Identification of predators at black-fronted tern *Chlidonias albostriatus* nests, using mtDNA analysis and digital video recorders

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Abstract: Predators at black-fronted tern (*Chlidonias albostriatus)* nests on the Wairau braided riverbed in Marlborough, New Zealand, were identified using (1) mtDNA analysis of 438 swabs from shell remains, nest contents, and carcass remains, and (2) digital video surveillance of 85 nests. DNA analysis suggested harriers (*Circus approximans*) were the main predator of tern eggs (171 of 192 shell samples containing predator DNA). Cats (*Felis catus*) and stoats (*Mustela erminea)* were the probable predators of the majority of adult terns killed (9 and 8 respectively, of swabs from 19 carcasses). Video results were broadly, though not entirely, consistent with the DNA results, and showed that harriers were the main predator of eggs (9 of 19 videoed predation events), followed by Southern black-backed gulls (*Larus dominicanus dominicanus;* 3/19); hedgehogs (*Erinaceus europaeus occidentalis;* 2/19*)*, ship rats (*Rattus rattus;* 2/19*),* pied oystercatchers (*Haematopus finschi;* 2/19*)* and stoats (1/19). DNA was analysed from nine of the 19 videoed nests but the only predator DNA obtained was from harriers (four nests). Sixty-four percent of depredated nests (683/1063) contained no eggshell remains at the next monitoring visit after predation. DNA analysis of nest material from 71 of these empty nests yielded only one predator result; video footage was therefore essential to identify the cause of 12 empty nests at 19 videoed nest predations. Terns removed the depredated egg remains from eight nests; black-backed gulls consumed eggs at three nests; and a stoat carried the eggs away from one nest. Hedgehog DNA was not found on shell remains from nests with videoed hedgehog predations. Analysing DNA from eggshell and carcass remains is a valuable new tool in wildlife research and management because it can identify predator species and indicate their relative importance. However, our results show that predator species are not equally detectable using this technique, leading to biases in the DNA results. This 'detectability bias' needs to be further quantified, and recognised when interpreting DNA results.

Keywords: New Zealand; predator saliva; Wairau River

Introduction

Introduced mammalian predators are the major cause of the continuing decline and range restrictions of native birds in New Zealand's remaining large forest tracts (Innes et al. 2010), and a major cause of declines in shorebird abundance and range in braided river systems (Dowding & Murphy 2001; Keedwell et al. 2002; Keedwell 2005; Murphy et al. 2004; Sanders & Maloney 2002). Avian predators, such as harriers (*Circus approximans*), Australian magpies (*Gymnorhina tibicen*), and Southern black-backed gulls (*Larus dominicanus dominicanus*), have also been recorded taking chicks and/or eggs of braided river birds (Guthrie-Smith 1936; Sanders & Maloney 2002; R. McClellan, Wildlands Consultants, Christchurch, New Zealand, pers. comm.). Predator management is needed to halt this decline (Innes et al. 2010). However, for predator control to be effective in terms of outcomes and resources, the relative impacts of different predators need to be quantified and appropriate control methods implemented.

The identity of predators that have preyed on eggs, chicks and adults at nests of various bird species has been inferred using various methods. These include carcass lesions (Keedwell et al. 2002), sign at nests (Moors 1983), teeth/bill marks on artificial eggs (Boulton & Cassey 2006), hair left on adhesive tape (Major 1991), and still and video photography (Brown et al. 1998; Sanders & Maloney 2002; Williams & Wood 2002; Stake et al. 2004). Video recordings provide the most definitive evidence of predator species identity and other causes of mortality. While video investigations in the field have previously been expensive and labour-intensive (Brown et al. 1998), digital camera technology has greatly reduced costs by extending recording time and reducing power requirements (Reif & Tornberg 2006; Parker et al. 2008). Observer effects, caused by cameras at nests, on predator and prey species are a potential danger with this method. However, such effects have been noted in some studies (Cutler & Swann 1999) but not others (Sanders & Maloney 2002).

A relatively recent development is the use of forensic analysis to identify predators. For example, predation wounds can be analysed for the presence of DNA from the predator (saliva) to determine predator species (Williams et al. 2003). Sundqvist et al. (2008) obtained DNA from saliva left close to the bite wounds on sheep *(Ovis aries)* and showed that all incidents could be attributed to a single dog *(Canis familiaris)*. Onorato et al. (2006) used another technique, mitochondrial DNA (mtDNA) analysis of hair and scat samples, to determine

the identity of the predator species at elk (*Cervus elaphus*) mortality sites.

