Occurrence of prey species identified from remains in regurgitated pellets collected from king shags, 2019 - 2020

Final Report

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EXECUTIVE SUMMARY

This report encompasses the first component of a project to deduce diet of king shags from analysis of prey remains from 225 regurgitated pellets collected in Marlborough Sounds during 2019 and 2020. Here we quantified the frequency of occurrence of prey taxa for a future comparison with the outcome of DNA analysis on the same pellets by Aimee van der Reis and Andrew Jeffs (Institute of Marine Science, School of Biological Sciences, University of Auckland).

This study represents the second published investigation of king shag diet from analysis of prey remains in pellets. We increased the biodiversity of prey from the first study in 1991 and 1992 with 10 taxa (two crustaceans and eight fishes) from 22 pellets at one site to this study with 26 taxa (two crustaceans, two cephalopods and 22 fishes) from 215 pellets at seven sites. The basic understanding of foraging and diet remains unchanged—king shags target bottom-dwelling fishes and flatfishes, particularly witch (*Arnoglossus scapha*), predominate.

Frequencies of occurrence deduced from prey remains analysis and DNA analysis provide a simple qualitative assessment of king shag diet through the presence/absence of taxa in pellets. In future a more thorough analysis of the prey remains in these pellets would quantify diet as an average daily biomass for each prey species. The equivalent in DNA analysis is more qualitative: relative read abundance, an assessment of the strength of DNA signatures, generates estimates for proportion of total biomass. Comparisons between results from these two analyses could facilitate calculation of indices to transform relative read abundances into real masses.

The key issue for future projects on king shag diet is to decide on the purpose and desired outcome of research and then select the appropriate methods and analyses before samples are collected.

INTRODUCTION

New Zealand king shags (king shags, *Leucocarbo carunculatus*) are designated as Nationally Endangered under the New Zealand Threat Classification System (Robertson *et al*. 2017) because they have a small range and very small population. Their distribution is restricted to the sea and small islands bounded within a 55 km by 35 km rectangle within Marlborough Sounds at the northern tip of South Island, New Zealand (Schuckard *et al.* 2018) (Figure 1). This equates to an at least 75% contraction of their more widespread prehistoric distribution that extended to the southern North Island and the northeastern tip of South Island (Rawlence *et al*. 2017). Records stretching back to 1773 indicate that king shags have not been more widespread or more numerous through the historical past and the present population is considered stable (BirdLife International 2020). Monitoring of the species is ongoing with censuses in 2020 producing estimates of 815 individuals in February prior to the breeding season (Bell *et al.* 2020), followed by 277 nests at nine colonies in May (Schuckard & Frost 2020) (Figure 1). King shags are exclusively marine foragers and fly an average of 6–10 km (maximum 24 km) from colonies or roosts (Schuckard 1994, 2006) to forage alone in depths of 20–60 m (Brown 2001).

Shags (Phalacrocoracidae) typically regurgitate daily a pellet containing prey remains that can provide a quantitative assessment of diet composition and daily intake—among the variety of methods to investigate diet, pellets provide the most comprehensive information for the lowest sampling effort and least disturbance (Seefelt & Gillingham 2006, Barrett *et al.* 2007, Oehm *et al.* 2016). However, pellet analysis suffers from biases due to the underestimation of prey that are digested completely and overestimation of prey with resilient remains. These biases potentially can be overcome by analysis of the DNA digested prey, a relatively new technique first applied in 2005 (Barrett *et al.* 2007). However, in common with analysis of prey remains, frequency of occurrence deduced from DNA analysis overestimates the importance of prey species taken frequently but only in small amounts (Deagle *et al.* 2019). This bias is rectified in prey remains analyses by assessing composition of the diet from masses (estimated original masses) of prey species. The equivalent to prey species mass in DNA analysis is relative read abundance, an assessment of the relative strength of species-specific DNA signatures (Deagle *et al.* 2019).

