Indirect effects on seabirds in northern North Island

POP2017-06

Identification of diet samples collected from seabirds

NORTHERN NZ SEABIRD TRUST

30 April 2019

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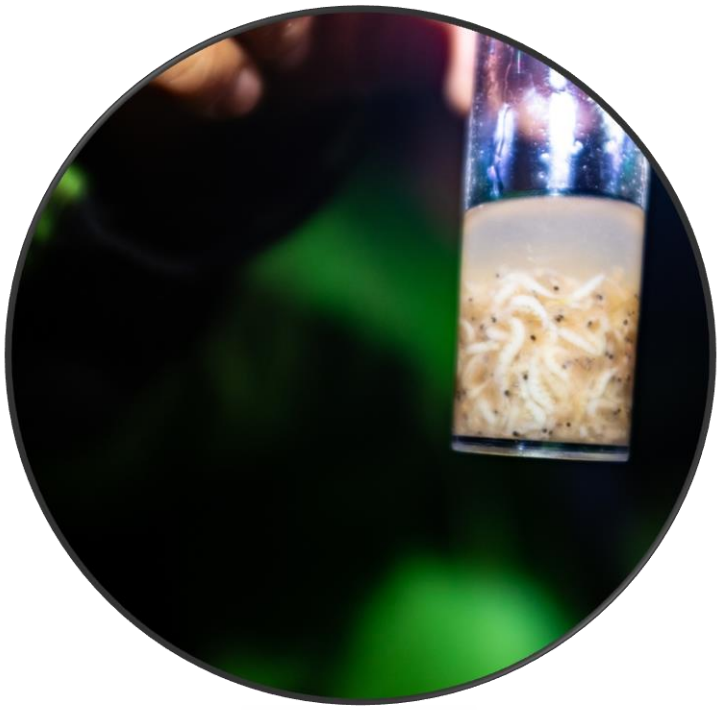


Cover photo: Australasian gannet feeding a chick, Mahuki Island. *Photo: Edin Whitehead*

Figure 1 (above). Regurgitate sample collected from a fairy prion, Tawhiti Rahi (Poor Knights Islands). *Photo: Edin Whitehead*

Introduction

This project (POP2017-06) builds on the findings of INT2016-04. (Indirect effects of commercial fishing on Buller's shearwater and red-billed gulls). A range of commercial fisheries target aggregations of surface shoaling fish. Purse seining is commonly used to capture these fish schools. The 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 what prey items are being made available to seabirds from fish work-ups. This has limited 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).

POP2017-06 aims to further our understanding of the diet, foraging ecology, breeding success and population status of these species that regularly forage in association with fish work-ups. The six species identified as feeding in association with fish schools in the northern north island region are red-billed gull, white-fronted tern, Australasian gannet, fairy prion, Buller's shearwater and fluttering shearwaters.

Gaskin (2018) outlined 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 were archived for later molecular analysis and potential identification to family, genus or species. For Australasian gannets, the report summarised the 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.

POP2017-06 Objective 2 which this report (Milestone 6) addresses covers the collection of samples of food fed to chicks of the six species (i.e. regurgitations, faecal, blood and feather samples) during the 2018-2019 season and subsequent analysis. Samples were collected as follows:

- **Fairy prion *Pachyptila turtur*** – faecal, regurgitation and feather samples were collected during incubation and chick-rearing stages on Tawhiti Rahi, Poor Knights Islands. Blood samples were collected during chick rearing stage only. Geolocators were deployed on 20 birds for late-breeding and post-breeding distribution (separate project).
- **Fluttering shearwater *Puffinus gavia*** – faecal, regurgitation and feather samples were collected during incubation and chick-rearing stages on Taranga (Hen Island) and Muriwhenua (Northwest Chickens Islands). Blood samples were collected during chick-rearing stage only.
- **Buller's shearwater *Puffinus bulleri*** – faecal, regurgitation and feather samples collected during pre-lay, incubation stages to date on Tawhiti Rahi, Poor Knights Islands. Blood samples were collected during incubation and chick-rearing stages. GPS loggers were deployed on breeding birds during mid to late chick-rearing stages (separate project).

- **Red-billed gull *Larus novaehollandiae scopulinus*** – faecal samples and pellets (regurgitations) collected during incubation and chick-rearing stages at Tiritiri Matangi Island, Tawharanui and Marsden Point Refinery.
- **Australasian gannet *Morus serrator*** – regurgitations and faecal, feather and preen gland samples collected during chick-rearing stage in December 2018 and January 2019 at Mahuki Island (Aotea Great Barrier Group) and Horuhoru (Gannet) Rock (Waiheke Group). GPS loggers were deployed in January to study foraging (separate project).
- **White-fronted tern *Sterna striata*** – faecal samples collected during incubation and chick-rearing stages at Tiritiri Matangi, Tawharanui and Horuhoru Rock. Photographs were also been taken of birds carrying prey items in their bills at Tawharanui and Horuhoru Rock.

NB: The collection of blood samples from Buller's shearwaters, fluttering shearwaters and fairy prions and GPS and GLS tracking of Buller's shearwaters, fairy prions and Australasian gannets support complementary research projects funded through the Foundation North G.I.F.T. Initiative and Birds New Zealand Research Fund in collaboration with University of Auckland and Unitec Institute of Technology. The results of these will be published separately.

Methods

Collection of samples

Faecal samples were collected opportunistically during handling, or in the case of some of the Buller's shearwater, fluttering shearwater, red-billed gull and white-fronted tern samples also collected fresh as possible from the ground within the colonies (figs. 2 & 5).

Regurgitations were collected both opportunistically during handling and using flushing technique (figs. 6 & 7). Pellets of red-billed gulls were collected in colonies.

Regurgitation samples were collected from Buller's shearwaters and fairy prions on Tawhiti Rahi Island, Poor Knights Islands in October and December 2018, and fluttering shearwaters in October, November and December 2018 on Taranga Hen Island and Muriwhenua Island using the flushing technique. A crop tube was used with saltwater fed from a syringe in increments. If the bird did not regurgitate, then the process was repeated up to the maximum set for each species (20ml fluttering shearwater, 30-40ml fluttering shearwater, 60ml Buller's shearwater).

All sampling was conducted under the DOC CSP project contract POP2017-06 and Wildlife Act Authority 70910-FAU.

Identification and DNA extraction

For methodology of identification and DNA extraction and sequencing of regurgitation samples refer to the individual reports appended here:

Appendix 1: L. Kozmian-Ledward, A. Jeffs & C. Gaskin (2019). Seabird regurgitation analysis. Report prepared for the Northern NZ Seabird Trust

Appendix 2: N. Adams (2019). Diet and trophic interactions of Australasian Gannet *Morus serrator* – samples collected 2018-2019. Report prepared for the Northern NZ Seabird Trust.

Appendix 3: E. Doyle & N. Adams (2019). DNA extraction and amplification of seabird regurgitates from Buller's Shearwater (*Puffinus bulleri*) and Fairy Prions (*Pachyptila turtur*). Report prepared for the Northern NZ Seabird Trust.

Appendix 4: E. Doyle & N. Adams (2019). DNA extraction and amplification of faecal samples from the White-fronted terns (*Sterna striata*). Report prepared for the Northern NZ Seabird Trust.

Photography

The importance of taking high quality images to chronicle all aspects of the POP2017-06 project is reinforced here with a set of images of birds carrying prey at colonies and feeding chicks (white-fronted tern and Australasian gannet) (cover image, figs. 11 & 12, Appendix 6). Also, birds catching prey at sea where the prey is clearly visible and identifiable (figs. 13 & 14, Appendix 6).

Related sampling and tracking

Funding from the Foundation North G.I.F.T. Initiative and Birds New Zealand allowed us to maximise the work undertaken on islands during the 2018-2019 season. This included collecting additional samples (bloods and feathers) as well as conducting two tracking trials (Buller's shearwater and Australasian gannet). Blood samples from Buller's shearwaters, fairy prions and fluttering shearwaters will be used for stable isotope analysis through contracts with the Ecological Stable Isotope Laboratory, NIWA. The collection of the additional samples and tracking were conducted under Wildlife Act Authority 70910-FAU.

Figure 2. Collecting faecal samples from Buller's shearwater using thermal imaging camera. A fresh deposit is circled. *Photo: NNZST*

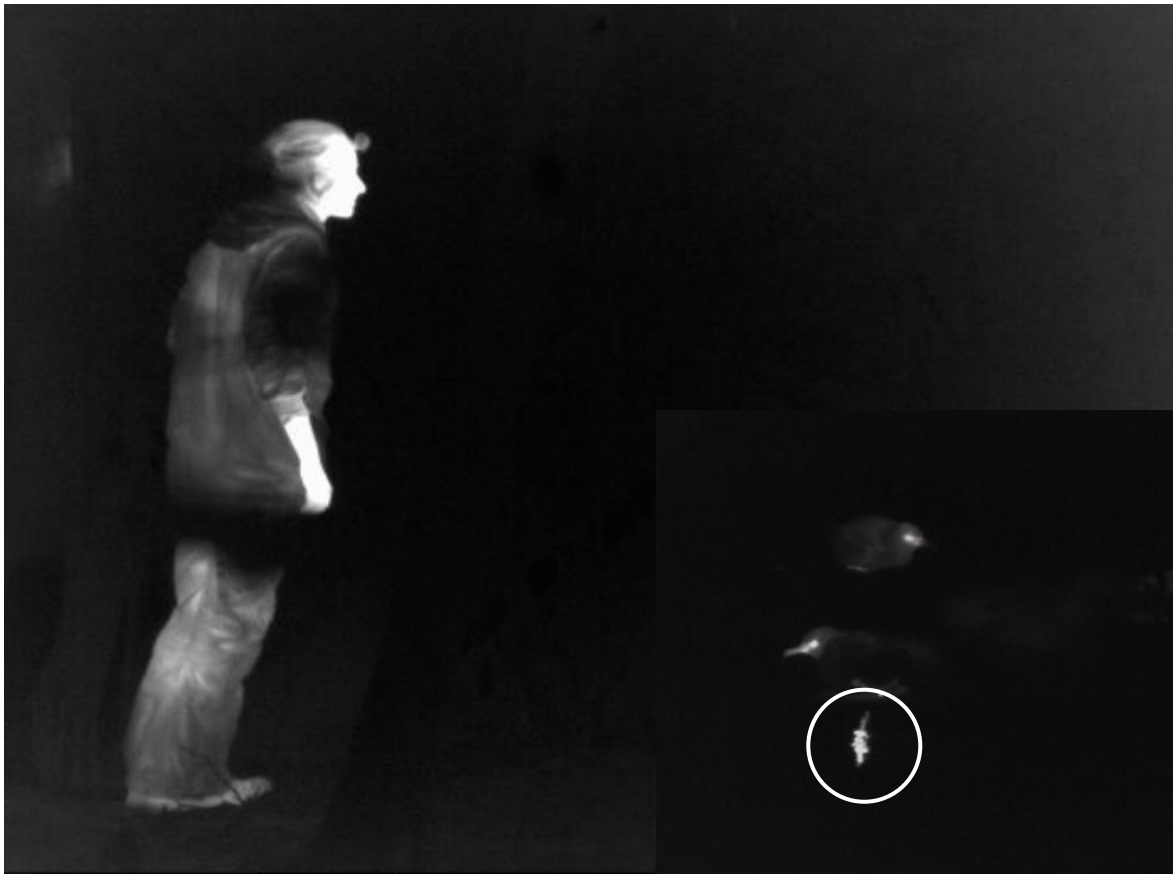


Figure 3. Faecal samples collected from fairy prions and Buller's shearwaters, 20-23 October 2018. *Photo: Chris Gaskin*

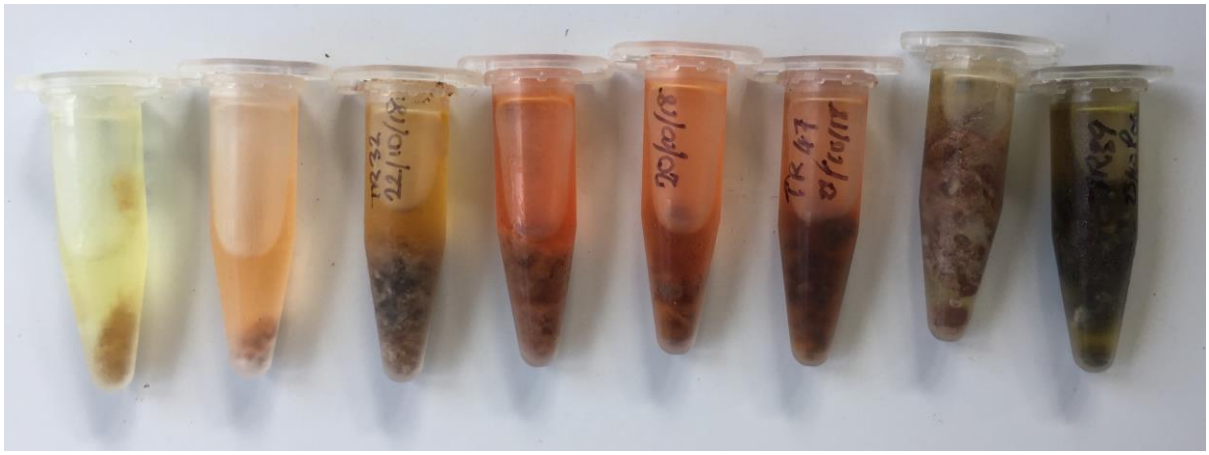


Figure 4. Part of the red-billed gull colony at Phoenix Rocks, Tawharanui, 13 November 2018. *Photo: Adélie Krellenstein*. Figure 5 (insert). Collecting red-billed gull faecal samples. *Photo: Andy McCall (Refining NZ)*

Figure 6. Flushing a fluttering shearwater on Muriwhenua, 18 December 2018. *Photo: Chris Gaskin*

Figure 7. Sample showing semi-digested euphausiids and euphausiid nauplii collected from a fairy prion on Tawhiti Rahi, Poor Knights islands, 22 October 2018, *Photo: Chris Gaskin*

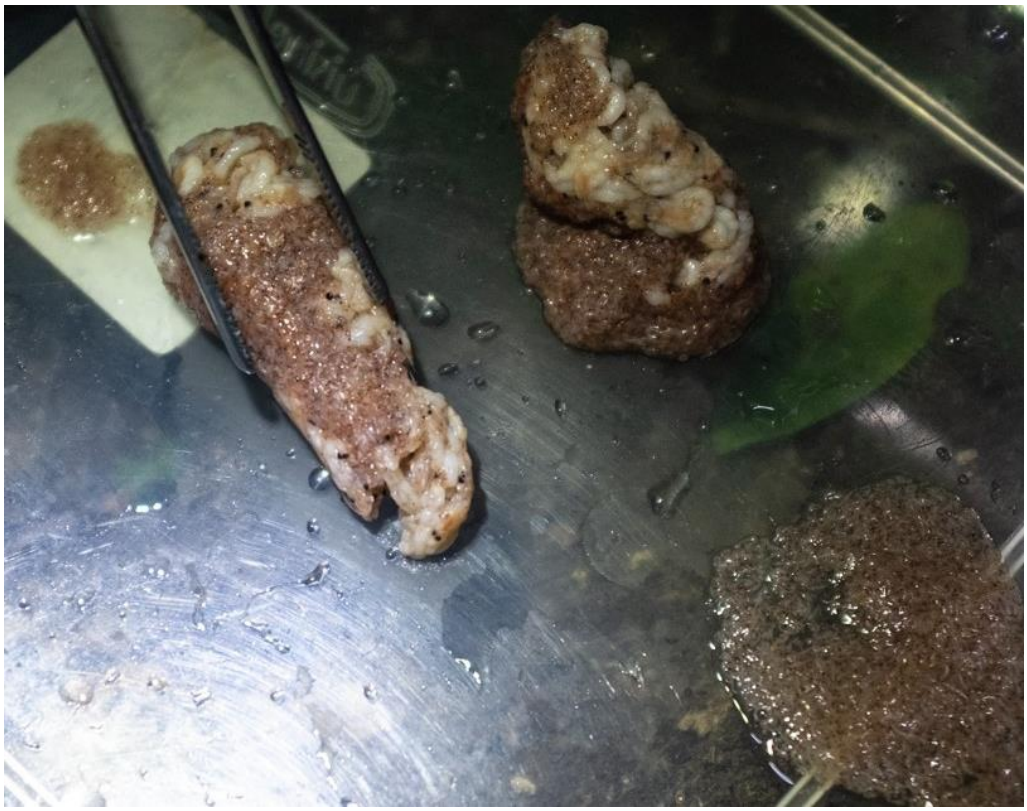


Figure 8. Obtaining a regurgitation sample from an Australasian gannet on Mahuki Island, 7 January 2019. *Photo: Chris Gaskin*

Figure 9 (insert). Jack mackerel regurgitate collected on Horuhoru Rock, 12 January 2019. *Photo: Edin Whitehead*

Figure 10 (lower). Flying fish collected on Mahuki Island, 17 December 2018. *Photo: Nigel Adams*

Figure 11. White-fronted tern with anchovy, Horuhoru Rock, 13 January 2019. *Photo: Edin Whitehead*

Figure 12. White-fronted tern with juvenile squid, Marine Triangle Tokatu Point, Tawharanui 30 December 2018. *Photo: Chris Gaskin*

Figure 13. Australasian gannet with a saury, caught in association with actively foraging common dolphins and flesh-footed shearwaters approximately midway between Marotere Chickens Islands and Mokohinau Islands. *Photo: Edin Whitehead*

Figure 14. An Australasian gannet, harassed by three others, attempting to swallow a large kahawai that it had caught on the fringes of a work up off Coppermine Island, Marotere Chickens Islands, 15 January 2019. *Photo: Karen Baird*

Figure 15. White-fronted tern dips for prey on the fringes of a trevally school near Tara Rocks, Marotere Chickens Islands, 26 October 2018. *Photo: Edin Whitehead*

Figure 16. Red-billed gulls, fairy prions and fluttering shearwaters feeding over an active mixed school of kahawai and trevally near Bream Islands, 17 December 2018. *Photo: Edin Whitehead*

Results

For identification of prey items for each species please refer to the appended reports and tables:

Appendix 1: L. Kozmian-Ledward, A. Jeffs & C. Gaskin (2019). Seabird regurgitation analysis. Report prepared for the Northern NZ Seabird Trust

Appendix 2: N. Adams (2019). Diet and trophic interactions of Australasian Gannet *Morus serrator* – samples collected 2018-2019. Report prepared for the Northern NZ Seabird Trust.