In this study, we used digital video recorders and DNA analysis of samples from eggshells, nest contents, and carcasses of chicks and adults of the endangered black-fronted tern (*Chlidonias albostriatus*) (IUCN 2008; Miskelly et al. 2008) to determine the relative impact of different predators at tern nests on the Wairau riverbed, Marlborough, New Zealand.

Methods

Study site and timing of the study

This study was carried out on the Wairau riverbed (Fig. 1), between St Ronans Stream (NZTopo50-BS25 924E 576N) and the Tuamarina Bridge (NZTopo50-BQ28 806E 119N), in conjunction with a larger study of black-fronted tern nesting success (to be reported elsewhere). In the larger study, 2275 tern nests at 80 colonies were monitored by regularly visiting them during the nesting season (October–January) over three consecutive years, 2007–2009.

A subset of these nests and colonies were used for the present study. Colonies were widely distributed along the Wairau River with the camera units distributed among 1–5 tern colonies at any one time. Of the 17 colonies where video units were set, four were 'mainland/peninsula' colonies (i.e. colonies not entirely surrounded by water), and the remaining 13 colonies were located on islands. DNA samples were collected from 438 nests between 2007 and 2009, and 85 nests were videoed in 2008 and 2009 (Table 1). Nine of these nests were both videoed and sampled for DNA.

Black-fronted terns usually lay two eggs, 40 mm long, which hatch after approximately 24 days (Heather & Robertson 1996; Keedwell 2005). Nests were monitored following the methods of Keedwell (2005). In summary, nests were marked with a small rock cairn 1–2 m upstream from the nest and the position recorded with a GPS unit. Colonies were visited usually every 2–4 days to check on progress of nests and to mark any new ones. Nests were monitored until eggs hatched or the nest had failed. All nests within a colony were monitored.

DNA sampling

DNA samples were collected from three sources: eggshell fragments left at the nest; adult/chick carcasses; and nest material from nests in which eggs or eggshell had been completely removed during or after predation. DNA was sampled from eggshell fragments and tern carcasses by swabbing the shell surface or in and around wounds, respectively, with a dry cotton bud. Nest material was sampled by thoroughly swabbing the nest material and the surrounding rocks surfaces. However, obtaining predator DNA from nest material proved problematic and this method was abandoned in the final year of the study.

Table 1. Numbers of DNA samples collected, nests videoed, and videoed nests where predation occurred and DNA samples were available on the Wairau riverbed.

Year	DNA samples	Videoed nests	Videoed predation events coupled with DNA analysis
2007	219	-	-
2008	167	50	7
2009	52	35	2
Total	438	85	9