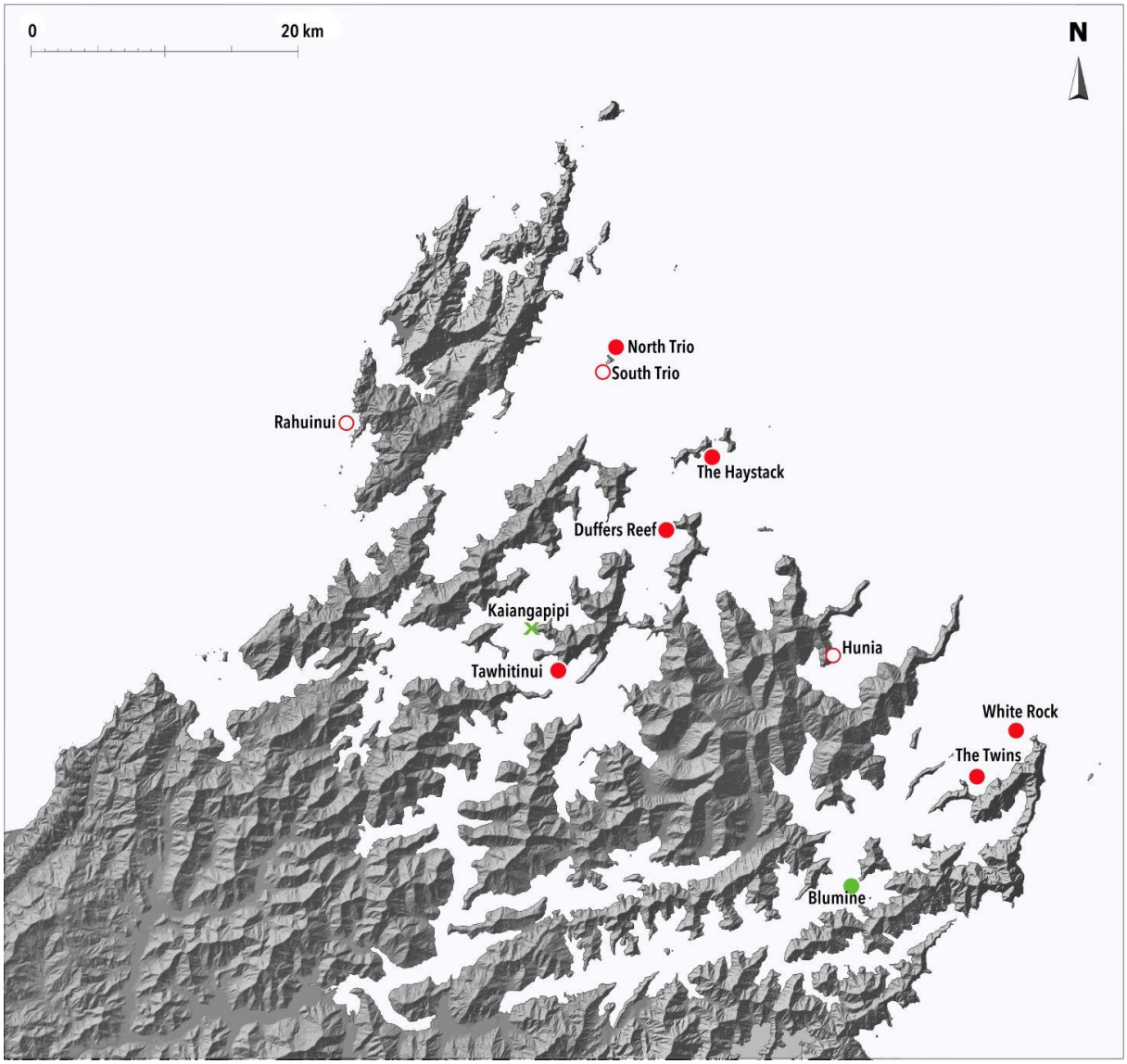

Figure 1: Map of Marlborough Sounds showing the nine breeding colonies (red circles) and one major roost site (green circle) of New Zealand king shags in 2019 and 2020. Pellets were collected at six colonies (solid red circles) and the one major roost site. The depicted roost site (Blumine) was the only site to average ≥10 individuals in a survey on 21 February 2020 by Bell *et al*. (2020) that was not recorded as a breeding colony in 2019 and/or 2020 by Schuckard & Frost (2020). Lalas & Brown (1998) collected pellets at Te Kaiangapipi (green cross), a currently unoccupied roost site.