Appendix 3: E. Doyle & N. Adams (2019). DNA extraction and amplification of seabird regurgitates from Buller's Shearwater (*Puffinus bulleri*) and Fairy Prions (*Pachyptila turtur*). Report prepared for the Northern NZ Seabird Trust.

Appendix 4: E. Doyle & N. Adams (2019). DNA extraction and amplification of faecal samples from the White-fronted terns (*Sterna striata*). Report prepared for the Northern NZ Seabird Trust.

Appendix 5: Samples collected from Buller's shearwater, fluttering shearwaters, fairy prions, Australasian gannet, red-billed gull and white-fronted tern.

Appendix 6: Photographs taken at sea and in colonies showing seabirds carrying prey.

Discussion

Euphausiids at both adult and nauplii stages dominate the diet of fluttering shearwaters and fairy prions through incubation and chick-rearing, from late-October to mid-December (Appendix 1). These results correspond directly with observations of their foraging where zooplankton was sampled at fish work ups, particularly observations of birds feeding in association with trevally and kahawai schools (L. Kozmian-Ledward et al unpubl.).

Two samples collected from Buller's shearwaters were identifiable by eye, euphausiids in one sample collected in October 2018 (Appendix 1), and squid in a second collected in April 2019 from a bird when retrieving a logger. In contrast to those from fluttering shearwaters and fairy prion these items were rarities in samples collected during a thorough collecting programme for this species as the birds were, for the most part, very reluctant to regurgitate. Other than these two, samples collected were homogeneous in appearance and appeared to contain little besides the salt water used for flushing. A selection of 15 of these were sent through for DNA extraction and amplification with mixed results (Appendix 3). DNA from five (5) samples was extracted and successfully amplified using a Chordata based primer. Accordingly, the chordate taxa within these samples could potentially be identified using the appropriate DNA sequencing methodology. Better preserved regurgitates from this set of 15 that could be identified visually contained euphausiids (Class malacostraca). Consequently, the inability to extract and amplify DNA from samples using a Malacostran primer was unexpected. The results suggest that preservation of samples using ethanol for most of the samples was inadequate to reliably preserve intact DNA of a suitable quality to amplify and/or the lack of suitability of the selected malacostraca primer. With respect to the squid regurgitation (April 2019) tracking of this bird (RK14) shows that it made a single 8-day foraging journey of just under 1500kms SE of the Poor Knights Islands, to an area 600km ENE of the Chatham Islands. Then, in five days, it flew back to its colony on the Poor Knights Islands where it was recaptured, and the logger downloaded

(Gaskin et al in prep.). The squid regurgitation was relatively fresh with copious squid ink suggesting the squid had been caught en route back to its colony.

Gannets sampled in December 2018 and January 2019, consistent with gannet regurgitation samples collected in the two preceding breeding seasons and from historical studies fed on a range of small to medium sized shoaling fish species and squid. Important fish species recovered in this review period were anchovy and jack mackerel (Outer Gulf; Mahuki Island and Inner Gulf: Horuhoru) and blue mackerel (Inner Gulf: Horuhoru). Arrow squid was important for birds breeding at Mahuki. Refer to Appendix 2 for further discussion.

Red-billed gull regurgitation (pellet) samples collected from two sites (Marsden Point Oil Refinery and Tawharanui) show differences to each other. The Tawharanui samples collected mid-November when birds were either on eggs or with small chicks, contained material that appeared to originate from the intertidal zone and/or from land. In contrast, the Marsden Point Oil Refinery samples collected mid-January, late in the breeding season, suggest birds there have a very cosmopolitan diet reflecting foraging both within the Whangarei Harbour environment and scavenging on land. However, neither sets of samples indicate birds feeding in association with work ups. This contrasts markedly with observations of red-billed gulls feeding en masse around trevally and particularly kahawai schools at Bream Islands, Marotere Chickens Islands, Mokohinau Islands and off Cape Rodney at Leigh Reef (Gaskin 2019). The most likely prey in those cases would have been euphausiids which were picked up in zooplankton trawls (Kozmian-Ledward et al unpub.). In the absence of breeding colonies at either Bream Islands or the Marotere Chickens Islands, the birds feeding around those islands were most likely to be from the Marsden Point Oil Refinery colony (one of Aotearoa New Zealand's largest), reinforced by the common sight of skeins of gulls flying overland from Ocean Beach to Whangarei Harbour late afternoon and at dusk (P. & C. Mitchell pers. comm.). While the small sample sizes may be a factor in not detecting prey associated with fish schools, it's possible that future collection of faecal samples and regurgitations at roost and colony sites closer to regular foraging areas (e.g. Coppermine and Whatupuke Islands, Maori Rocks, Hawere Goat Island) during the height of chick-rearing would yield more accurate results for this species.

Photographs of white-fronted terns carrying prey (Appendix 6) suggest a diet dominated by small fish which is supported by the literature (Mills 2013). Successful amplification of DNA extracted from some faecal samples using a Chordata primer would be consistent with fish in the diet. Confirmation of this from faecal samples would be dependent on sequencing of the DNA to establish more specific identifications and eliminate the possibility that a positive indication of Chordata in the sample was due to the presence of DNA from white fronted terns. However, one bird carrying juvenile squid to the colony at Tawharanui appears to be a new diet record for this species. In the case of a white-fronted tern photographed feeding on the fringes of a mixed trevally kahawai school (fig 14), while no prey could be identified in the tern's bill, the zooplankton sampling on that occasion (26 October 2018) was predominantly euphausiids (L. Kozmian-Ledward et al unpublish.).

Difficulties with respect to preserving some samples in the field for some of the species led to mixed results for DNA extraction and sequencing, something that needs to be addressed with future sampling efforts. However, despite this, the variety of prey that has been identified from samples collected for the six species in 2018-2019 further indicates a complex suite of feeding and

foraging associations for these six seabird species. While the feeding associations that catch the most attention are those relating directly to highly-visible tightly-packed shoaling fish schools of trevally and kahawai and work ups featuring cetaceans, the associations with other prey fish species targeted by the purse seine fishery also need to be better understood. These include jack mackerel, blue mackerel, saury, pilchards, sardines, anchovies and especially skipjack tuna, for which a close association with Buller's shearwaters has already been signaled (Gaskin 2019). If changes in the distribution and/or abundance of fish work-ups and other fish school activity are driving seabird population changes (population status and annual breeding success), then further examination of fish school dynamics across all those fish species and seabird associations relating to each will be required. In addition, at least three species in this study have been shown to be feeding on squid: Australasian gannet on arrow squid (multiple samples), white-fronted tern on juvenile squid sp. (photograph of bird in colony), and Buller's shearwater (a single regurgitation of unidentified squid).

Next stages – final reporting

The final reporting on POP2017-06 will draw all the components of the project together. That is, comparing the availability of food species in fish shoals and how those items are represented in different seabird diets in the region (Milestone 7) and summarising new information on range of seabird populations in northern New Zealand and how these might have changed over time.

Acknowledgements

This project was largely funded by the Conservation Services Programme, Department of Conservation project POP2017-06. Additional funding was made available through Birds New Zealand Research Fund and the Foundation North G.I.F.T. Initiative. Ngatiwai approved access to and monitoring on the Poor Knights, Marotere Chickens and Taranga Islands and we are grateful for their support of this research. Marsden Point Oil Refinery staff facilitated a collection trip to the red-billed gull colony there. Likewise, Auckland Parks supported fieldwork at Tawharanui Open Sanctuary and for providing accommodation for two of our volunteers. The fieldwork on islands throughout this project has been made possible by a hard-working and dedicated team: Cathy and Peter Mitchell, Edin Whitehead, Nigel Adams, Karen Baird, Catherine Geoffroy, Kerry Lukies, Spencer McIntyre, Adélie Krellenstein, Ilse Corkery, James Ross, Jane Gardiner, Keith Hawkins, Rob Chappell, Graeme Loh, Lorna Deppe, Eva Fernandez and Josh Adams. Special thanks to DOC Whangarei staff for managing island visits and maintaining strict biosecurity controls on visits to islands; to Dive Tutukaka for their support for seabird research on the Poor Knights Islands and for transferring field teams to and from islands; Trevor Jackson for both day and overnight boat transfers to the Marotere Chickens Islands. Thanks to Edin Whitehead and Karen Baird for photographs of birds with prey caught at sea and in colonies; to Andrew Jeffs and the University of Auckland Leigh Marine Laboratory for supporting the identification of some of the regurgitations collected in colonies; to Lily Kozmian-Ledward, Olivia Lord and Alyssa Ward for their work in the lab; to Edin Whitehead and John Stewart for collecting tern and gull samples. Finally, to Graeme Taylor, Ian Angus, Igor Debski, Shannon Weaver and the rest of the Conservation Services Programme team for their support and encouragement throughout the two-year term of the POP2017-06 contract.

References

Gaskin, C.P. 2018. Summary of activities carried out to collect samples from seabirds 2017-2018 (Milestone 3). Report for Indirect effects on seabirds in northern North Island POP2017-06. Conservation Services Programme, Department of Conservation, Wellington.

Gaskin, C.P. 2019. Summary of activities carried out to collect samples from fish shoals 2018 (Milestone 5) & overall project update. Report for Indirect effects on seabirds in northern North Island POP2017-06. Conservation Services Programme, Department of Conservation, Wellington.

Mills, J.A. 2013. White-fronted tern. *In* Miskelly, C.M. (ed.) *New Zealand Birds Online* www.nzbirdsonline.org.nz

Appendix 1

Seabird regurgitation analysis

Lily Kozmian-Ledward (Sea Lily Ltd.), with Associate Professor Andrew Jeffs (University of Auckland) and Chris Gaskin (NNZST)

30 April 2019

Aim

Regurgitation samples were collected in colonies from fluttering shearwater, fairy prion, Buller's shearwater, red-billed gull and white-fronted tern for POP2017-06 – see Appendix 4. Of these a selection was made of samples that could potentially be identified under a dissecting microscope. This report presents the results of this identification process.

Methods and results

Regurgitations were analysed from five seabird species as follows:

- Fourteen fluttering shearwaters (FLSH) at Muriwhenua, NW Chickens between 29 October 2018 and 18 December 2018.
- Eighteen fairy prions (FAPR) at Tawhiti Rahi, Poor Knights between 20 October 2018 and 13 December 2018.
- One Buller's shearwater (BUSH) at Tawhiti Rahi on 8 December 2018.
- One white-fronted tern (WFTE) at Horuhoru Rock, Waiheke on 11 January 2019.
- Fifteen red-billed gulls (RBGU); 7 of these at Phoenix Rocks, Tawharanui on 14 November 2018, and 8 at the Marsden Point refinery colony on 18 January 2019.

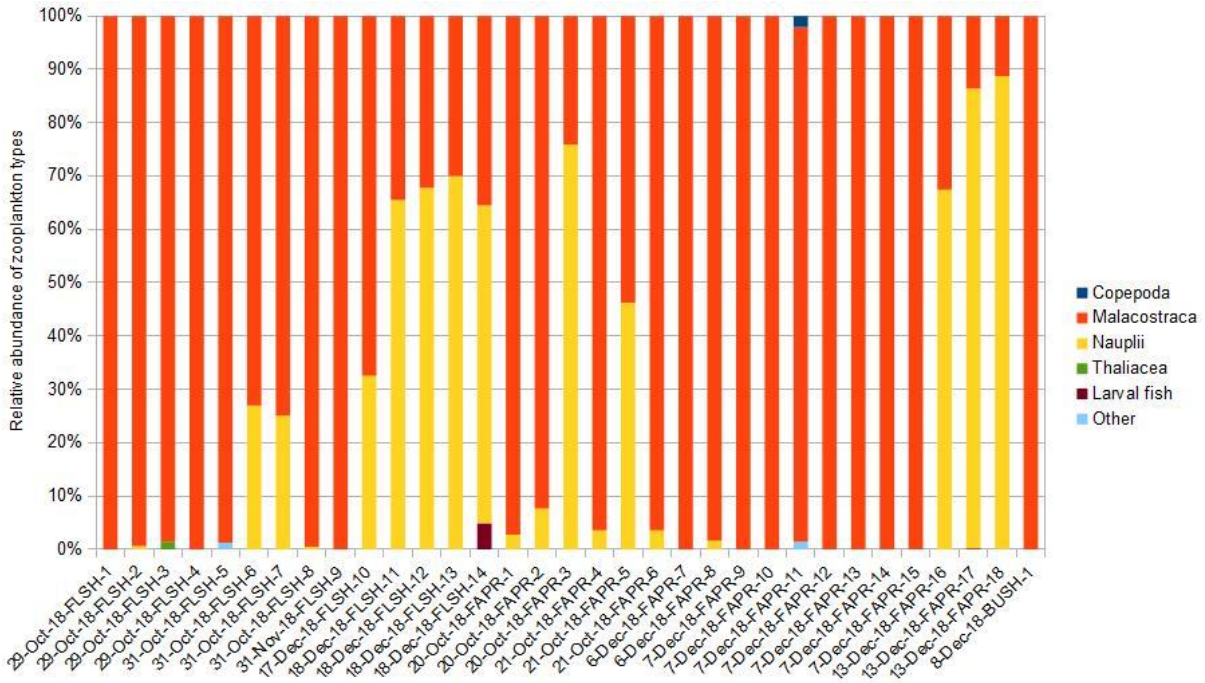
NB: Details of the collection methods of these regurgitation samples and discussion of results are presented in the main report.

The regurgitations from the FLSH, FAPR and BUSH were predominantly comprised of zooplankton. Regurgitation samples which contained many organisms were subsampled using an 8-way zooplankton splitting device (Taylor, 1991) with one quarter or one half of the sample retained for counting. Zooplankton were identified and counted in a Bogorov tray under a dissecting microscope. Six groups of zooplankton were identified and counted: Copepoda, Malacostraca, Thaliacea, Osteichthyes, Larval crustaceans (early stages such as euphausiid nauplii and crab zoeae) and "Other" which consisted of any other zooplankton types. The WFTE sample consisted of a single fish which was measured and photographed. The RBGU regurgitations were in the form of a pellet. These were pulled apart in a petri dish using tweezers and forceps, viewed under the microscope and the contents described qualitatively.

