate way (e.g., frozen on collection), then UOA collaborators would investigate the microbiome of the different Procellariiform species. The microbiome is the term for the microbial communities living on or in a multicellular organism or host. Microbiomes can be screened to learn about the health of individuals, as the microbial community can contain both beneficial and harmful micro-organisms (Loudon et al., 2014, Acevdeo-Whitehouse et al 2010). Changes in the characteristics of the microbiome over time, such as diversity, might also be indicative of changes in the quality of the social or broader environment (Amato et al., 2013; Tung et al., 2015), and can be significantly differentiated amongst individuals within a population (Klein-Jöbstl et al. 2015). Another aspect is to investigate whether direct transmission of oral and gut microbes from parent to offspring convey some benefit if the young are faithful to their parents' diet. This may be due to some functional aspects of the microbiome, such as assistance in digestion of different compounds (e.g., Zhu et al 2011). The microbiome could be investigated by either targeted amplification of 16S genes found in microbes, or by direct sequencing of the faecal or vomit sample (Sanders et al 2015, Srivathsan et al 2016). The latter method would allow the diet and microbiome to be concurrently characterized.

Additional species data

Observations made at sea during collection trips for Objective 1 and surveys conducted in Objective 4 (both aerial and ground surveys) will include collection of data for species other than those targeted for this contract. For example: photographs taken during the aerial survey for gannet and red-billed gull colonies showed the extent of Buller's albatross nesting on Rosemary

Rock, Three Kings Islands (38 nests). The same photographs revealed a roosting sooty tern amongst red-billed gulls on the summit of the same stack.

Figure 15 & 16 (inset). Nesting Buller's albatross on Rosemary Rock, Three Kings Islands. Single birds (red circles), two birds (yellow circles). Photo: Richard Robinson during gannet and red-billed gull aerial survey 23 November 2017

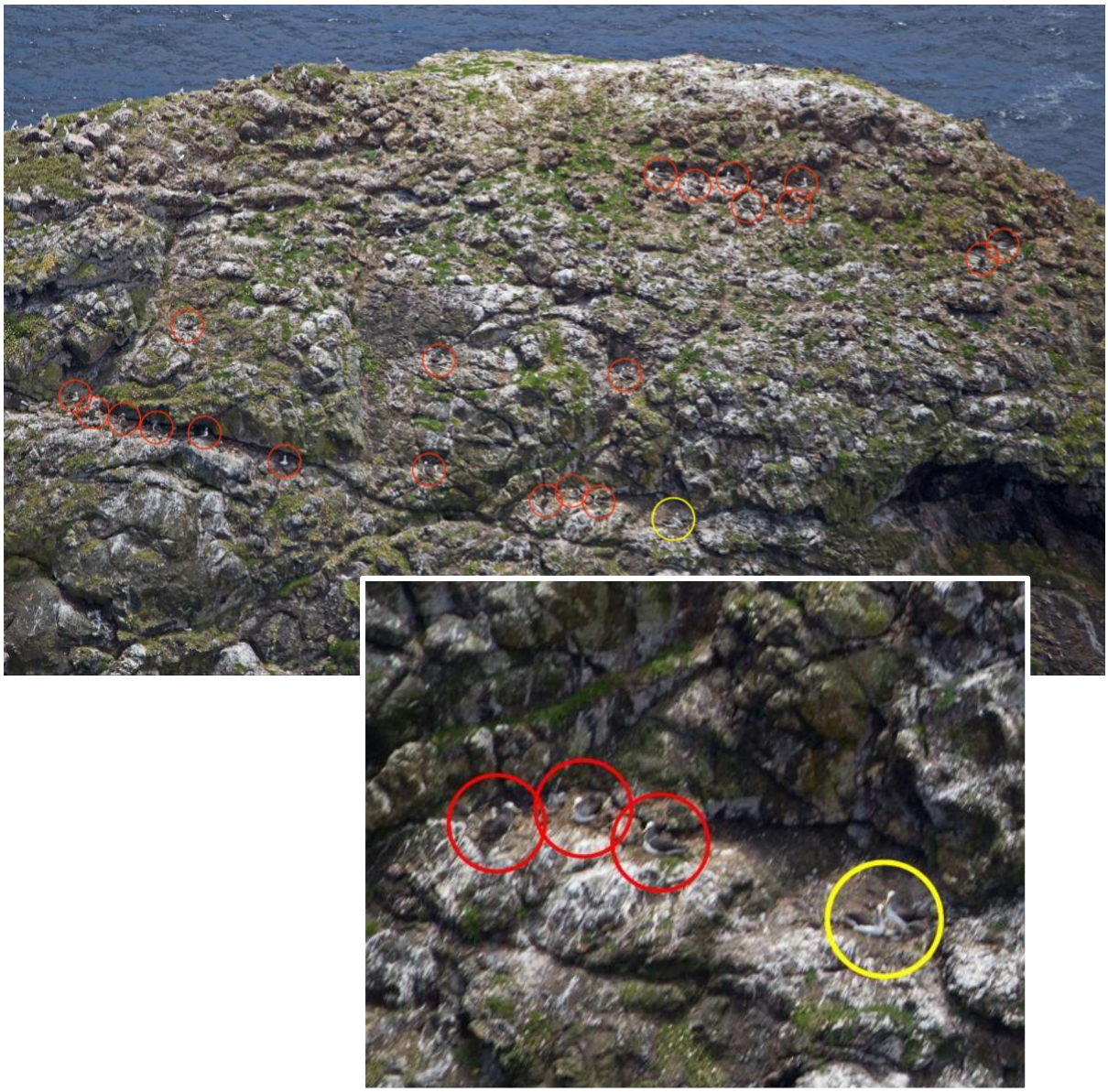


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Figure 17. Prions and shearwaters over active trevally school, Simpsons Rock, Mokohinau Group. Photo: Edin Whitehead.