The purpose of this report is to present frequency of occurrence of remains of prey species in king shag pellets for comparison with DNA analysis of the same pellets as presented by van der Reis and Jeffs (2020). The outcome of this comparison will compare efficacies of these two analyses and highlight similarities, differences and anomalies. Prey remains analysis of pellets can provide a quantitative estimate for daily intake as a total biomass of prey items. The equivalent in DNA analysis is more qualitative: relative read abundance, an assessment of the strength of DNA signatures, generates estimates for proportion of total biomass. Comparisons between results from these two analyses could facilitate calculation of indices to transform relative read abundances into real masses. This outcome is an unexpected bonus that could make a major contribution to the methodology for quantification of DNA analysis. In particular, pellets with only one taxon identified among prey remains could provide the clearest outcomes.

The only previous assessment of the diet of King Shags has been for birds based at Te Kaiangapipi, a roost site within Pelorus Sound (Figure 1). Here prey remains found in 22 pellets collected in 1991 and 1992 contained 10 taxa, all bottom-dwelling species dominated by witch (*Arnoglossus scapha*), a flatfish (Lalas & Brown 1998). Our present study encompasses over 10 times the number of pellets from seven sites and will lead to insight into spatial differences in diet of king shags.

METHODS

Source and analyses of pellets

King shag regurgitated pellets were collected by Mike Bell (Wildlife Management International Ltd) and Dan Palmer (Department of Conservation) from seven sites during monitoring of the species in Marlborough Sounds. Each site was sampled on one or two occasions from March 2019 to March 2020. Pellets were individually coded, stored in alcohol and sent to Andrew Jeffs and Aimee van der Reis (Institute of Marine Science, School of Biological Sciences, University of Auckland) who undertook DNA analyses. Partially sorted pellets stored in alcohol were then forwarded to us (Lalas & Schuckard) with the contents of each pellet subdivided into 2–4 pottles. We sorted and analysed prey remains found in pellets in October 2020. Details for the location, date of collection and contents of each pellet are given in the Appendix Excel file (can be requested from csp@doc.govt.nz).

Identification of prey species from prey remains

We teased apart pellets in water and retained all diagnostic prey remains identified from a comprehensive reference collection held by Chris Lalas. Diagnostic remains differ among taxa: jaws from polychaete worms; pieces of exoskeleton (especially claws and carapaces; often de-calcified) from crustaceans; beaks from cephalopods; tunic (gelatinous tube) from salps; mouthparts from hagfish and lampreys; teeth, body thorns, dorsal spine sheaths and (cartilaginous) vertebrae from sharks and rays; and otoliths (sagittal otoliths), jaws and other dentition, caudal vertebrae and some other species-specific bones for teleost fishes. Otoliths for all the genera and most of the teleosts we encountered are illustrated in Schwarzhans (1984, 1999); Smale *et al.* (1995); or Furlani *et al*. (2007). Jaws and some other fish bones we encountered are illustrated in Leach (1997). For the taxonomy and nomenclature (common and scientific names) of New Zealand fishes we follow Roberts *et al.* (2015).

Analyses of prey remains in taxa

We restricted quantified analysis of prey remains in pellets to the occurrence (presence or absence) of each prey taxon. Our precision of presentation of taxon reflected our assessment of reliability in identification of remains: typically to species, but to genus and on occasion family in cases where multiple closely-related species occur at Marlborough Sounds. We quantified frequency of occurrence for each prey taxon as the number (FOO) and proportion (%FOO) of pellets for each site that contained that taxon. We did not attempt to differentiate between primary prey items (items caught by king shags) and secondary prey items (items originating from the digestive tract of primary prey items).

Here we present results for frequency of occurrence for each site and for totals from all seven sites. Accurate identification of remains required careful inspection of each sample. Consequently, we capitalised on this effort by estimating the minimum number of prey items per taxon. For fishes we typically did this by halving the number of otoliths. These data were entered into spreadsheets and used to indicate occurrence in pellets (Appendix).

Fate of prey remains

We dried all diagnostic prey remains and retained them for future further analysis. Each pellet was allocated an A4 ziplock bag containing handwritten details on A4 paper and remains retained in labelled, species-specific, small ziplock bags.

RESULTS

Number of pellets analysed

Analyses were derived from a total of 225 king shag pellets collected from seven colonies during 2019 and 2020 (Table 1). We analysed 215 (96%) of the total 225 pellets. The other 10 pellets were not analysed for a number of reasons and another seven pellets contained anomalies; details about these 17 pellets are presented in the Appendix. Both types of diet analyses—prey remains and DNA—were performed on 184 (82%) of the total pellets, and represent a large sample size for future comparisons between outcomes of the two methods. These are huge sample sizes relative to the size of the species total population, equivalent to one pellet for every four individuals (where 215/815 = 26% and 184/815 = 23%), and near 10-fold the sample size of 22 pellets in the previous analysis of king shag diet by Lalas & Brown (1998).