Relative abundance of zooplankton groups in FLSH, FAPR and BUSH regurgitations

The regurgitation samples varied in the extent to which they had been digested but the individual zooplankton were generally identifiable. The larger zooplankton such as euphausiids (Malacostraca) and juvenile fish (Osteichthyes) were sometimes in pieces and the number of whole organisms was estimated conservatively. Where a sample was more deteriorated, the counts are likely under estimated. Across the FLSH, FAPR and BUSH regurgitation samples, the most common zooplankton types were Malacostraca and larval crustaceans (Fig. 1). Small numbers of Copepoda, Thaliacea, Osteichthyes and other zooplankton types were also found. Further details are given below for each bird species.

Figure 1. Relative abundance of zooplankton groups in fluttering shearwater (FLSH), fairy prion (FAPR) and Buller's shearwater (BUSH) regurgitations. Sample ID given on x-axis showing date sample collected, bird species code and sample number.



Fluttering shearwater

The most common zooplankton type in FLSH regurgitation samples that were taken during October and November 2018 was Malacostraca and these were predominantly euphausiids (Fig. 2 & 5). Samples taken during December 2018 mostly had the Larval crustacean group as the most common type. These comprised mainly of euphausiid nauplii (Fig. 4). Euphausiid eggs (Fig. 3) were also present in these samples which would have been attached to the female and then become dislodged. Euphausiid eggs were not added to the counts. No Copepoda were found. Only one individual from the Thaliacea group was found; a salp in sample 3. An estimated 27 juvenile fish (Osteichthyes) were found in sample 14 (Fig. 6); none of which was a whole fish. Two organisms from the Other group were found, both nematode worms in sample 5.

Figure 2. Relative abundance of zooplankton groups in fluttering shearwater (FLSH) regurgitations

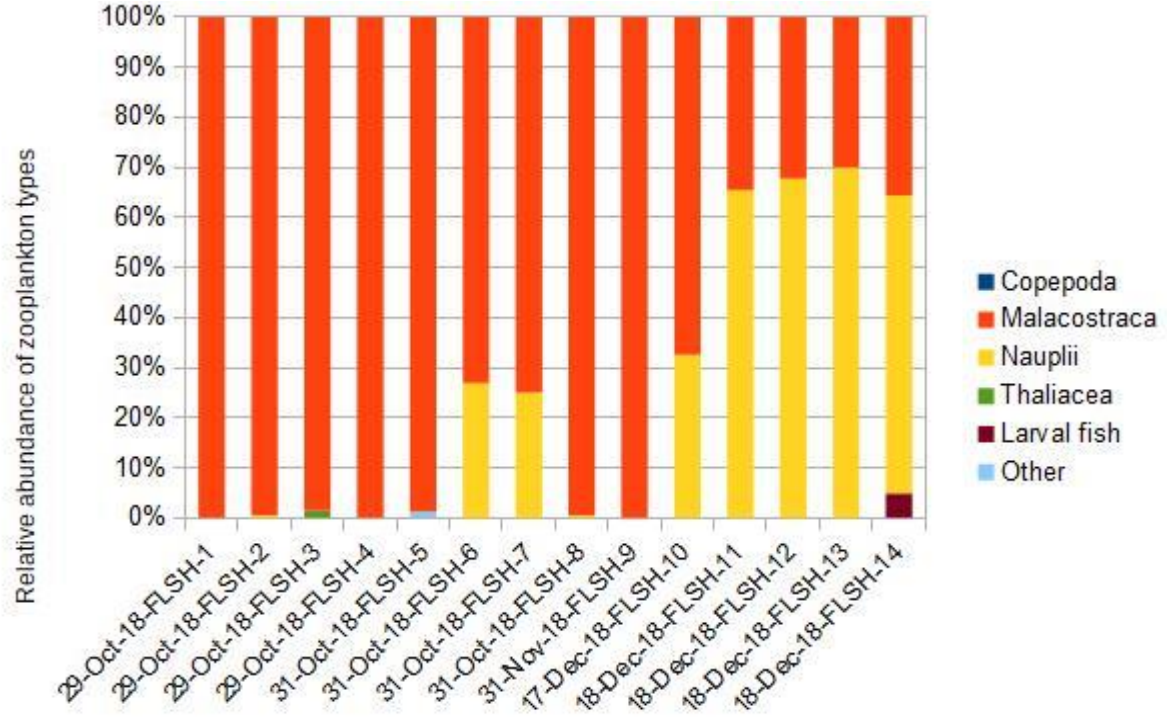


Figure 3. Euphausiid female with eggs attached, from FLSH regurgitation, NW Chickens. Egg diameter approx. 0.3 mm.

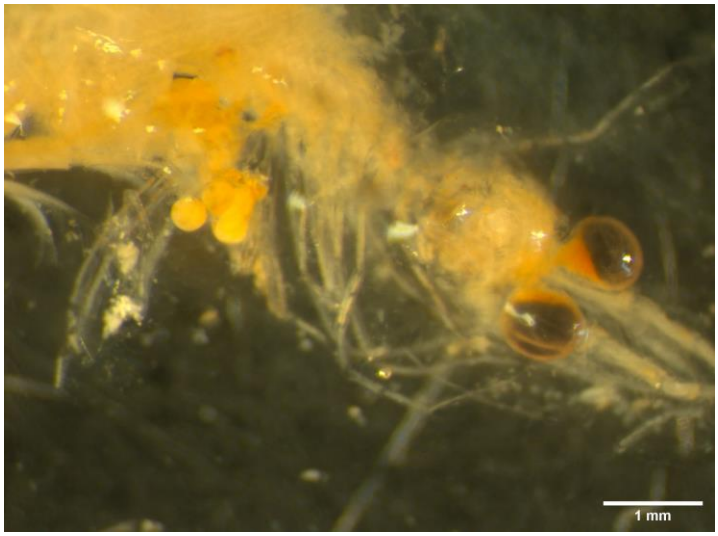


Figure 4. Euphausiid nauplii, from FLSH regurgitation, NW Chickens

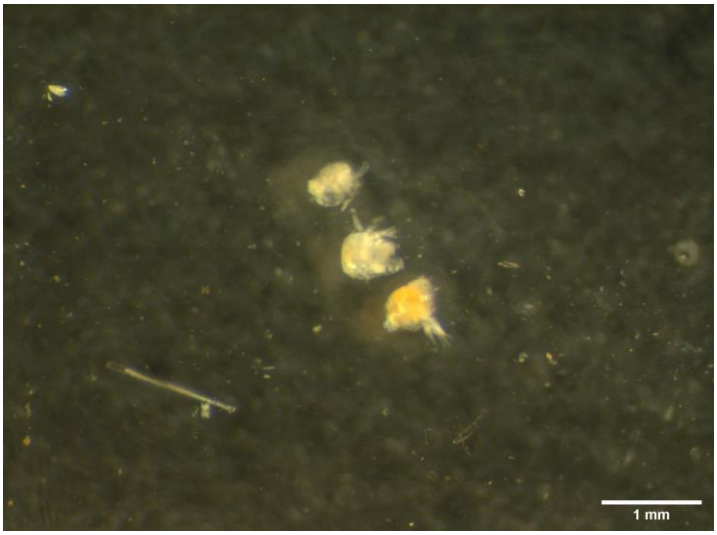


Figure 5. Euphausiid from FLSH regurgitation, NW Chickens.

Figure 6. Partial fish from FLSH regurgitation, NW Chickens.

Fairy prion regurgitations

The FAPR regurgitations were generally more digested/fragmented than those of the FLSH which made the identification and counting more difficult. The most common zooplankton type in the FAPR regurgitation samples was Malacostraca, comprising mostly of euphausiids (Fig. 10), (predominately adults but also some juveniles) with the occasional crab megalopa (Fig. 7). Nauplii were common in two of the October 2018 samples and all of the samples from 13 December 2018. The Nauplii counts were comprised predominantly of euphausiid nauplii (Fig. 8) and crab zoeae (Fig. 9). Copepods were found in low numbers in sample number 6. No Thaliacea were observed in any of the 18 samples. One piece of larval fish was found in sample 3 and two unidentified fish were found in sample 17 (Fig. 11). Within the Other type, four ostracods were found in sample 11 and a flatworm in sample 18.

Figure 7. Relative abundance of zooplankton groups in fairy prion (FAPR) regurgitations.

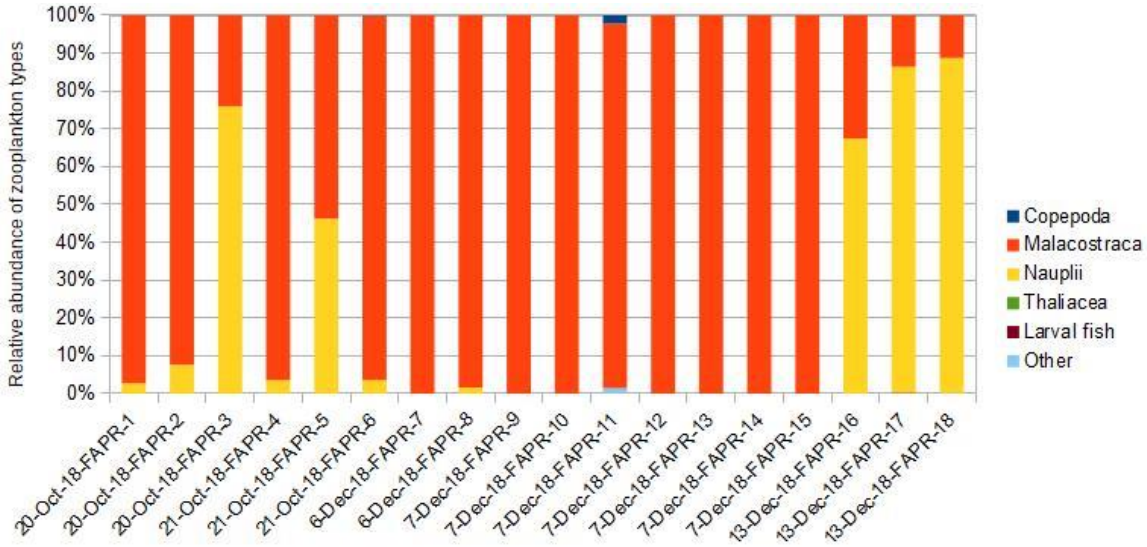


Figure 8. Euphausiid nauplii from FAPR regurgitation, Poor Knights.

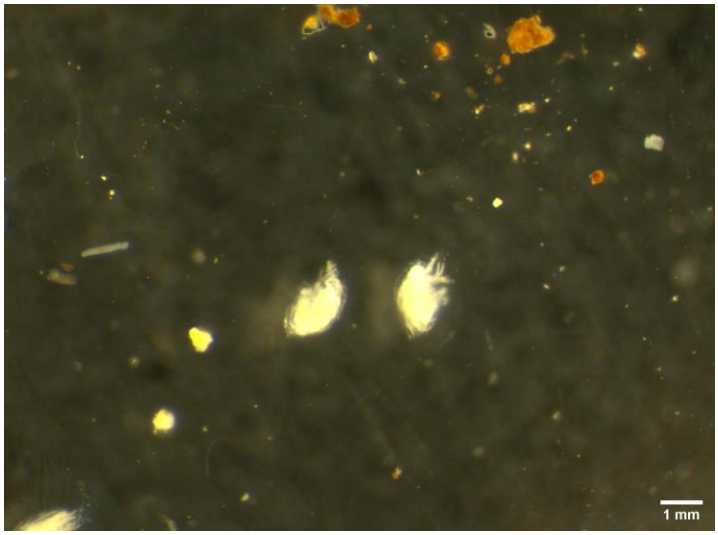


Figure 9. Hermit crab zoeae from FAPR regurgitation, Poor Knights.

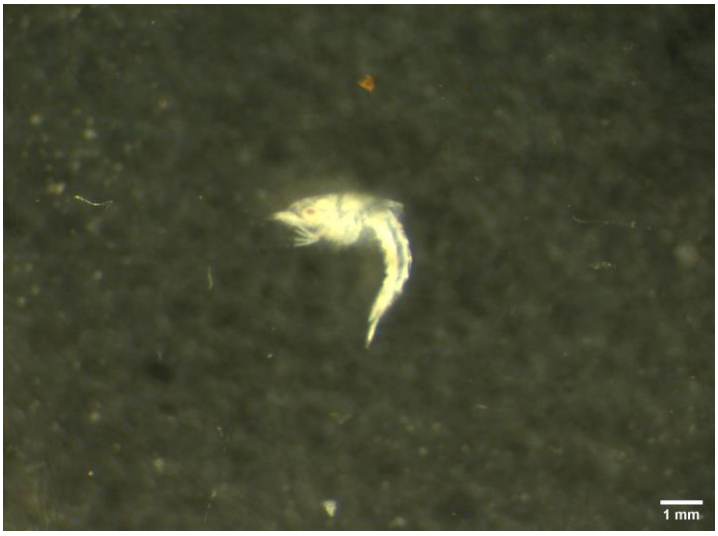
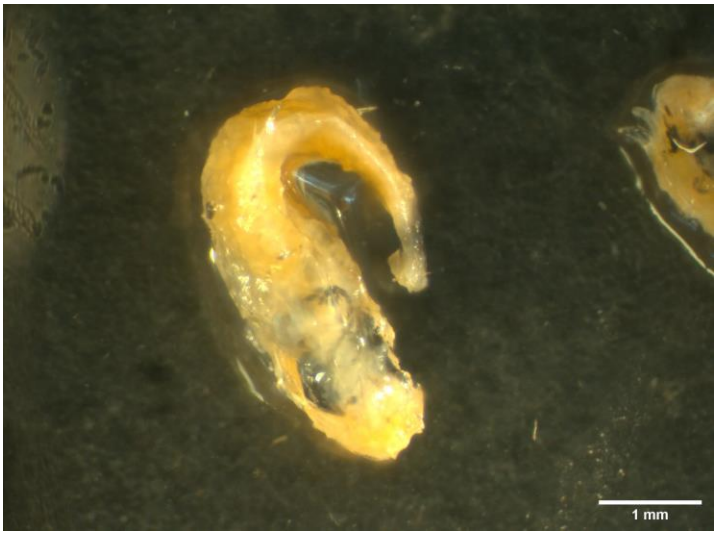


Figure 10. Euphausiid from FAPR regurgitation, Poor Knights.

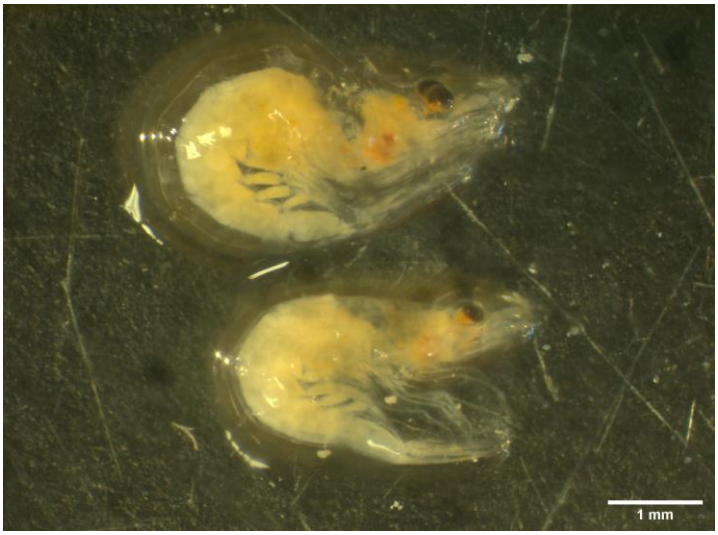
Figure 11. Larval fish from FAPR regurgitation, Poor Knights.



Buller's shearwater regurgitation

Only one BUSH regurgitation was analysed, the contents of which was entirely euphausiids (Fig. 12).

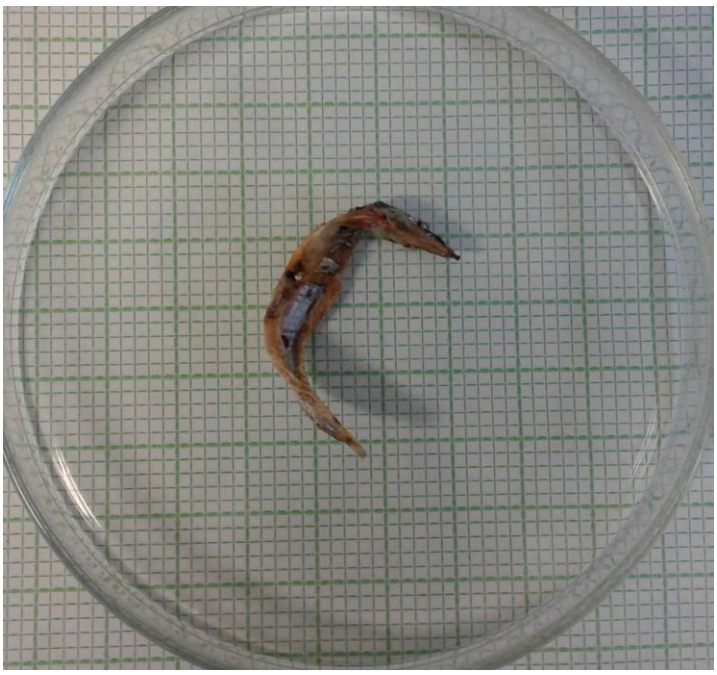
Figure 12. Euphausiids from BUSH regurgitation, Poor Knights. Approx. Length (top and bottom): 6.6, 5.6 mm.