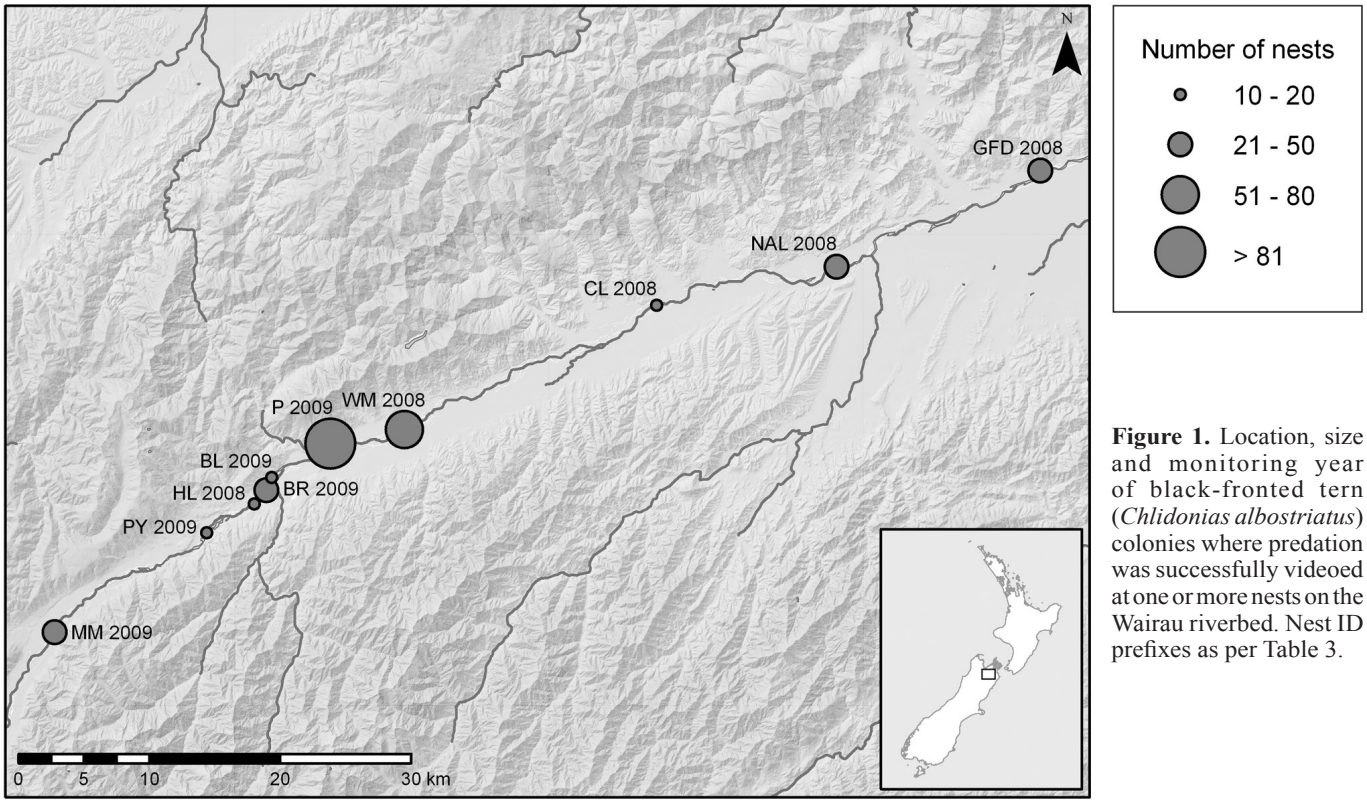

Figure 1. Location, size and monitoring year of black-fronted tern (*Chlidonias albostriatus*) colonies where predation was successfully videoed at one or more nests on the Wairau riverbed. Nest ID prefixes as per Table 3.

DNA samples were generally collected within 4 days and in the case of videoed nests within 48 h of predation. In the field we used fresh gloves for each sample to avoid cross-contamination. In the first year of the study cotton-bud swabs were air-dried at room temperature at the field base before being stored at 4°C in plastic bags. DNA detection overall was improved in the second and third years by: (1) collecting eggshell remains (using sterile forceps) into plastic bags and sending them to be swabbed in the laboratory rather than swabbing them in the field; (2) attempting to visit nests more frequently (i.e. within 3 days) to minimise the time between predation and DNA analysis; and (3) immediately placing swabs into plastic bags with filter paper (to absorb moisture) for storage at 4°C at the field base, rather than air-drying first. These refinements resulted in a reduction in the number of samples from which no DNA was obtained, from 84 in 2007 to 11 in 2008 and none in 2009.

In the laboratory, cotton buds were dipped in Tissue Digest (DXT) (Qiagen) prior to swabbing over eggshell fragments. These were incubated at 56°C overnight in 420 µl of Tissue Digest (DXT) and 4.2 µl of DX Digest enzyme. DNA was extracted using the Corbett X-tractor Gene (Qiagen) automated standard swab protocol, following the manufacturer's instructions. DNA was then eluted in 50 µl of elution buffer.

Polymerase Chain Reaction (PCR) was undertaken using the universal mtDNA primers CB-J-10612 and CB-N-10920 targeting a highly conserved region of the cytochrome b gene (*CYTB*) common across a wide range of vertebrates (Kocher et al. 1989). PCR amplifications were performed on a GeneAmp 9700 thermocycler (Applied Biosystems) in 25-µl reactions containing 5 µl of DNA extract, 2.5 µl of FastStartTaq DNA Polymerase PCR Buffer with $MgCl_2$, 2.5 µl dNTPs (2 mM), 1 µl of each primer (10 pm ul^{-1}), 1µl of BSA (10 mg ml^{-1}) and 1.5U of FastStartTaq DNA Polymerase (Roche Diagnostics). Cycles were as follows: 95°C for 4 min; 40 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 1 min; 72°C for 10 min. Amplification products were visualised under UV using ethidium-bromide-stained agarose gels. To avoid DNA contamination, genomic mtDNA and PCR products were kept separate and negative controls were used extensively.