A problem with alcohol denaturing otoliths

We encountered two problems attributable to storage of otoliths in alcohol. First, otoliths were dehydrated. Otoliths typically became opaque and lost their internal detail meaning that the internal growth rings and earlier shape of otoliths were often difficult to see. This loss of clarity could compromise the accuracy of species identification, especially for eroded otoliths. Second, otoliths were difficult to clean because grime often adhered to the surface – this could mask the surface and compromise judgements of the degree of erosion of otoliths (important when deriving estimates for prey size) and sometimes species identifications.

Table 1: Summary of results for the number of king shag pellets collected from seven sites in 2019 and 2020, and for their contents deduced from analysis of prey remains. Three sites were in Queen Charlotte Sound (Charlotte), three in Pelorus Sound (Pelorus) and one in Admiralty Bay (Admiralty). Sites are depicted in Figure 1. Number of nests are from Schuckard & Frost (2020) and number of individuals from Bell *et al*. (2020).

Criterion	Total	White Rocks	The Twins	Blumine	The Haystack	Duffers Reef	Tawhitinui	North Trio
Features of sites in 2020 (see Figure 1):								
Location	-	Charlotte	Charlotte	Charlotte	Pelorus	Pelorus	Pelorus	Admiralty
Number of nests (% total 277)	213 (77%)	24 (9%)	13 (5%)	0 (0%)	20 (7%)	83 (30%)	29 (10%)	44 (16%)
Number of individuals (% total 815)	564 (69%)	46 (6%)	43 (5%)	55 (7%)	16 (2%)	207(25%)	78 (10%)	119 (15%)
Number of pellets:								
Collected	225	23	24	28	10	51	42	47
Analysed for prey remains	215	22	22	28	10	46	42	45
Analysed for DNA	183	23	23	28	10	33	33	33
Analysed for DNA & remains	180	22	22	28	10	33	33	32
Mean (range) per pellet:								
Number of prey taxa	3.2 (1-9)	2.8 (1-9)	3.0 (1-7)	2.5 (1-6)	3.4 (1-7)	3.5 (1-7)	3.6 (1-6)	3.0 (1-7)
Minimum number of prey items	19 (2-114)	15 (4-36)	19 (4-64)	15 (4-48)	17 (5-66)	18 (3-69)	25 (2-114)	20 (5-57)
Pellets with particular taxa FOO% (proportion of pellets):								
Contain witch (*Arnoglossus scapha*)	77%	95%	68%	93%	50%	70%	69%	84%
Contain flatfishes (Pleuronectiformes)	86%	95%	82%	93%	60%	80%	88%	89%
Contain only flatfishes	20%	14%	18%	43%	10%	11%	12%	29%
Do not contain any flatfishes	14%	5%	18%	7%	40%	20%	12%	11%
Pellets with only one taxon FOO (number of pellets):								
Witch (*Arnoglossus scapha*)	20	3	2	5	0	1	3	6
Leatherjacket (*Meuschenia scaber*)	4	0	2	0	1	0	0	1
Gurnard (Triglidae)	1	0	0	0	0	0	0	1
Wrasse (Labridae)	1	0	0	0	0	1	0	0
Total with only one taxon	26	3	4	5	1	2	3	8

We need to emphasise that analysis of compromised otoliths had two detrimental impacts on this project. First, the duration required to deduce frequency of occurrence by about a third from an average of about one hour to about 1 h 20 min. Second, the reliability of some species identifications was compromised.

Prey taxa represented in prey remains

Analysis of prey remains from 215 pellets generated averages of 3.2 (range 1–9) prey taxa and a minimum 19 (range 2–114) prey items per pellet (Table 1). Here we further consider the frequency of occurrence of prey taxa. Minima for number of prey items per taxon for each pellet are presented in the Appendix.