White-fronted tern regurgitation

The single sample from a WFTE consisted of a single fish of an unidentified species (Fig. 13). This was amongst the WFTE colony and most likely dropped by a parent bird, or during display between adult birds.

Figure 13. Fish from WFTE regurgitation. Approx. length: 55 mm



Prey size

Fluttering shearwater:

- Mature euphausiid: 6.34 – 7.37 mm (5 measured)
- Euphausiid egg (not included in counts): 0.35 mm (1 measured)
- Euphausiid nauplii: 0.43 – 0.62 mm (6 measured)

Fairy prion:

- Hermit crab zoeae (included in 'nauplii' counts): 1.48 – 2.64 mm (6 measured)
- Euphausiid nauplii: 0.67 – 0.77 mm (2 measured)
- Euphausiid calytopsis (included in 'nauplii' counts): 1.11 mm (1 measured)

White-fronted tern:

- Unidentified juvenile fish: 55 mm (n=1)

Red-billed gull pellets

The seven samples from RBGU's at Phoenix Rocks, Tawharanui were generally firm pellets containing material that appeared to originate from the intertidal zone and/or from land. Pellet components included fragments of shell that may have been from gastropod molluscs and crabs (Figs. 14 & 15), plant/algal matter (Fig. 17), white powdery lumps (possibly calcium carbonate), arthropod/insect looking legs (Fig. 16) and other body parts. Fleshy material was relatively rare.

The eight samples from RBGU's at the Marsden Point refinery colony generally were in the form of dense, fibrous pellets. The fibres appeared to be fine feathers (Fig.18) and possibly some animal fur. The origins of the pellet components again appeared to come from terrestrial and intertidal areas. Five out of the eight samples contained small pieces of rubbish, i.e., plastic (hard and soft) glass, polystyrene, paper and foil (Figs. 20 & 21). One sample comprised mainly of seven fish bones which were approx. 60 mm long (Fig. 19). Other components of the pellets included: shell, pebbles, possible seeds, plant/algal matter, wood, crab parts and white powdery lumps.

Figure 14. Shell pieces from RBGU regurgitation, Tawharanui.

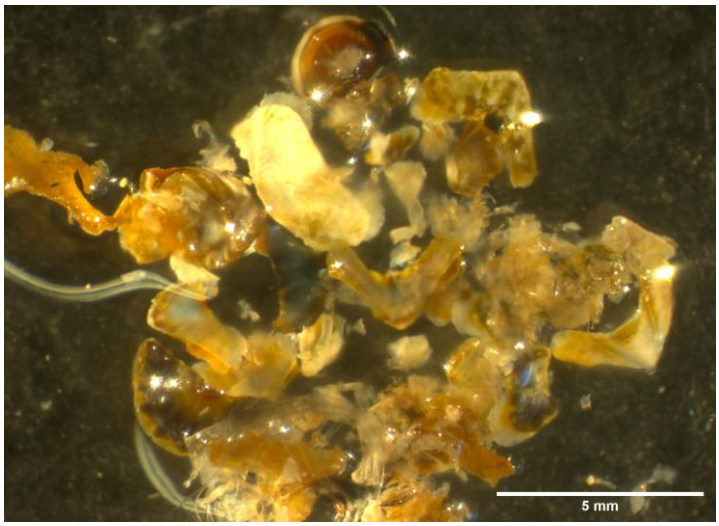


Figure 15. Close up of shell pieces. Possible arthropod carapace sections.

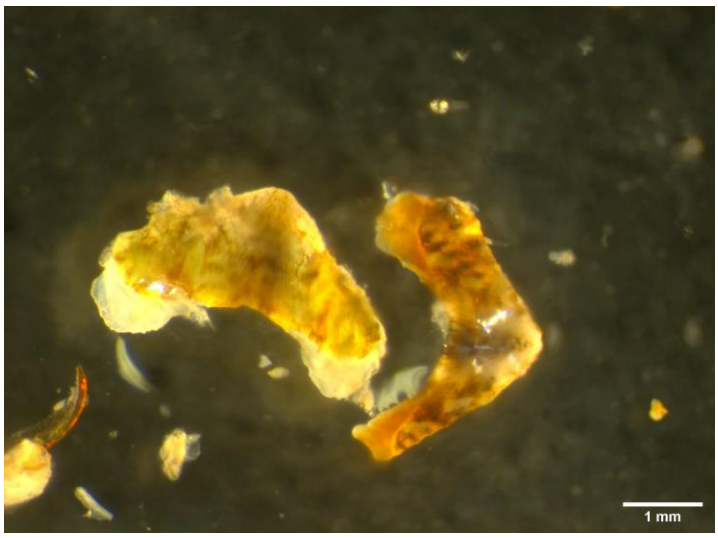


Figure 16. Unidentified legs from RBGU regurgitation, Tawharanui.

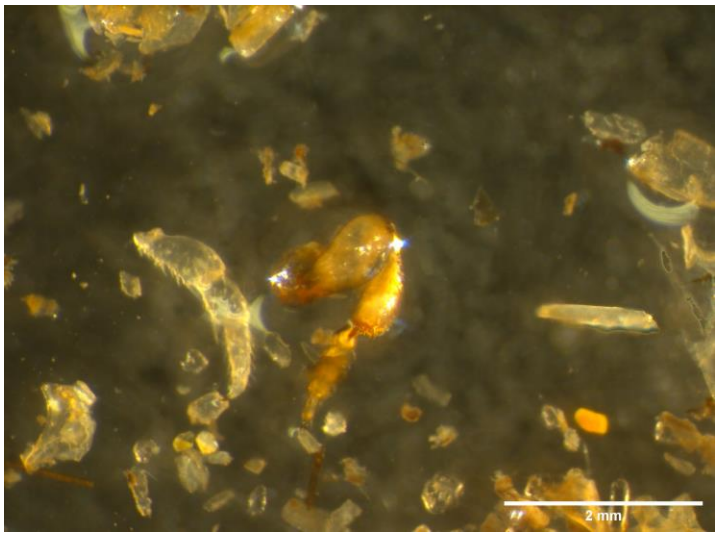


Figure 17. Plant material from RBGU regurgitation, Tawharanui.

Figure 18. Fine feathers and plant material in RBGU regurgitation, Marsden Point.

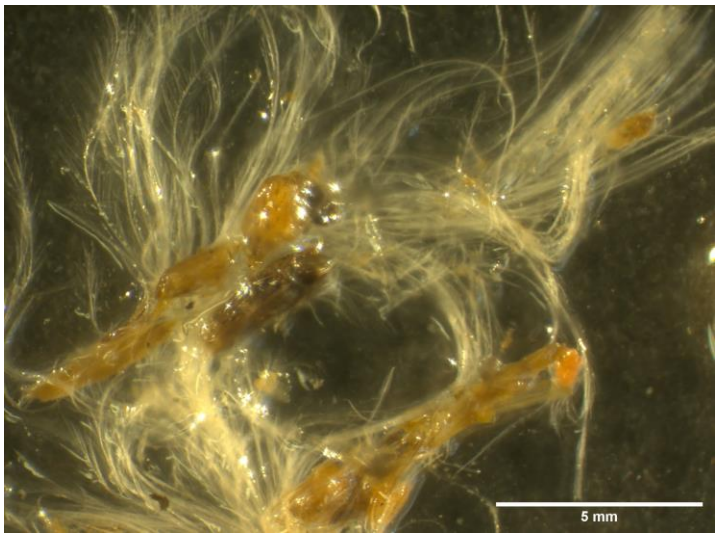


Figure 19. Fish bones from RBGU regurgitation, Marsden Point.

Figure 20. From top, clockwise: polystyrene, plastic and foil from RBGU regurgitation, Marsden Point.

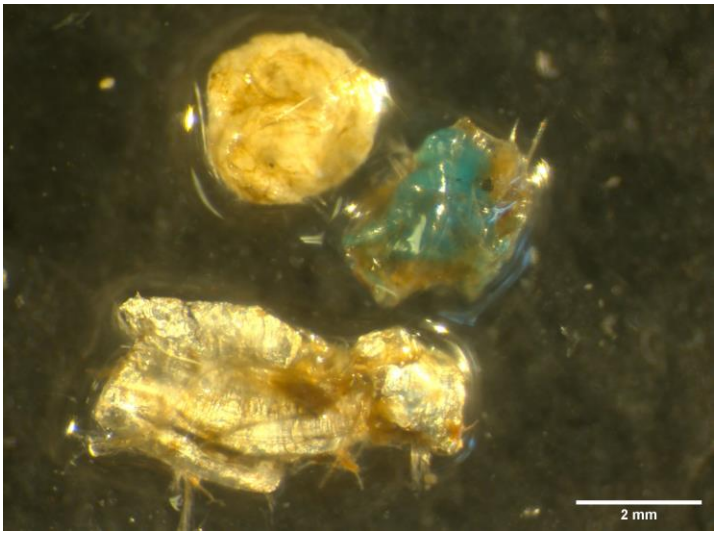
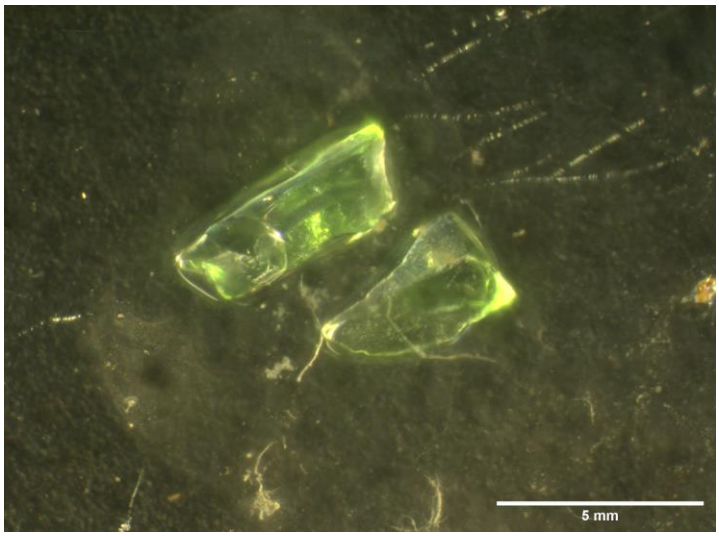


Figure 21. Glass pieces from RBGU regurgitation, Marsden Point.



Acknowledgements

Many thanks go to volunteers Olivia Lord and Alyssa Ward for assistance with processing the regurgitation samples.

Reference

Taylor, R. B. (1991). Effects of *Notolabrus celidotus* (Labridae) predation on motile macroalgal epifauna. Unpublished MSc. University of Auckland.

APPENDIX 2

Diet and trophic interactions of Australasian Gannet *Morus serrator* – samples collected 2018-2019

Nigel Adams, Unitec Institute of Technology NZ

Introduction

Seabirds are highly visible and often easily accessible top predators in marine ecosystems and depend for their survival on a complex underpinning food web. Both natural and human induced environmental perturbations of marine systems can affect the diversity and distribution of marine organisms that make up this web of interacting organisms.

Studying appropriate aspects of seabird feeding biology is likely to indicate changes energy flows within marine communities caused by natural and human induced impacts. One aspect of seabird feeding biology that can be measured is the diet composition of birds returning to feed chicks at land-based colonies. The relative abundance of items in the diet may be closely linked to its availability in the surrounding marine environment, although prey selection made to meet both macro and micro nutrient needs is also likely to pay a role.

As the basis to understanding of key trophic relationships with a coastal marine ecosystem, and hence a potentially important indicator of ecosystem function, this study focusses on describing the diet and trophic interactions of Australasian Gannets *Morus serrator* that utilize the Hauraki Gulf, North Island New Zealand. The Hauraki Gulf is a partly enclosed marine water body surrounded by highly modified terrestrial habitats. Auckland, the major urban area of New Zealand, is situated on the southern end of the Gulf. Accordingly, the gulf is subject to a range of environmental perturbations and challenges that potentially impact on ecosystem function. The specific nature of these are likely to change from the shallower, more turbid inner gulf to the deeper, less turbid waters of the outer gulf.

The diet of Australasian gannets in New Zealand waters, including at localities in the Hauraki Gulf, has previously been described from morphological analysis of intact prey recovered from regurgitated crop contents. These indicate a limited range of surface shoaling fish and squid to be common in the diet. Consistent with historical studies, gannet diet in January 2017 and January 2018 included a range of surface shoaling fish and squid. Most notably these included arrow squid *Nototodarus gouldi*, anchovy *Engraulis australis*, pilchard *Sardinops sagax*, saury *Scomberesox saurus*, redbait *Emmelichthys nitidus* and Jack mackerel *Trachurus spp.*

Particularly notable in 2017 was the importance of arrow squid in the diet of gannets at Mahuki Island. This was replaced by saury, redbait and anchovy in 2018. There were also differences

between the two sampling sites (Mahuki Island: outer gulf and Horuhoru Rock: Inner Gulf). Arrow squid, Red bait and saury were more frequently recovered from gannets breeding at Mahuki Island whereas pilchard was as an important species at Horuhoru (2018). These differences in diets between the two breeding sites suggests some 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.

The outcome of sampling described in this report (December 2018 and January 2019) builds on samples collected during the two preceding previous breeding seasons (January 2017 and January 2018). Diet sampling was combined with GPS tracking of a small number of foraging birds attending chicks (results to be described elsewhere).

The research study outlined here was conducted jointly by Unitec Institute of Technology and the Northern New Zealand Seabird Trust. The research was conducted under Wildlife Act Authority 38016-FAU (Variation).

Methods

Field protocols have been described in an earlier report (POP 2017-06m Milestone 3). This report reviews the diets of adults returning to feed their chicks at Mahuki Island in the outer gulf and Horuhoru Rock in the inner gulf during the 2018/2019 breeding season. In brief, gannets were captured on arrival using a modified shepherd's hook. Capture and handling caused most birds to regurgitate food. The regurgitates, collected into plastic bags, were chilled at the study colony and then frozen for later analysis. Back at the laboratory, fish and squid were identified to species using appropriate guides. The mass of regurgitates and their consistent species were weighed and for fully intact prey caudal length (fish) and mantle length (squid) were measured.

The primary diet of gannets was analysed as the number of prey items of each taxon recovered across all samples (Frequency Abundance), number of times a taxon was recorded as present across all samples (Frequency of occurrence) and as the accumulated mass of each taxon recorded across all samples.

Preliminary results

We obtained regurgitates from a total of 67 birds. Twenty-one samples were collected at Mahuki Island during December 2018 (17/12/2018-19/12/2018) and 26 samples were collected during January 2019 (05/01/2019-08/01/2019). At Horuhoru Rock we collected 21 samples (10/01/2017-15/01/2019). GPS trackers were attached to birds attending chicks at Mahuki and Horuhoru during the January sampling trip.

Regurgitation samples

Like previous years, the mean mass of regurgitate recovered from gannets was 239 ± 131.2 g (mean ± standard deviation) (n = 67). The maximum regurgitated mass recovered was 600 g. Individual regurgitate samples were most commonly homogeneous with most containing only one species.

Composition

Consistent with samples from previous years, the diet of gannet is dominated by shoaling fish and squid. Important species identified during the review period included anchovy *Engraulis australis,* arrow squid *Nototodarus gouldi*, Jack mackerel *Trachurus spp* and Blue mackerel *Scomber australasicus* (see below)

Frequency Abundance

Analysis of diet composition by frequency abundance highlights the numerical abundance of prey in the diet. Anchovy and arrow squid were numerically abundant in regurgitations from Mahuki in December 2018. In January 2019 Jack mackerel increased in abundance to an abundance equivalent to that of anchovy. Blue mackerel was the most commonly recovered prey at Horuhoru (Fig 1) followed by anchovy, Jack mackerel and pilchard. There were substantially fewer arrow squid recovered at Horuhoru than Mahuki.