Witch, a lefteye flounder (Bothidae), was the most frequently-encountered prey species; recorded in 77% of the total 215 pellets, with a range of 50–95% among the seven sites (Table 1). Following witch in frequency were three genera of southern righteye flounders (Rhombosoleidae): lemon sole (*Pelotretis*), sole (*Peltorhamphus*) and flounder (*Rhombosolea*). The predominance of flatfishes is exemplified by the paucity of pellets that did not contain any flatfishes: no flatfish were found in only 14% of the total 215 pellets, with a range of 5–40% among the seven sites (Table 1). A total of 26 taxa (two crustaceans, two cephalopods and 22 fishes) were recorded from the 215 pellets (Table 2). Other than flatfishes, five taxa can be regarded as important prey (recorded in ≥ 10% of the 215 pellets): jock stewart (*Heliocolenus percoides*), gurnard (Triglidae), opalfish (*Hemerocoetes* cf. *monopterygius*), triplefin (Tripterygiidae) and leatherjacket (*Meuschenia scaber*) (Table 2).

Twenty-six (12%) of the 215 pellets contained only one taxon: 20 with only witch; four with only leatherjacket; and one each with only red gurnard or wrasse (Labridae) (Table 1).

For wrasse, the entry "*Notolabrus* & *Pseudolabrus* 4 spp" in Table 2 represented a minimum of 29 fish of which 16 were tentatively identified to species: two spotty (*N. celidotus*), seven girdled wrasse (*N. cinctus*), one banded wrasse (*N. fucicola*) and six scarlet wrasse (*P. miles*). For gurnard, the entry "Triglidae 1-3 spp." In Table 2 represented a minimum of 108 fish, all identified from eroded otoliths. The only specific identifications were for three scaly gurnard (*Lepidotrigla brachyoptera*) identified by their distinctive parasphenoid (the bone that forms the midline base of skull).

Table 2: Frequency of occurrence of prey taxa deduced from analysis of prey remains found in king shag pellets collected from seven sites in 2019 and 2020. Results for occurrence of each taxon are presented in two formats: first, occurrence as the number (FOO) and proportion (%FOO) of the total 215 pellets; and second, occurrence as the number of sites (1–7) and the range in %FOO among sites.

Prey taxon recorded from prey remains (taxonomic listing)		Total 7 sites		Range among sites	
		FOO	%FOO	Number	%FOO
Witch	*Arnoglossus scapha*	166	77.2%	7	50–95%
Opalfish	*Hemerocoetes* cf. *monopterygius*	82	38.1%	7	10–64%
Lemon sole	*Pelotretis flavilatus*	60	27.9%	6	0–41%
Triplefin	Tripterygiidae spp.	55	25.6%	7	5–41%
Leatherjacket	*Meuschenia scaber*	54	25.1%	6	0–35%
Jock stewart	*Heliocolenus percoides*	47	21.9%	7	7–36%
Flounder	*Rhombosolea* 1-3 spp.	47	21.9%	6	0–38%
Sole	*Peltorhamphus* 1-3 spp.	45	20.9%	7	10–28%
Gurnard	Triglidae 1-3 spp.	37	17.2%	7	10–32%
Wrasses	*Notolabrus* & *Pseudolabrus* 4 spp.	20	9.3%	6	0–19%
Midget octopus	*Octopus* cf. *huttoni*	12	5.6%	6	0–30%
cf. Red cod	*Pseudophycis* 1-3 spp.	12	5.6%	6	0–20%
Red swimming crab	*Nectocarcinus antarcticus*	8	3.7%	5	0–10%
Butterfly perch	*Caesioperca lepidoptera*	8	3.7%	6	0–11%
Red scorpionfish	*Scorpaena papillosa*	7	3.3%	3	0–40%
Blue cod	*Parapercis colias*	5	2.3%	3	0–10%
Pillbox crab	cf. *Halicarcinus* sp.	4	1.9%	3	0–5%
cf. Twosaddle rattail	cf. *Coelorinchus biclinozonalis*	2	0.9%	2	0–5%
Arrow squid	*Nototodarus gouldi*	1	0.5%	1	0–5%
Silver conger	*Gnathophis habenatus*	1	0.5%	1	0–4%
Ling	*Genypterus blacodes*	1	0.5%	1	0–2%
Rock cod	*Lotella rhacina*	1	0.5%	1	0–2%
Dwarf cod	*Notophycis marginata*	1	0.5%	1	0–2%
Common roughy	*Paratrachichthys trailli*	1	0.5%	1	0–5%
Greenbone	*Odax pullus*	1	0.5%	1	0–5%
Stargazer	Leptoscopidae sp.	1	0.5%	1	0–2%

We did not record any gelatinous organisms (salps or jellyfish), polychaete worms or cartilaginous fishes. We did record two non-prey crustacean taxa likely to appear in DNA analyses: parasitic isopods (Cymothoidae, 6 pellets) and hermit crabs (Paguridae, 18 pellets). We also recorded trace remains of molluscs that are unlikely to appear in DNA, gastropod and bivalve shells (typically broken pieces), gastropod opercula; and chiton valves.

Fishes underrepresented in prey remains

Tiny otoliths (< 1 mm) are unlikely to survive digestion and appear as prey remains in pellets. Fish will be underrepresented in pellets if they not only have tiny otoliths but also lack resilient diagnostic bones and teeth. Two families of fishes fulfil these criteria as potential prey of king shags: pipefishes and seahorses (Syngnathidae); and tommyfishes (Creediidae), a close relative of opalfishes (Percophidae). In contrast, another two families have tiny otoliths but resilient diagnostic bones and so are likely to be represented realistically in pellets: dories (Zeidae) have distinctive resilient articular and maxilla (jaw bones, not recorded in pellets); articularbellowsfishes (Macroramphosidae) have a resilient dorsal spine (none recorded in pellets): and leatherjackets (Monacanthidae) have a resilient dorsal spine and resilient enamel teeth (regularly recorded in pellets).

DISCUSSION

This is the second published investigation of king shag diet from analysis of prey remains in pellets. We increased the number of reported prey taxa from 10 (two crustaceans and eight fishes) from 22 pellets (Lalas & Brown 1998) to 26 (two crustaceans, two cephalopods and 22 fishes) from 215 pellets.

We confined output to the frequency of occurrence of prey taxa because the allocated time and funding of our contracts were grossly inadequate. For methods we have given the example that the use of alcohol to ensure high quality samples for DNA analysis unfortunately downgraded their quality for analysis of prey remains—this problem could have been resolved at the planning stage of the project. Potential outcomes of prey remain analysis and DNA analysis are compared in Table 3.

Table 3: Expected outcomes of king shag diet studies derived from regurgitated pellets: comparison between prey remains analysis and prey DNA analysis.

Outcome	Prey remains analysis	DNA analysis
Definitive differentiation between primary and secondary prey	No	No
Detection of prey species that lack robust remains	Inconsistent	Yes
Number of prey species per pellet	Yes	Yes
Number of prey items per pellet & proportion of diet by number	Yes	No
Lengths of prey items	Yes	No
Biomass of prey items	Yes	No
Proportion of diet by biomass of prey species per pellet	Yes	RRA
Total biomass of all prey per pellet = daily intake	Yes	Not yet

Biomass = original mass of prey.
RRA = relative read abundance, an indicator of the relative importance of species.

RECOMMENDATIONS

1. The next step for frequency of occurrence of prey taxa is to compare outcomes between prey remains analysis and DNA analysis for the same 180 pellets. We are particularly keen to fully analyse (following Table 3) the 26 pellets that contained only one prey taxon. This would facilitate calculations to quantify relative read abundances into real masses and broaden the applicability of DNA analysis as a tool to deduce diet.
2. The key issue for future projects on king shag diet is to decide on the purpose and desired outcome of research and then select the appropriate analyses before samples are collected (Table 3).
3. Statistical advice is critical to deduce the minimum valid number of pellets required to satisfy intended diet analyses; e.g., the extreme range in the present project was for North Trio with 45 pellets (too many pellets) collected on 29 November 2019 and six pellets (too few pellets) collected on 11 March 2020 (Appendix).
4. The timing and sites of pellet collections need to be selected to satisfy intentions; e.g., collections must be six months apart for a study to detect seasonal differences from two samples.

5. Any future work involving DNA analysis and prey remains analysis on the same pellets must resolve the problem of degradation of otoliths stored in alcohol.

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REFERENCES

Barrett RT, Camphuysen CJ, Anker-Nilssen T, Chardine JW, Furness RW, Garthe S, Hüppop O, Leopold MF, Montevecchi WA Veit RR. 2007. Diet studies of seabirds: a review and recommendations. *ICES Journal of Marine Science* 64: 1675–1691.