Frequency of Occurrence

Analysis of diet by frequency of occurrence essentially identifies the number of birds that encountered a particular species within the sampling period. The general pattern of the importance of anchovy, arrow squid and Jack mackerel evident from frequency abundance (Fig 1) was also reflected in this measure of diet composition. (Fig 2). However, there were shifts in the relative importance of these. Jack mackerel was the species most frequently encountered in January 2019 at Mahuki and at Horuhoru.

Accumulated mass

The accumulated mass of prey species is likely to be a reasonable approximation of the energy value of a particular prey species to gannets. The high accumulated mass of Jack mackerel at both localities and across the December and January sampling period reflects the combination of larger size of individual specimens ingested by gannets compare to other prey species (Fig 3,4) as well its relatively high numerical abundance of the species in gannet diet at Mahuki Island in January 2019. (Fig 3). Arrow squid and anchovy were also important species by mass Mahuki in December. The reduced importance of anchovy (Mahuki December 2018 and January 2019) compared to other species is consistent with the smaller individual size of these prey (Fig 4).

While relatively small proportion of prey over this sample period the recovery of saury and flying fish from Mahuki but their absence from Horuhoru and the restriction of blue mackerel to gannets at Horuhoru was notable.

Discussion

Consistent with that of historical studies at colonies around New Zealand and those of gannets sampled in the two previous seasons gannet diet in December 2018 and January 2019 at Mahuki and Horuhoru continue to be dominated by small to medium sized (Fork length 5 – 30 cm) schooling fish and squid species common in coastal waters. The range of species available to gannets is constrained by their ability to forage dive to only relatively shallow depths (most dives 2.5-4 m) (Machovsky Capuska et al. 2011) and their general restriction to coastal waters with breeding birds foraging within 60 km of their colonies. (Machovsky-Capuska et al. 2014). Within these limits the diets of gannets are likely to reflect the local availability of particular prey and selection for species to fulfil particular macro and micro nutritional needs.

The most consistent signal in the diet samples across all years and between colonies has been the persistence of Jack mackerel and anchovy. A range of other species may be particularly important in one season or locality but not the next. For example, we have previously we have noted the importance of arrow squid in the diet particularly at Mahuki Island in January 2017 and reduction in January 2018.

The collection of samples at Mahuki during December 2018 and January 2019 was an attempt to gauge the shorter-term variability in diet within a single season at a particular site. The signal for anchovy, arrow squid and saury were similar between months, however, there were substantial difference in the importance of jack mackerel. Relatively few but large fish being recovered in December but larger number of smaller fish being recorded in January.

Differences in diets between Mahuki and Horuhoru in January 2019 were less different than that recorded in previous years with the most obvious difference being the appearance of blue mackerel and the absence of saury at Horuhoru still suggestive of some separation of foraging areas beyond what might be expected on the basis of the flight performance and foraging trip durations. Another indication in the diet of different foraging strategies by gannets is suggested by the distinction between regurgitates that contain a larger number of smaller fish and those that containing a single large fish. Wells et al. (2016) has suggested that gannets utilizing coast waters of southeast Australia may foraging in open or more pelagic waters in temporary congregations of birds that target shoals of fish often in association with other marine predators or foraging based around searching of shallow inshore waters for large single prey. Diet samples from gannets in the Hauraki Gulf are suggestive of a similar split in foraging strategies. Typically, many of the larger single fish recoveries were Jack mackerel with anchovy typically being substantially smaller and a number of individuals being retrieved from a single regurgitation. Expansion of GPS tracking of a large sample of birds and along with simultaneous monitoring of gannet diet should allow these hypotheses to be tested more fully.

References

Machovsky Capuska GE, Vaughn RL, Würsig B, Katzir G, Raubenheimer D (2011) Dive strategies and foraging effort in the Australasian gannet *Morus serrator* revealed by underwater videography. Mar Ecol Prog Ser 442:255-261.

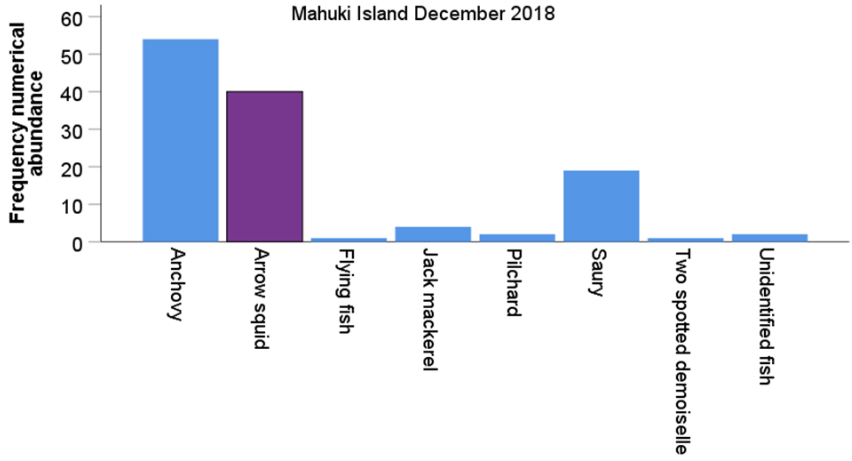
Machovsky-Capuska, G. E., Hauber, M. E., Dassis, M., Libby, E., Wikelski, M. C., Schuckard, R., Melville, D.S., Cook W., Houston, M,, Raubenheimer D (2014). Foraging behaviour and habitat use of chick-rearing Australasian Gannets in New Zealand. Journal of Ornithology, 155(2), 379-387.

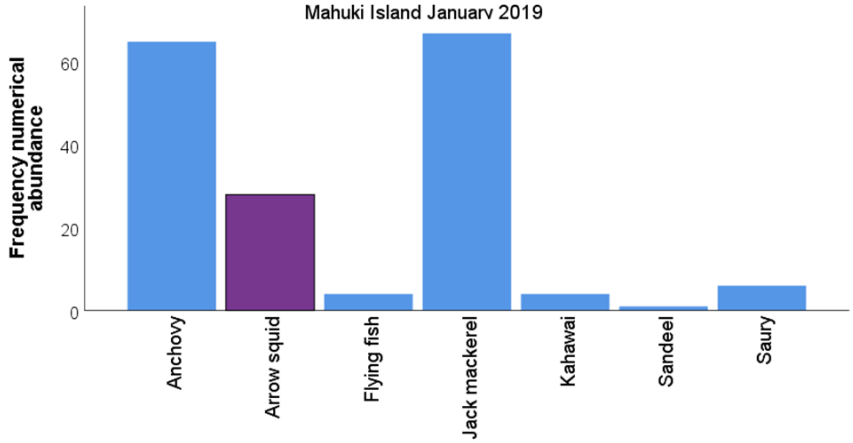
Robertson, D.A. 1992 Diet of the Australasian gannet *Morus serrator* (G.R. Grey) around New Zealand. New Zealand Journal of Ecology 16(2): 77-81

Schuckard, R., Melville, D., Cook, W. I. L. L. I. E., & Machovsky-Capuska, G. E. (2012). Diet of the Australasian gannet (Morus serrator) at Farewell spit, New Zealand. Notornis, 59, 66-70.

Wells, M. R., Angel, L. P., & Arnould, J. P. Y. (2016). Habitat-specific foraging strategies in Australasian gannets. Biology Open, 5(7), 921–927. http://doi.org/10.1242/bio.018085

Figure 1. Numerical abundance of prey recovered from gannets attending chicks at Mahuki Island and Horuhoru Rock, December 2018 and January 2019.





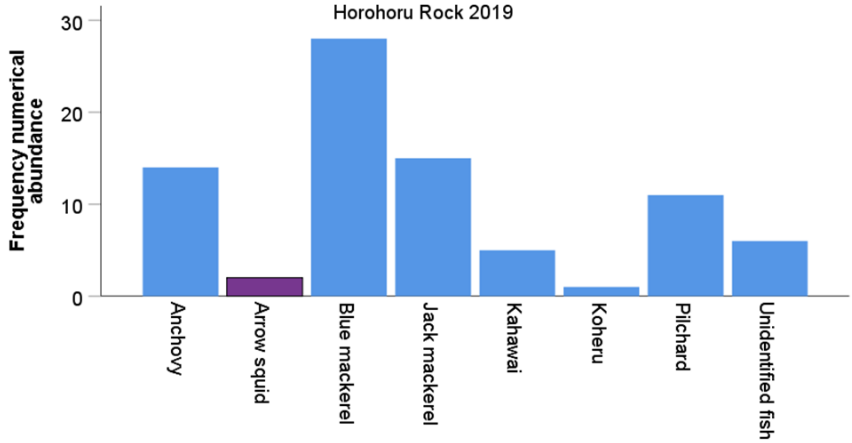
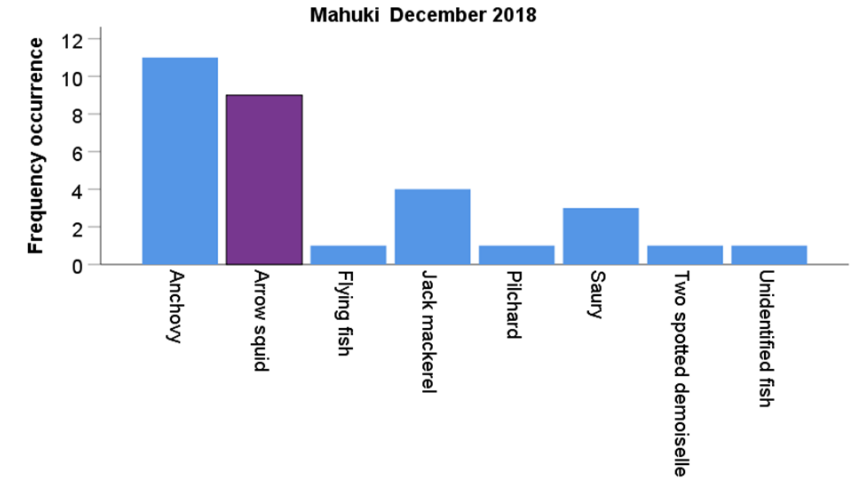
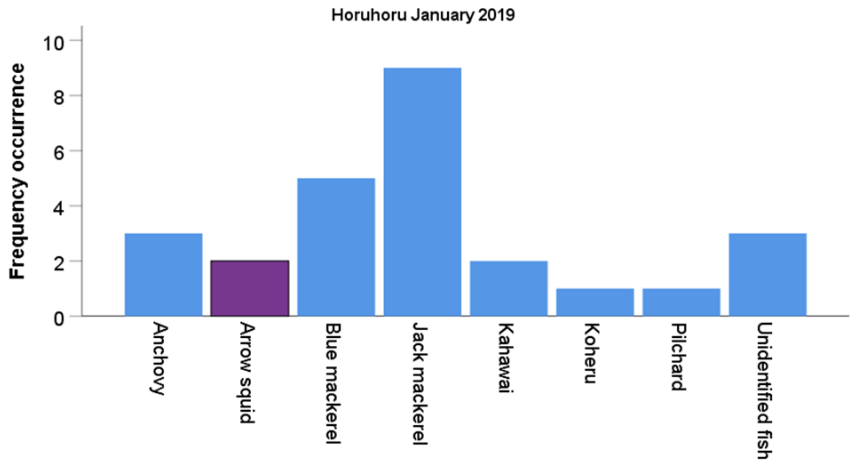


Figure 2. Frequency of occurrence of prey recovered from gannets attending chicks at Mahuki Island and Horuhoru Rock, December 2018 and January 2019.





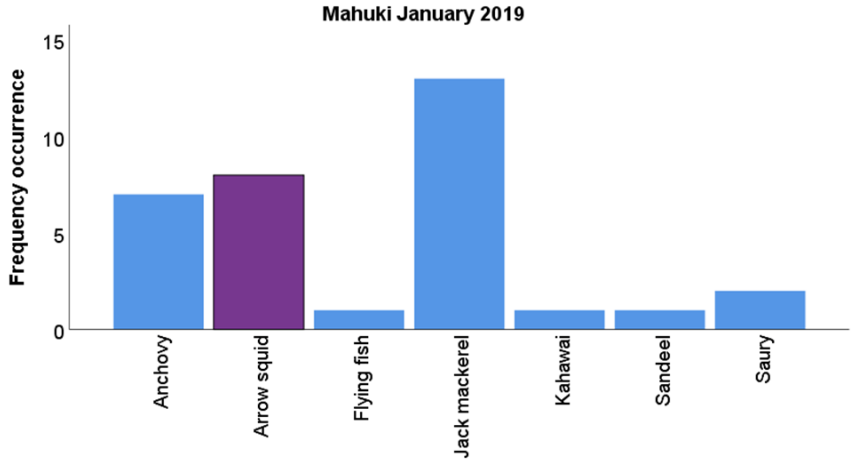


Figure 3. Accumulated mass of prey recovered from gannets attending chicks at Mahuki Island and Horuhoru Rock, December 2018 and January 2019.

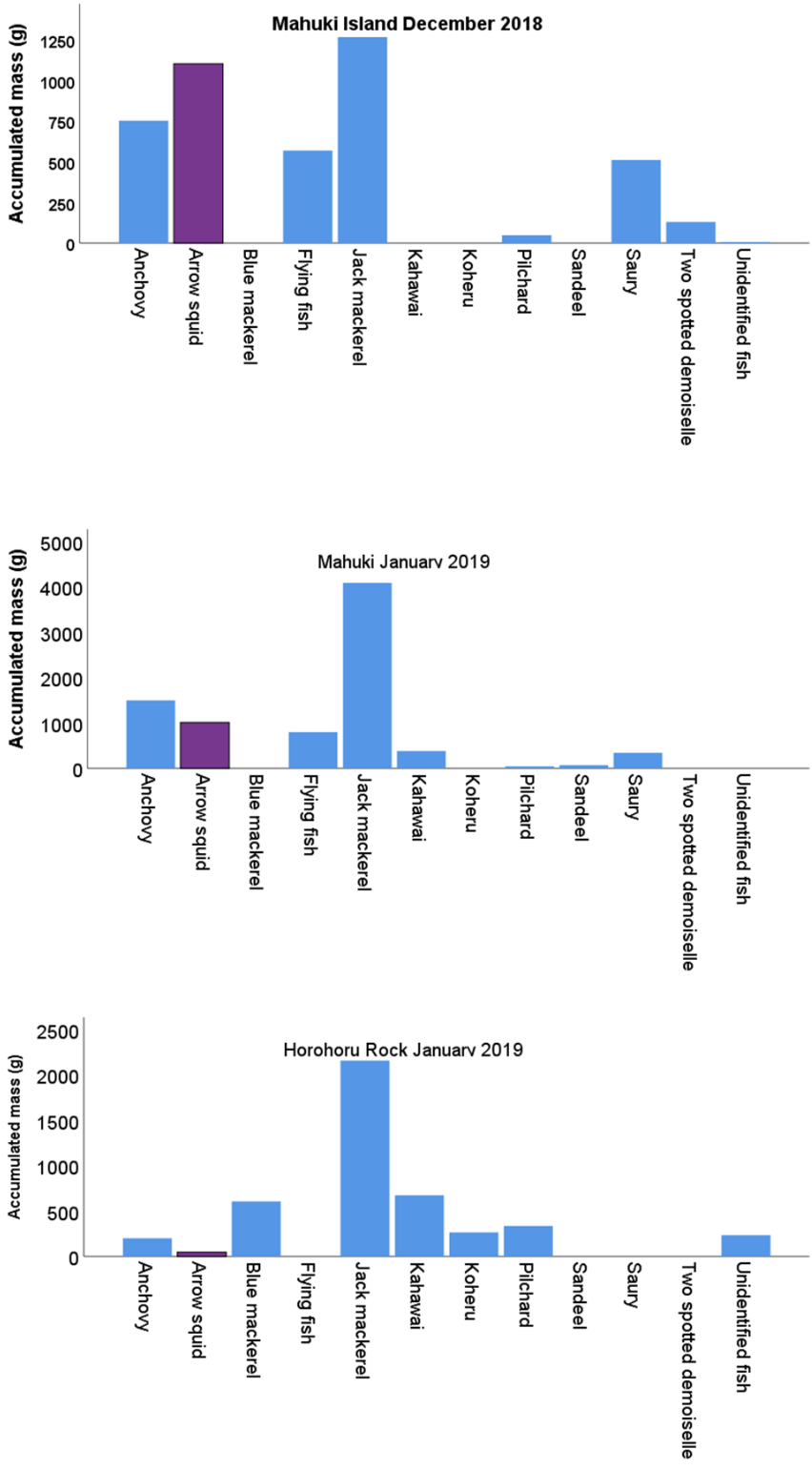
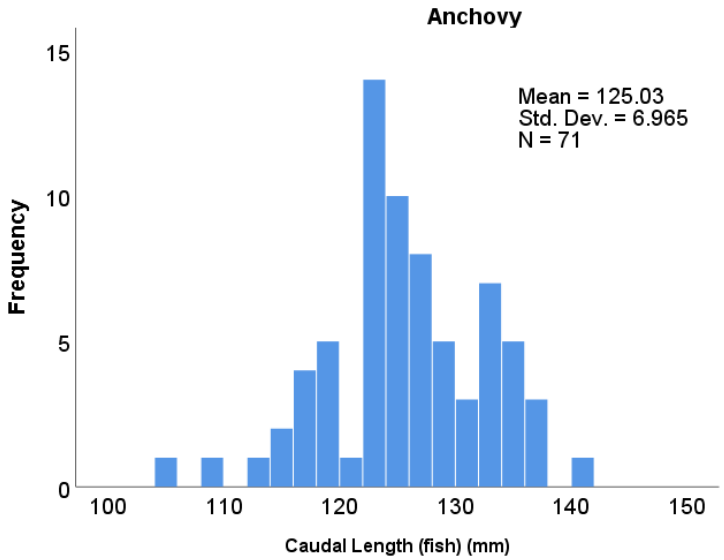
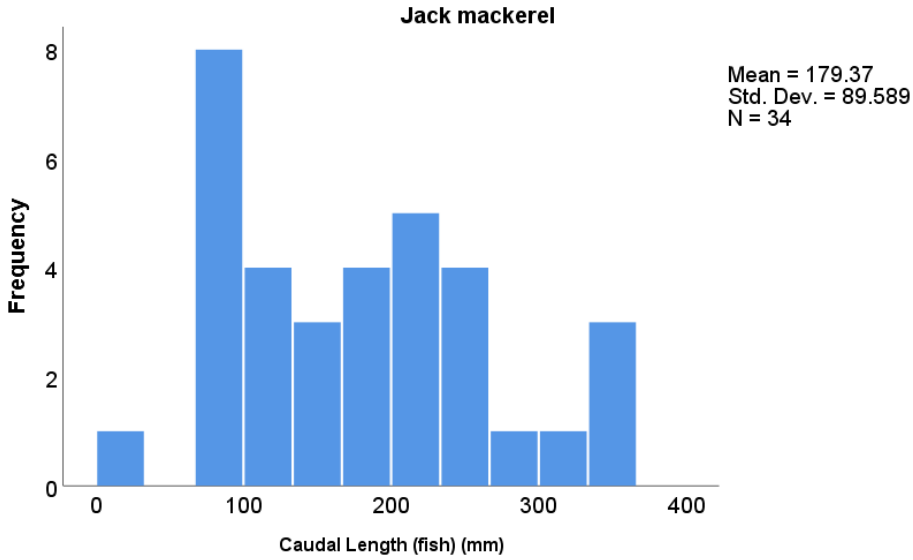


Figure 4. Size frequency distributions of Jack mackerel and anchovy recovered from gannet regurgitations.





APPENDIX 3

DNA extraction and amplification of seabird regurgitates from Buller's Shearwater (*Puffinus bulleri*) and Fairy Prions (*Pachyptila turtur*)

Erin Doyle & Nigel Adams, Unitec Institute of Technology New Zealand.

Summary

Research which aims to identify the prey items found in the diet of seabirds by analysing stomach contents, faeces, and other remains strongly biased towards detecting organisms which are slow to digest, while soft bodied prey may be fully digested and remain undetected. DNA-based methods of prey item identification have been used in a number of studies to address this issue. The intention of this investigation was to further test the feasibility of applying DNA analysis techniques to ethanol preserved regurgitate samples for the purposes of DNA extraction. DNA was extracted from 18 regurgitate samples collected from Buller's Shearwaters *Puffinus bulleri* and Fairy prions (*Pachyptila turtur*). PCR amplification was then used to selectively amplify short sections of the 16S gene which can be used for identification. PCR products were run on an agarose gel, as a success/fail test for amplification. DNA was successfully amplified in five of the eighteen samples using primers targeting Chordata.

Introduction

In a previous study originating from this laboratory we were able to obtain amplifiable DNA from regurgitate and faecal samples collected from adult Australasian gannets (*Morus serrator*) in the Hauraki Gulf, and identify the contents of these samples using next generation sequencing. The current study continues from this work and tested the feasibility of extracting DNA from regurgitation samples collected from birds at the breeding colonies. test the potential for applying this technique to samples from other sea birds.

Method

Regurgitate samples were collected from Buller's shearwaters (*Puffinus bulleri*) and Fairy prions (*Pachyptila turtur*) and then stored in 70% ethanol until extraction. The samples ranged in volume and consistency. The samples were visually assessed and placed into one of five categories depending on their consistency:

1. Small amount of well digested solid matter
2. Medium amount of well solid matter
3. White liquid material
4. Large volume well digested solid matter with white liquid material
5. Large volumes of intact or near-intact krill

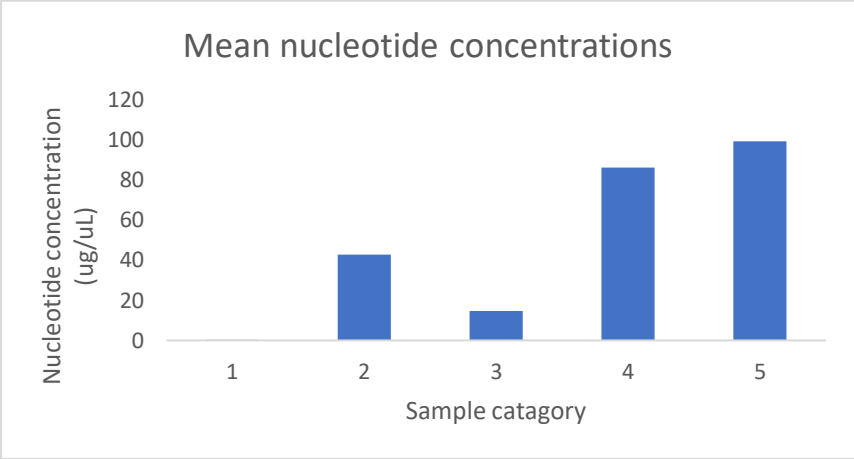
DNA was extracted from 18 randomly selected samples, with 3-5 samples from each of the categories. Prior to DNA extraction the ethanol was evaporated off. Extractions were carried out using the Qiagen® DNeasy kit. The nucleotide concentration of each sample was measured by spectrophotometer.

A 155bp section of the 16S gene in Chordata was PCR amplified 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) (Deagle et al., 2009) in 20µL PCR reactions containing of 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 0.2 µL MgCl (25mM), 3µL of DNA template, and 5.2µl of MQ water. A negative control without DNA, in which 3µL of MQ water was substituted for the template DNA, was included in addition to a positive control using 3µL of whitebait (*Galaxis sp.*). A second PCR reaction was carried out using the primers Mala_16S1F (5'-TGA CGA TAA GAC CCT) and Mala_16S2R (5'- CGC TGT TAT CCC TAA AGT AAC T) (Deagle et al., 2005), which target a 200bp section of the 16S gene in Malacostraca. These PCR reactions had the same composition as those using Chordata primers and incorporated three positive controls using 3µL each of shrimp (*Decapoda*), squid (*Teuthida*), and mussel (*Bivalvia*) DNA, in addition to a negative control using 3µL of MQ water. All PCRs were conducted in a Surecycler 8800 (Agilent Technologies). An initial denaturation period of 15 minutes at 94°C was followed by 33 cycles of denaturation for 20 seconds at 94°C, annealing for 90 seconds at 48.7°C, and extension for 45 seconds at 72°C, followed by a final extension for 2 minutes at 72°C. PCR products were run on a 2% agarose gel and visualised in an UVIDOC HD6 Touch (UVITEC Cambridge), to determine the amplification success of each of the samples.

Results

None of the samples could be amplified using the Malacostraca primers, however five of the eighteen regurgitate samples were successfully amplified using the Chordata primers. These samples were from categories 1, 2, and 3, and a mean nucleotide concentration of 0.22µg/µL, significantly lower than the mean nucleotide concentration of all DNA samples combined, 42.96µg/µL (fig. 1)).

Figure 1: Mean nucleotide concentrations of DNA extractions from samples from each of five categories of sample consistency.



Discussion

The ability to amplify DNA isolated from samples which were categorised as having only small amounts of well digested material using the Chordata primers was encouraging. DNA successfully amplified may have originated from a combination of DNA from the birds itself as well as from food items regurgitated by the birds. Sanger sequencing would not be expected to resolve this question, as the samples are likely to contain DNA from multiple sources, resulting in multiple signals in the sequence data, regardless of the presence of bird DNA.

Fifteen of the eighteen samples were selected for testing were from Buller's Shearwaters, and the remaining three were from Fairy prions. The three samples tested from category 5 all were from Fairy prions regurgitations. These samples contained large amounts of undigested material, primarily krill and yielded the highest nucleotide concentrations, with a mean concentration of 99.23 µg/µL. However, these failed to amplify using either of the two primers tested (Chordata and Malacostraca specific primers).

Given the expectation that food items of both species may include krill, the ability to amplify DNA using the Chordata primer but not the Malacostraca primer was unexpected. Our results suggest that preservation of samples using ethanol for most samples was inadequate to preserve intact DNA of a suitable quality to amplify.

References

Deagle, B. E., Jarman, S. N., Pemberton, D., Gales, N. J. (2005). Genetic screening for prey in the gut contents from a giant squid (Architeuthis sp.). *Journal of Heredity* 96(4), 417-423.

Deagle, B. E., Gales, N. J., Jarman, S. N., Robinson, S., Trebilco, R., & Hindell, M. A. (2007). Studying seabird diet through genetic analysis of faeces: A case study of macaroni penguins (Eudyptes chryolophus). *PLoS One, 9*.

Deagle, B. E., Kirkwood, R., & Jarman, S. N. (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in feces. *Molecular Ecology 18*, 2022-2038.

APPENDIX 4

DNA extraction and amplification of faecal samples from the White-fronted terns (*Sterna striata*)

Erin Doyle & Nigel Adams, Unitec Institute of Technology New Zealand

Summary

This report describes the success of DNA extraction and amplification of fresh faecal samples collected from White Fronted Tern *Sterna striata* in the field. Sample handling involved immediate placement in ethanol field followed by freezing at -20°C a few hours later on the day of collection. DNA was extracted from 10 faecal samples collected from birds (*Sternula nereis*). PCR using primers targeting Chordata was then used to selectively amplify short sections of the 16S gene which can be used for prey identification. PCR products were run on an agarose gel, as a success/fail test for amplification. DNA was successfully amplified in four of the ten samples. Attempts to amplify DNA using primers targeting Malacostraca and a second pair of PCR primers, targeting a 180-270bp section of the 16S gene of multiple taxa, were unsuccessful.

Introduction

Our laboratory has successfully obtained amplifiable DNA from regurgitate and faecal samples collected from adult Australasian gannets (*Morus serrator*) in which the samples had been initially chilled and then frozen on the day of collection. Attempts to extract and amplify DNA recovered from faecal samples (preserved in ethanol) from Buller's shearwaters *Puffinus bulleri* and fairy prions *Pachyptila turtur* have been unsuccessful.

Many variables affect the outcomes of faecal DNA analysis, particularly sample degradation and contamination. Modifications made to sample collection methods, the extraction techniques used, and changes to the PCR protocol used can help to mitigate some of the issues which arise and affect the outcome of DNA amplification. Here, we report on the success of DNA extraction and amplification of faecal samples collected from white-fronted terns (*Sterna striata*). In contrast to samples from Buller's Shearwater and fairy prions immediate ethanol preservation of faecal samples was followed by freezing of the samples at -20°C later the same day.

Method

Samples were collected fresh and stored in 70% ethanol followed by freezing and storage at -20°C until DNA extractions. DNA was extracted from a total of ten faecal samples, sourced from White Fronted Terns, using two different methods, an isopropanol extraction technique and the Qiagen® DNA Stool kit.

A 155bp section of the 16S gene in Chordata was PCR amplified 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) (Deagle et al., 2009) in 20µL PCR reactions containing of 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 0.2 µL MgCl (25mM), 3µL of DNA template, and 5.2µl of MQ water. A negative control without DNA, in which 3µL of MQ water was substituted for the template DNA, was included in addition to a positive control using 3µL of whitebait (*Galaxis sp.*). A second PCR reaction was carried out using the primers Mala_16S1F (5'-TGA CGA TAA GAC CCT) and Mala_16S2R (5'- CGC TGT TAT CCC TAA AGT AAC T) (Deagle et al., 2005), which target a 200bp section of the 16S gene in Malacostraca. These PCR reactions had the same composition as those using Chordata primers and incorporated three positive controls using 3µL each of shrimp (*Decapoda*), squid (*Teuthida*), and muscle (*Bivalvia*) DNA, in addition to a negative control using 3µL of MQ water.
The DNA samples which were extracted using the Qiagen® DNA Stool kit were amplified in a second round of PCR reactions, using a modified protocol in an effort to reduce the presence of primer dimers in the PCR product. Samples were amplified in 20µL reactions containing 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 2µL of DNA template, and 6.4µl of MQ water.

In all reactions, thermal cycling was conducted in a Surecycler 8800 (Agilent Technologies). An initial denaturation period of 15 minutes at 94°C was followed by 33 cycles of denaturation for 20 seconds at 94°C, annealing for 90 seconds at 48.7°C, and extension for 45 seconds at 72°C, followed by a final extension for 2 minutes at 72°C. PCR products were run on a 1% agarose gel and visualised in an UVIDOC HD6 Touch (UVITEC Cambridge), to determine the amplification success of each of the samples.

A second pair of PCR primers, targeting a 180-270bp section of the 16S gene of multiple taxa, was also trailed. DNA samples were PCR amplified using the primers 16S1F (GACGAKAAGACCCTA) and 16S2R (CGCTGTTATCCCTADRGTAACT) (Deagle, 2007) in 20µL PCR reactions containing of 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 1.0 µL BSA, 0.2 µL MgCl (25mM), 3µL of DNA template, and 4.2µl of MQ water. A negative control without DNA, in which 3µL of MQ water was substituted for the template DNA, was included in addition to a positive control using 3µL of whitebait (*Galaxis sp.*). Testing of this primer pairs efficacy prior to this trial showed that it would amplify DNA from fish, but not from the other taxa of interest, therefore only whitebait DNA was included as a positive control.

Results

Of the samples extracted using the isopropanol technique, no DNA amplification was detected seen in the 10 samples using the Malacostraca primers. One sample amplified using the Chordata

primers. With both primers, electrophoresis examination of the PCR products showed the presence of significant amounts of primer dimers in the reactions.

One the same set of samples we then used the Qiagen® DNA Stool kit to extract DNA followed by amplification in a PCR reaction that excluded additional MgCl. The samples in which we used the Malacostraca primers did not amplify successfully, however the success rate of the samples amplified using the Chordata primers increased from one in ten (isopropanol extraction), to four in ten (Qiagen® DNA Stool kit extraction). A significant reduction in the presence of primer dimers in the electrophoresis gel was observed. The 16S1F/16S2R primers, while successful in amplifying fish DNA from a test sample of fish tissue, were not effective when used on the faecal samples, giving no positive results.

Discussion

While the optimal method to use for DNA extraction may vary with the oil content of each individual sample, in this study the use of the Qiagen® DNA Stool kit improved the PCR amplification success rate from 10% to 40%. This suggests this approach is the better option for use with faecal samples collected from the White Fronted Terns,

An attempt was made to improve amplification success by trialing new PCR primers and modifying the protocol from what work had been done previously. While the use of the new 16S1F/16SR primers failed to produce the desired result, the removal of additional MgCl in the reaction did eliminate the presence of primer dimers, which would be expected to led to cleaner results were the samples to be sequenced. It should be noted, that the GoTaq® PCR Master Mix used in the PCR contains MgCl, thus the change made represented a reduction in total MgCl, not the complete removal of it from the reaction.

The successful amplification of 4 of 10 samples using the Chordata primers, but none using the Malacostraca primers, is in line with expectations of the largely fish-based diet of white-fronted terns (Mills 2013).

References

Deagle, B. E., Jarman, S. N., Pemberton, D., Gales, N. J. (2005). Genetic screening for prey in the gut contents from a giant squid (Architeuthis sp.). *Journal of Heredity* 96(4), 417-423.