BirdLife International. 2020. IUCN Red List for birds. Species factsheet: *Leucocarbo carunculatus*. Downloaded from http://www.birdlife.org on 06 November 2020.

Bell M. 2019. King Shag research project: Year One update report. Unpublished Wildlife Management International Technical Report to the Marine Farming Association and Seafood Innovations Limited.

Bell M, Frost PG, Melville DS. 2020. Population assessment during the non-breeding season of King Shag in the Marlborough Sounds, February 2020. Unpublished Technical Report to New Zealand King Salmon.

Brown D. 2001. Dive duration and some diving rhythms of the New Zealand king shag (*Leucocarbo carunculatus*). *Notornis* 48: 177–178.

Deagle BE, Thomas AC, McInnes JC, Clarke LJ, Vesterinen EJ, Clare EL, Kartzinel TR, Eveson JP. 2019. Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology* 28: 391–406.

Furlani D, Gales R, Pemberton D. 2007. *Otoliths of common Australian temperate fish*. CSIRO Publishing, Collingwood, Australia.

Lalas C, Brown D. 1998. The diet of New Zealand king shags (*Leucocarbo carunculatus*) in Pelorus Sound. *Notornis* 45: 129–139.

Leach F. 1997. *A guide to the identification of fish remains from New Zealand archaeological sites*. New Zealand Journal of Archaeology Special Publication, Wellington.

Oehm J, Thalinger B, Eisenkölbl S, Traugott M. 2017. Diet analysis in piscivorous birds: What can the addition of molecular tools offer? *Ecology and Evolution* 7: 1984–1995.

Rawlence NJ, Till CE, Easton LJ, Spencer HG, Schuckard R, Melville DS, Scofield RP, Tennyson AJD, Rayner MJ, Waters JM, Kennedy M. 2017. Speciation, range contraction and extinction in the endemic New Zealand King Shag complex. *Molecular Phylogenetics and Evolution* 115: 197–209.

Roberts CD, Stewart AL, Struthers CD. Editors. 2015. *The fishes of New Zealand*. In 4 volumes. Te Papa Press, Wellington.

Robertson HA, Baird K, Dowding JE, Elliott GP, Hitchmough RA, Miskelly CM, McArthur N, O'Donnell CFJ, Sagar PM, R. Scofield P, Taylor GA. 2017. Conservation status of New Zealand birds, 2016. New Zealand Threat Classification Series 19. Department of Conservation, Wellington.

Schuckard R. 1994. New Zealand Shag (*Leucocarbo carunculatus*) on Duffers Reef, Marlborough Sounds. *Notornis* 41: 93–108.

Schuckard R. 2006. Distribution of New Zealand king shags (*Leucocarbo carunculatus*) foraging from the Trio Is and Stewart I colonies, Marlborough Sounds, New Zealand. *Notornis* 53: 291–296.

Schuckard R, Bell M, Frost P, Taylor G, Greene T. 2018. A census of nesting pairs of the endemic New Zealand king shag (*Leucocarbo carunculatus*) in 2016 and 2017. *Notornis* 65: 59–66.

Schuckard R, Frost P. 2020. Assessment of the breeding population of New Zealand King Shag, *Leucocarbo carunculatus*, in the Marlborough Sounds, May–June 2020. Client Report Department of Conservation.

Schwarzhans W. 1984. Fish otoliths from the New Zealand Tertiary. *New Zealand Geological Survey Report* 113: 1–269.

Schwarzhans W. 1999. *A comparative morphological treatise of recent and fossil otoliths of the order Pleuronectiformes*. Verlag Dr. Friedrich Pfeil, München, Germany.

Seefelt NE, Gillingham JC. 2006. A comparison of three methods to investigate the diet of breeding double-crested cormorants (*Phalacrocorax auratus*) in the Beaver Archipelago, northern Lake Michigan. *Hydrobiologia* 567: 57–67.

Smale MJ, Watson G, Hecht T. 1995. Otolith atlas of southern African marine fishes. *Ichthyological Monographs* 1: 1–253.

van der Reis A, Jeffs, A. 2020. Determining the diet of New Zealand king shag using DNA metabarcoding. Report for Department of Conservation.

APPENDIX

Frequency of occurrence and minimum number of prey items per taxon for prey identified from prey remains in king shag pellets are presented for each location in an attached Excel Workbook. This can be requested from csp@doc.govt.nz.