Deagle, B. E., Gales, N. J., Jarman, S. N., Robinson, S., Trebilco, R., & Hindell, M. A. (2007). Studying seabird diet through genetic analysis of faeces: A case study of macaroni penguins (Eudyptes chryolophus). *PLoS One, 9*.

Deagle, B. E., Kirkwood, R., & Jarman, S. N. (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in feces. *Molecular Ecology 18*, 2022-2038.

Mills, J.A. 2013. White-fronted tern. *In* Miskelly, C.M. (ed.) *New Zealand Birds Online*. www.nzbirdsonline.org.nz

Appendix 5

Samples collected

Table 3. Samples collected from Buller's shearwaters on Tawhiti Rahi, Poor Knights 2018-2019. BUSH = Buller's shearwater; Y = yes, N = No; NR = not recorded.

Date	Species	Band #	Sample #	Mass (g)	Faecal	Regurgitate	Feathers (4x belly)	Blood
23 October 2018	BUSH	H42246	TR35		N	Y	Y	N
23 October 2018	BUSH	H42247	TR36		N	Y	Y	N
23 October 2018	BUSH	H42248	TR37		N	Y	Y	N
23 October 2018	BUSH	H42249	TR38		Y	N	Y	N
23 October 2018	BUSH	H42250	TR39		N	Y	Y	N
23 October 2018	BUSH	H40146	TR40		N	Y	Y	N
23 October 2018	BUSH	H40147	TR41		N	Y	Y	N
23 October 2018	BUSH	H40148	TR42			Y	Y	N
23 October 2018	BUSH	H40149	TR43		Y	N	Y	N
23 October 2018	BUSH	H40150	TR44		Y	Y	Y	N
23 October 2018	BUSH	H42362	TR45		N	Y	Y	N
23 October 2018	BUSH	H42363	TR46		N	Y	Y	N
23 October 2018	BUSH	H42364	TR47		Y	Y	Y	N
8 December 2018	BUSH	H42190	H190	415	Y	Y	Y	Y
8 December 2018	BUSH	H42191	H191	395	Y	Y	Y	Y
8 December 2018	BUSH	H42192	H192	448	Y	Y	Y	Y
8 December 2018	BUSH	H42193	H193	420	N	Y	Y	Y
8 December 2018	BUSH	H42194	H194	450	Y	Y	Y	Y
8 December 2018	BUSH	H26712	H712	485	N	Y	Y	Y
8 December 2018	BUSH	H26713	H713	430	N	Y	Y	Y
8 December 2018	BUSH	H26714	H714	440	N	Y	Y	Y
8 December 2018	BUSH	H26715	H715	415	N	Y	Y	Y
8 December 2018	BUSH	H26716	H716	380	Y	Y	Y	Y
8 December 2018	BUSH	H26717	H717	398	Y	Y	Y	Y
9 December 2018	BUSH	H26718	H718	415	N	N	Y	Y
9 December 2018	BUSH	H26719	H719	465	N	Y	Y	Y
9 December 2018	BUSH	H26720	H720	415	N	Y	Y	Y
9 December 2018	BUSH	H26721	H721	360	Y	Y	Y	Y
9 December 2018	BUSH	H26722	H722	415	N	Y	Y	Y
9 December 2018	BUSH	H26723	H723	425	N	N	Y	Y
9 December 2018	BUSH	H26725	H725	425	N	Y	Y	Y
9 December 2018	BUSH	H26726	H726	425	N	Y	Y	Y
9 December 2018	BUSH	H26727	H727	380	Y	Y	Y	Y
9 December 2018	BUSH	H26728	H728	400	N	Y	Y	Y

9 December 2018	BUSH	H26729	H729	410	N	Y	Y	Y
9 December 2018	BUSH	H26730	H730	425	N	N	Y	Y
9 December 2018	BUSH	H26731	H731	375	N	Y	Y	Y
9 December 2018	BUSH	H26732	H732	430	N	Y	Y	Y
9 December 2018	BUSH	H40147	H147	410	N	Y	Y	Y
10 December 2018	BUSH	H26733	H733	435	N	Y	Y	Y
10 December 2018	BUSH	H26734	H734	455	N	Y	Y	Y
10 December 2018	BUSH	H26736	H736	415	Y	Y	Y	Y
10 December 2018	BUSH	H40148	H148	375	N	Y	Y	Y
10 December 2018	BUSH	H42365	H365	500	N	N	Y	Y
10 December 2018	BUSH	H42366	H366	530	N	Y	N	Y
10 December 2018	BUSH	H42367	H367	420	N	Y	N	Y
10 December 2018	BUSH	H42368	H368	435	N	Y	N	Y
10 December 2018	BUSH	H42369	H369	475	N	N	N	Y
10 December 2018	BUSH	H42370	H370	365	Y	Y	N	Y
10 December 2018	BUSH	H42371	H371	505	N	Y	N	Y
10 December 2018	BUSH	H42372	H372	395	N	Y	N	Y
10 December 2018	BUSH	H42373	H373	410	N	Y	N	Y
10 December 2018	BUSH	H42374	H374	360	N	Y	N	Y
10 December 2018	BUSH	H42375	H375	465	Y	Y	N	Y
10 December 2018	BUSH	H42376	H376	480	N	N	N	Y
10 December 2018	BUSH	H42377	H377	385	NR	NR	N	NR
23 March 2019	BUSH	H43601	TR1901	475	N	N	Y	Y
23 March 2019	BUSH	H43602	TR1902	440	N	N	N	Y
23 March 2019	BUSH	H43603	TR1903	390	N	N	Y	N
23 March 2019	BUSH	H43604	TR1904	500	N	N	N	Y
23 March 2019	BUSH	H43605	TR1905	430	N	N	N	Y
23 March 2019	BUSH	H-43606	TR1906	430	N	N	N	Y
24 March 2019	BUSH	H-43607	TR1907	450	N	N	N	Y
24 March 2019	BUSH	H-43630	TR1908	465	N	N	N	Y
25 March 2019	BUSH	H-43620	TR1908 .25	440	N	N	N	Y
25 March 2019	BUSH	H-43621	TR1909	405	N	N	Y	Y
28 March 2019	BUSH	H-43635	TR1913	480	N	N	N	Y
30 March 2019	BUSH	H-43866	TR1914	485	N	N	N	Y
30 March 2019	BUSH	H-43867	TR1915	500	N	N	N	Y
30 March 2019	BUSH	H-43868	TR1916	450	N	N	N	Y
30 March 2019	BUSH	H-43862	TR1917	540	N	N	N	Y
30 March 2019	BUSH	H-43869	TR1918	460	N	N	N	Y
30 March 2019	BUSH	H-43647	TR1919	550	N	N	N	Y
9 April 2019	BUSH	H-43605	RK16	500	N	N	N	Y
10 April 2019	BUSH	H-43855	RK14	475	N	Y	N	Y
10 April 2019	BUSH	H43646	RK07	460	N	N	N	Y
13 April 2019	BUSH	H-43602	RK10	460	N	N	N	Y
13 April 2019	BUSH	H-43652	RK05	555	N	N	N	Y
14 April 2019	BUSH	H-43601	RK20	430	N	N	N	Y
14 April 2019	BUSH	H-43644	RK18	555	N	N	N	Y

14 April 2019	BUSH	H-43649	RK02	450	N	N	N	Y
15 April 2019	BUSH	H-43655	RK22	505	N	N	N	Y

Table 4. Samples collected from fairy prions on Tawhiti Rahi, Poor Knights 2018. FAPR = fairy prion; Y = yes, N = No; NR = not recorded.

Date	Species	Band #	Sample #	Mass (g)	Faecal	Regurgitate	Feathers (4x belly)	Blood
20 October 2018	FAPR	D191669	TR2	NR	N	Y	Y	N
20 October 2018	FAPR	D191668	TR1	NR	Y	Y	Y	N
20 October 2018	FAPR	D191667	TR3	NR	N	Y	Y	N
20 October 2018	FAPR	D191666	TR4	NR	Y	Y	Y	N
20 October 2018	FAPR	D191665	TR5	NR	N	Y	Y	N
20 October 2018	FAPR	D191664	TR6	NR	N	N	Y	N
20 October 2018	FAPR	D191663	TR7	NR	N	Y	Y	N
20 October 2018	FAPR	D191662	TR8	NR	Y	Y	Y	N
20 October 2018	FAPR	D191660	TR9	NR	Y	Y	Y	N
20 October 2018	FAPR	D191661	TR10	NR	Y	Y	Y	N
20 October 2018	FAPR	D191659	TR11	NR	N	Y	Y	N
21 October 2018	FAPR	D191658	TR12	NR	Y	N	Y	N
21 October 2018	FAPR	D191657	TR13	NR	N	Y	Y	N
21 October 2018	FAPR	D191656	TR14	NR	Y	N	Y	N
21 October 2018	FAPR	D191655	TR15	NR	N	N	Y	N
21 October 2018	FAPR	D191671	TR16	NR	N	Y	Y	N
21 October 2018	FAPR	D191672	TR17	NR	N	Y	Y	N
21 October 2018	FAPR	D191673	TR18	NR	Y	Y	Y	N
21 October 2018	FAPR	D191674	TR19	NR	N	N	Y	N
21 October 2018	FAPR	D191675	TR20	NR	Y	Y	Y	N
21 October 2018	FAPR	D191676	TR21	NR	Y	N	Y	N
22 October 2018	FAPR	D191677	TR22	NR	Y	Y	Y	N
22 October 2018	FAPR	D191678	TR23	NR	N	Y	Y	N
22 October 2018	FAPR	D191679	TR24	NR	N	Y	Y	N
22 October 2018	FAPR	D191680	TR25	NR	Y	Y	Y	N
22 October 2018	FAPR	D191681	TR26	NR	N	N	Y	N
22 October 2018	FAPR	D191682	TR27	NR	N	N	Y	N
22 October 2018	FAPR	D191683	TR28	NR	Y	N	Y	N
22 October 2018	FAPR	D191684	TR29	NR	N	Y	Y	N
22 October 2018	FAPR	D191685	TR30	NR	Y	Y	Y	N
22 October 2018	FAPR	D191686	TR31	NR	Y	N	Y	N
22 October 2018	FAPR	D191687	TR32	NR	Y	N	Y	N
22 October 2018	FAPR	D191688	TR33	NR	N	N	Y	N
22 October 2018	FAPR	D191679	TR34	NR	N	Y	Y	N
6 December 2018	FAPR	D191719	719	122	Y	Y	Y	Y
6 December 2018	FAPR	D191720	720	120	N	Y	Y	Y
6 December 2018	FAPR	D191721	721	133	N	Y	Y	Y

6 December 2018	FAPR	D191722	722	134	Y	Y	Y	Y
6 December 2018	FAPR	D191723	723	121	N	N	Y	Y
6 December 2018	FAPR	D191724	724	148	N	Y	Y	Y
6 December 2018	FAPR	D191727	727	122	Y	N	Y	Y
6 December 2018	FAPR	D191728	728	210	N	N	Y	Y
6 December 2018	FAPR	D191729	729	123	N	N	Y	Y
6 December 2018	FAPR	D191730	730	129	Y	N	Y	Y
6 December 2018	FAPR	D191731	731	125	N	N	Y	Y
7 December 2018	FAPR	D191732	732	120	N	N	Y	Y
7 December 2018	FAPR	D191655	655	112	N	N	Y	Y
7 December 2018	FAPR	D191733	733	121	N	Y	Y	Y
7 December 2018	FAPR	D191734	734	112	N	N	Y	Y
7 December 2018	FAPR	D191746	746	111	N	Y	Y	Y
7 December 2018	FAPR	D191747	747	120	N	Y	Y	Y
7 December 2018	FAPR	D191748	748	150	Y	Y	Y	Y
7 December 2018	FAPR	D191749	749	126	Y	Y	Y	Y
7 December 2018	FAPR	D191750	750	128	Y	Y	Y	Y
7 December 2018	FAPR	D191751	751	143	N	Y	Y	Y
7 December 2018	FAPR	D191752	752	140	Y	Y	Y	Y
7 December 2018	FAPR	D191753	753	118	Y	Y	Y	Y
7 December 2018	FAPR	D191754	754	122	N	N	Y	Y
7 December 2018	FAPR	D191757	757	118	N	N	Y	Y
7 December 2018	FAPR	D191756	756	122	N	N	Y	Y
7 December 2018	FAPR	D191755	755	129	Y	N	Y	Y
7 December 2018	FAPR	D191758	758	138	N	Y	Y	Y
7 December 2018	FAPR	D191759	759	118	Y	Y	Y	Y
7 December 2018	FAPR	D191760	760	128	N	Y	Y	Y
8 December 2018	FAPR	D191761	761	151	N	Y	Y	Y

Table 5. Samples collected from fluttering shearwaters on Taranga and Muriwheuna (Marotere Chickens Islands) 2018. FLSH = fluttering shearwater; Y = yes, N = No; NR = not recorded.

Date	Species	Band #	Sample #	Faecal	Regurgitate	Feathers (4x belly)	Blood
1 October 2018	FLSH	NA	TA01	y	n	y	n
1 October 2018	FLSH	NA	TA02	y	n	y	n
1 October 2018	FLSH	NA	TA03	n	n	y	n
1 October 2018	FLSH	NA	TA04	n	n	y	n
1 October 2018	FLSH	NA	TA05	y	n	y	n
1 October 2018	FLSH	NA	TA06	y	n	y	n
1 October 2018	FLSH	NA	TA07	y	n	n	n
1 October 2018	FLSH	NA	TA08	n	n	y	n
2 October 2018	FLSH	NA	MW01	y	n	n	n

4 October 2018	CODP	NA	MW02	n	n	y	n
4 October 2018	FLSH	NA	MW03	y	n	y	n
4 October 2018	FLSH	NA	MW04	y	n	y	n
4 October 2018	FLSH	NA	MW05	y	n	y	n
4 October 2018	FLSH	NA	MW06	y	n	y	n
4 October 2018	FLSH	NA	MW07	y	n	n	n
4 October 2018	FLSH	NA	MW08	y	n	y	n
4 October 2018	FLSH	NA	MW09	y	n	y	n
4 October 2018	FLSH	NA	MW10	y	n	y	n
4 October 2018	FLSH	NA	MW11	n	n	y	n
4 October 2018	FLSH	NA	MW12	y	n	n	n
4 October 2018	FLSH	NA	MW13	n	n	y	n
4 October 2018	FLSH	NA	MW14	y	n	y	n
29 October 2018	FLSH	NA	MW01	y	n	y	n
29 October 2018	FLSH	NA	MW02	y	y	y	n
29 October 2018	FLSH	NA	MW03	y	y	y	n
29 October 2018	FLSH	NA	MW04	n	2	y	n
29 October 2018	FLSH	NA	MW05	y	y	y	n
29 October 2018	FLSH	NA	MW06	y	n	y	n
29 October 2018	FLSH	NA	MW08	y	n	y	n
29 October 2018	FLSH	NA	MW09	y	y	y	n
29 October 2018	FLSH	NA	MW10	n	y	y	n
29 October 2018	FLSH	NA	MW11	n	y	y	n
29 October 2018	FLSH	NA	MW12	y	y	y	n
31 October 2018	FLSH	NA	MW13	y	y	y	n
31 October 2018	FLSH	NA	MW14	n	y	y	n
31 October 2018	FLSH	NA	MW15	y	y	y	n
31 October 2018	FLSH	NA	MW16	n	y	y	n
31 October 2018	FLSH	NA	MW17	y	y	y	n
31 October 2018	FLSH	NA	MW18	y	n	y	n
31 October 2018	FLSH	NA	MW19	n	y	y	n
31 October 2018	FLSH	NA	MW20	y	y	y	n
31 October 2018	FLSH	NA	MW21	y	y	y	n
17 December 2018	FLSH	NA	MW01	y	y	y	y
17 December 2018	FLSH	NA	MW02	y	y	y	y
17 December 2018	FLSH	NA	MW03	y	y	y	y
17 December 2018	FLSH	NA	MW04	y	y	y	y
17 December 2018	FLSH	NA	MW05	y	y	y	y
17 December 2018	FLSH	NA	MW06	y	y	y	y
17 December 2018	FLSH	NA	MW07	y	y	y	y
17 December 2018	FLSH	NA	MW08	y	y	y	y
17 December 2018	FLSH	NA	MW09	n	y	y	y
17 December 2018	FLSH	NA	MW10	y	n	y	y
17 December 2018	FLSH	NA	MW11	y	y	y	y
18 December 2018	FLSH	NA	MW12	y	n	y	y
18 December 2018	FLSH	NA	MW13	y	y	y	y

18 December 2018	FLSH	NA	MW14	y	n	n	n
18 December 2018	FLSH	NA	MW15	y	n	n	n
18 December 2018	FLSH	NA	MW16	n	y	y	y
18 December 2018	FLSH	NA	MW17	y	n	y	y
18 December 2018	FLSH	NA	MW18	y	n	y	y
18 December 2018	FLSH	NA	MW19	y	y	y	y
18 December 2018	FLSH	NA	MW20	y	y	y	y
18 December 2018	FLSH	NA	MW21	y	y	y	y
18 December 2018	FLSH	NA	MW22	n	y	y	y
18 December 2018	FLSH	NA	MW23	n	y	y	y
18 December 2018	FLSH	NA	MW24	y	n	y	y

Table 5. Samples collected from Australasian gannets on Mahuki Island and Horuhoru Rock December 2018 and January 2019.

Date	sample ID	Band	Adult/chick	Colony	Bird Mass (kg)	Faecal sample	Feather Sample	Preen sample	Total regurgitate (g)	Prey ID initial
17 December 2018	MI1	NA	Adult	Mahuki	2.25	Y	Y	Y	140	Squid, anchovy
17 December 2018	MI2	NA	Adult	Mahuki	2.38	Y	Y	Y	315	Jack mackerel
17 December 2018	MI3	NA	Adult	Mahuki	2.47	Y	Y	Y	570	Flying fish
17 December 2018	MI4	NA	Adult	Mahuki	2.45	Y	Y	Y	130	Unident
17 December 2018	MI5	NA	Adult	Mahuki	2.34	Y	Y	Y	140	Anchovy, squid
18 December 2018	MI6	NA	Adult	Mahuki	2.25	Y	Y	Y	130	Squid
18 December 2018	MI7	NA	Adult	Mahuki	2.22	Y	Y	Y	190	Anchovy x3 pilchard
18 December 2018	MI8	NA	Adult	Mahuki	2.42	Y	Y	Y	105	Unident
18 December 2018	MI9	NA	Adult	Mahuki	2.49	Y	Y	Y	10	Anchovy
18 December 2018	MI10	NA	Adult	Mahuki	2.72	Y	Y	Y	300	Anchovy
18 December 2018	MI11	NA	Adult	Mahuki	2.56	Y	Y	Y	255	Anchovy
18 December 2018	MI12	NA	Adult	Mahuki	2.34	Y	Y	Y	230	Squid
18 December 2018	MI13	NA	Adult	Mahuki	NR	Y	Y	Y	290	Anchovy, squid, Jack mackerel
18 December 2018	MI14	NA	Adult	Mahuki	2.61	Y	Y	Y	100	Squid
18 December 2018	MI15	NA	Adult	Mahuki	2.62	N	Y	Y	520	Jack mackerel
18 December 2018	MI16	NA	Adult	Mahuki	2.49	Y	Y	Y	200	Jack mackerel
18 December 2018	MI17	NA	Adult	Mahuki	2.27	Y	Y	Y	260	Saury
19 December 2018	MI18	NA	Adult	Mahuki	2.37	Y	Y	Y	140	anchovy, new sp
19 December 2018	MI19	NA	Adult	Mahuki	2.19	Y	Y	Y	230	Anchovy, squid
19 December 2018	MI20	NA	Adult	Mahuki	2.06	Y	Y	Y	545	Anchovy, squid
19 December 2018	MI21	NA	Adult	Mahuki	2.47	Y	Y	Y	200	Anchovy
5 January 2019	MI23	M85251	Adult	Mahuki	2.35	Y	Y	Y	53	squid
6 January 2019	MI24	M84264	Adult	Mahuki	2.27	Y	Y	Y	520	Jack mackerel

6 January 2019	MI25	M85265	Adult	Mahuki	2.21	Y	Y	Y	210	Anchovy
6 January 2019	MI26	M85266	Adult	Mahuki	2.47	Y	Y	Y	505	Anchovy
6 January 2019	MI27	M85267	Adult	Mahuki	2.42	Y	Y	Y	230	Anchovy, squid, eel
6 January 2019	MI28	M85268	Adult	Mahuki	NR	Y	Y	Y	120	Anchovy, squid, eel
6 January 2019	MI29	M85269	Adult	Mahuki	2.03	Y	Y	Y	175	Anchovy, squid, eel
6 January 2019	MI30	M85252	Adult	Mahuki	2.39	Y	Y	Y	390	Jack macakerel GPS bird
6 January 2019	MI31	M85270	Adult	Mahuki	2.59	Y	Y	Y	225	Well digested
6 January 2019	MI32	M85271	Adult	Mahuki	2.24	Y	Y	Y	225	Jack mackerel
6 January 2019	MI33	M85272	Adult	Mahuki	2.14	Y	Y	Y	150	Squid
6 January 2019	MI34	M85262	Adult	Mahuki	2.7	Y	Y	Y	250	Jack mackerel
6 January 2019	MI35	M85254	Adult	Mahuki	2.49	Y	Y	Y	130	Jack mackerel
6 January 2019	MI36	M85256	Adult	Mahuki	2.19	Y	Y	Y	220	Jack mackerel
7 January 2019	MI37	M85273	Adult	Mahuki	NR	Y	Y	Y	475	Jack mackerel, squid, fish head (discard)
7 January 2019	MI38	M85274	Adult	Mahuki	2.64	Y	Y	Y	380	Squid, saury
7 January 2019	MI39	M85275	Adult	Mahuki	2.4	Y	Y	Y	200	Anchovy
7 January 2019	MI40	M85276	Adult	Mahuki	2.31	Y	Y	Y	250	Jack mackerel
7 January 2019	MI41	M85277	Adult	Mahuki	2.22	Y	Y	Y	225	Anchovy
7 January 2019	MI42	M85278	Adult	Mahuki	2.57	Y	Y	Y	230	Anchovy
8 January 2019	MI43	M85279	Adult	Mahuki	2.16	Y	Y	Y	395	Squid, saury, jack mackerel
8 January 2019	MI44	M85280	Adult	Mahuki	2.68	Y	Y	Y	150	Jack mackerel
8 January 2019	MI45	M85281	Adult	Mahuki	1.9	Y	Y	Y	145	Squid
8 January 2019	MI46	M85282	Adult	Mahuki	2.52	Y	Y	Y	255	Jack mackerel
8 January 2019	MI47	M85283	Adult	Mahuki	2.57	Y	Y	Y	230	Jack mackerel
8 January 2019	MI48	M85284	Adult	Mahuki	2.6	Y	Y	Y	280	Anchovy, saury
10 January 2019	HH0TK05	NA	Adult	Horuhoru	NR	Y	Y	N	140	Jack mackerel, anchovy
10 January 2019	HH1	NA	Chick	Horuhoru	NR	N	N	N	195	Unidentif
10 January 2019	HH2	M85285	Adult	Horuhoru	2.25	Y	Y	N	145	Blue mackerel
10 January 2019	HH3	NA	Chick	Horuhoru	NR	N	N	N	205	Jack mackerel
10 January 2019	HH4	M85286	Adult	Horuhoru	2.35	Y	Y	N	250	Kahawai
10 January 2019	HH5TK08	M85302	Adult	Horuhoru	2.7	Y	Y	N	110	Jack mackerel, anchovy
10 January 2019	HH6TK15	M85294	Adult	Horuhoru	2.66	Y	Y	N	90	Unident
11 January 2019	HH10	M85288	Adult	Horuhoru	2.67	Y	Y	N		Unidentif
11 January 2019	HH11	M85289	Adult	Horuhoru	2.3	Y	Y	N	170	Jack mackerel
11 January 2019	HH12	M85290	Adult	Horuhoru	2.52	Y	Y	N	335	Anchovy, unident
11 January 2019	HH13	NA	Chick	Horuhoru	NR	N	N	N	170	Jack mackerel
11 January 2019	HH14	M85291	Adult	Horuhoru	2.45	Y	Y	N	230	Anchovy
11 January 2019	HH15	M85292	Adult	Horuhoru	2.21	Y	Y	N	420	Intact unident
11 January 2019	HH16	M85293	Adult	Horuhoru	2.24	Y	Y	N	275	Kahawai, squid,anchovy
12 January 2019	17HH	M89295	Adult	Horuhoru	2.52	Y	Y	N	600	Jack mackerel
12 January 2019	18HH	M85296	Adult	Horuhoru	2.45	Y	Y	N	80	Squid, pilchard (?)

12 January 2019	19HH	NA	Chick	Horuhoru	NR	N	N	N	345	Jack mackerel
12 January 2019	HH20	M85297	Adult	Horuhoru	2.62	Y	Y	N	295	Jack mackerel
12 January 2019	HH21	NA	Chick	Horuhoru	NR	N	N	N	250	Unidentif
12 January 2019	HH22	M85298	Adult	Horuhoru	2.36	Y	Y	N	360	Jack mackerel
12 January 2019	HH23	M85299	Adult	Horuhoru	NR	Y	Y	N	265	Jack mackerel
12 January 2019	HH24TK13	M85301	Adult	Horuhoru	2.43	Y	Y	N	40	Garfish (?)

Table 6. Regurgitate samples collected from red-billed gull colonies 2018-2019. R = samples collected at Phoenix Rocks, Tawharanui, MP for Marsden Point Oil Refinery.

Sample date	Sample label	Contents
14 November 2018	R1	Mostly fragments of shell – gastropod mollusc and possible crab. Intertidal not zooplankton. Seaweed fragments. Very little 'flesh'. Quite a few of the same looking shells which might be identifiable – crab? Few UnID legs – Arthropoda looking
14 November 2018	R2	Calcium carbonate looking white powdery lumps. Large pellet, firm, dense. Hard to pull apart. Majority plant matter. Small shell fragments. Large sand grains? 1 piece of red coral looking structure. Cant see any 'flesh'
14 November 2018	R3	Firm pellet. Majority plant matter. Some shell pieces. Similar to R2
14 November 2018	R4	Crumbly pellet. Sand/shell grains. UnID legs – Arthro looking. Small amounts of plant matter. Ca carbonate. No 'fleshy bits'
14 November 2018	R5	Firm pellet. Mixture of plant matter and sand/shell fragments (small). Small amounts of 'fleshy' matter. No crab legs seen
14 November 2018	R6	Small mass. Bit crumbly. Mostly plant matter. Some fleshy pieces. Some Arthropod looking shell pieces. Insect parts? Beetle carapace potentially. Pieces like exoskeleton segments. Head with large mandibles – millipede? Some legs
14 November 2018	R7	Small/med pellet. Mostly plant matter. Some large shell fragments. Some softer unID matter – flesh? Piece of bone/cartilage x 2. Some unID legs
18 January 2019	MP1	'Crispy' bits. Fibrous matter (fine feathers) with seeds (?) Occasional bit of shell.
18 January 2019	MP2	Firm pellet. Mostly fine feather forming fibrous matter – appear like string. Small bit of plastic. Shell fragments. Small pieces of foil – pie shell x 2. Some plant matter. Small bit of polystyrene?
18 January 2019	MP3	Firm, fibrous pellet. Clear cellophane/plastic. Fine feathers plus fur? Some plant matter. White plastic pieces. Small bits blue plastic.
18 January 2019	MP4	Crumbly pieces, white Ca carb? Quite a lot. Unable to distinguish anything, crumbling to dust. Red matter – algae?
18 January 2019	MP5	Predominately fish bones x 7 ~ 60 mm long. Multilegged organisms – no heads. Crab claw. Fish scales.
18 January 2019	MP6	Firm pellet. Fibrous. 1 x piece soft plastic. Green and clear glass pieces – sharp. Fine feathers. Shell/stone pieces. Wood? Hard opaque plastic.
18 January 2019	MP7	Large, firm, fibrous pellet. Paper – from bags - e.g. pie bags. Mostly feather. Some plant/algal matter, shell, pink plastic, stone/gravel
18 January 2019	MP8	Firm, fibrous pellet. Mostly feather (and/or fur?). Some glass, gravel/stone, plant, shell, plastic (partially digested?)

Table 7. Samples collected from white-fronted terns at Tiritiri Matangi in December 2018 and Horuhoru Rock in January 2019.

Date	Species	location	sample #	Faecal
11 January 2019	WFTE	Horuhoru Rock	HH01	Y
11 January 2019	WFTE	Horuhoru Rock	HH02	Y
11 January 2019	WFTE	Horuhoru Rock	HH03	Y
11 January 2019	WFTE	Horuhoru Rock	HH04	Y
11 January 2019	WFTE	Horuhoru Rock	HH05	Y
11 January 2019	WFTE	Horuhoru Rock	HH06	Y
11 January 2019	WFTE	Horuhoru Rock	HH07	Y
11 January 2019	WFTE	Horuhoru Rock	HH08	Y
11 January 2019	WFTE	Horuhoru Rock	HH09	Y
11 January 2019	WFTE	Horuhoru Rock	HH10	Y
11 January 2019	WFTE	Horuhoru Rock	HH11	Y
11 January 2019	WFTE	Horuhoru Rock	HH12	Y
11 January 2019	WFTE	Horuhoru Rock	HH13	Y
11 January 2019	WFTE	Horuhoru Rock	HH14	Y
12 January 2019	WFTE	Horuhoru Rock	HH15	Y
12 January 2019	WFTE	Horuhoru Rock	HH16	Y
12 January 2019	WFTE	Horuhoru Rock	HH17	Y
12 January 2019	WFTE	Horuhoru Rock	HH18	Y
13 January 2019	WFTE	Horuhoru Rock	HH19	Y
13 January 2019	WFTE	Horuhoru Rock	HH20	Y
13 January 2019	WFTE	Horuhoru Rock	HH21	Y
13 January 2019	WFTE	Horuhoru Rock	HH22	Y
13 January 2019	WFTE	Horuhoru Rock	HH23	Y
13 January 2019	WFTE	Horuhoru Rock	HH24	Y
13 January 2019	WFTE	Horuhoru Rock	HH25	Y
13 January 2019	WFTE	Horuhoru Rock	HH26	Y
13 January 2019	WFTE	Horuhoru Rock	HH27	Y
13 January 2019	WFTE	Horuhoru Rock	HH28	Y

APPENDIX 6

Identification of prey from photographs

Table 1. Prey caught by seabirds identified from photographs taken at sea

Date	Species	Location	Activity	Prey item	Photographer
23 April 2018	FFSH	Between Poor Knights and Marotere Chickens Islands	Several FFSAH fighting over a fish – no associated activity	Likely saury	Edin Whitehead
26 October 2018	WFTE	Tara Rocks, Marotere Chickens Islands	On fringes of trevally school	Likely krill	Edin Whitehead
26 October 2018	AUGA	Northwest of Marotere Chickens Islands	Common dolphins, FFSH and AUGA actively feeding	Squid	Edin Whitehead
3 January 2019	WFTE	Kawau Bay	LIPN, FLSH and WFTE feeding with kahawai	Possible pilchard	Karen Baird
3 January 2019	WFTE	Kawau Bay	LIPN, FLSH and WFTE feeding with kahawai	Krill or tongue of the bird?	Karen Baird
15 January 2019	AUGA AUGA	Off Coppermine Island, Marotere Chickens Islands	Caught near edge of kahawai school	Kahawai	Karen Baird
5 February 2019	AUGA	Approx. midway between Mokohinau and Marotere Chickens Islands	Common dolphins, FFSH and AUGA actively feeding	Saury	Edin Whitehead
5 February 2019	AUGA	Approx. midway between Mokohinau and Marotere Chickens Islands	Common dolphins, FFSH and AUGA actively feeding	Saury	Edin Whitehead
5 February 2019	GRNO	Maori Rocks, Mokohinau Islands	Mixed trevally and kahawai school	Larval fish	Edin Whitehead

Table 2. Prey caught by seabirds identified from photographs taken within colonies.

Date	Species	Location	Activity	Prey item	Photographer
11 January 2019	WFTE	Horuhoru Rock, Waiheke Group	Chick feeding	Anchovy	Edin Whitehead
11 January 2019	WFTE	Horuhoru Rock, Waiheke Group	Chick feeding	Anchovy	Edin Whitehead
28 January 2019	WFTE	Tokatu Point, Tawharanui	Display between adults/chick feeding	Juvenile squid - Sepioteuthis bilineata (S. australis)	Chris Gaskin
28 January 2019	WFTE	Tokatu Point, Tawharanui	Chick feeding	Anchovy	Chris Gaskin
28 January 2019	WFTE	Tokatu Point, Tawharanui	Chick feeding	Sardine	Chris Gaskin