Direct sequencing of purified products was carried out with BigDye™ Terminator Version 3.1 (Applied Biosystems) following the manufacturer's protocol. Sequences were analysed on an Applied Biosystems 3130xl genetic analyser using DNA Sequencing Analysis Software Version 5.3.1 (Applied Biosystems).

DNA sequences were compared and edited manually using the programme Sequencher 4.6 (Gene Codes). Sequence results usually consisted of mixed profiles that had to be separated out in order to determine whether any potential predator DNA was present. The previously known 'host' mtDNA *CYTB* sequence, which is available on GenBank, was first subtracted from the mixed profile. If a mixed profile was still evident, then another likely source of DNA contamination, human mtDNA *CYTB* sequence, was removed. When a single profile was obtained that was neither the 'host' nor human mtDNA *CYTB* sequence, the BLAST (Basic Local Alignment Search Tool) algorithm was used to search for the most closely matched sequences within the National Center for Biotechnology Information (NCBI) database, GenBank.

Video study

The video protocols we used were similar to those used by Sanders and Maloney (2002) except that the use of digital storage media obviated the need for daily visits to change tapes and batteries (although frequent equipment failure meant that the units in the field were often checked daily). Sanders and Maloney (2002) found no evidence that videoing at black-fronted tern nests influenced nest fate or predator behaviour (but see Cutler & Swann 1999).

Nests were videoed from October to December in 2008 and 2009 using black and white, IR-sensitive video cameras mounted on tripods (0.3 – 0.5 m high), 1–1.5 m from the nest. A cable ran (2–5 m) from the camera to a time-lapse digital 'security' video recorder QV3094. The recorder was also connected to one 17.2-Ah 12-V battery. Two- to four-gigabyte (GB) SD cards were used to record footage. Up to nine camera units were in use at any one time.

Digital video recorders were operated on time-lapse mode, recording two frames per second, medium-quality-footage video of size 352 × 280. Audio was off. At these settings, approximately 5.5 days of data could be recorded. Batteries were changed every 48 h and SD cards changed every 2–4 days. Time spent by staff at each camera unit was kept to a minimum, usually <10 min. Video footage revealed that incubating terns returned to the nest usually within 5 min of staff leaving the nest.

Colonies at which cameras were located were not selected on the basis of number of nests because this changed constantly. The smallest colony comprised two nests at the time of camera placement (down from 10 nests, due to predation and flooding). Camera units within a colony were set as far apart as possible to minimise the disturbance caused by camera maintenance.

After a nest was preyed upon, the camera unit was moved to a new nest. If eggs hatched at a nest, the camera unit was moved to a new nest only after the chicks had left the immediate vicinity of the nest bowl (usually 1–2 days). Camera units were also moved to new nests and/or new colonies if the colony had been flooded or if predation was occurring at a higher rate at another colony, in order to maximise the probability of recording predation events.

Each SD card was viewed on a laptop (Windows Media Player), by playing back at the maximum play speed of ×16 until a predation event took place. All subsequent footage was viewed to identify any scavenging. Picture quality was generally good during the day (it was important to face the camera away from the rising/setting sun), but at night it varied among cameras, mainly because of condensation on the lens.

Results

DNA results

A total of 438 samples from black-fronted tern nest material, eggshell, and chick and adult carcasses were collected for mtDNA analysis on the Wairau River from 2007 to 2009 (Table 2). Most samples were taken from eggshell (72%; 316/438) while relatively few were taken from adult or chick carcasses. A total of 71 samples were taken from nest material but these yielded only one sample containing predator DNA. Consequently this method was discontinued in the final year of the study (Table 2).

Seventy-eight percent of samples (343/438) yielded DNA, and 49% (215/438) contained DNA from potential predators. Twenty-nine percent of samples (128/438) contained DNA from the terns themselves or from non-predatory species, such as humans (most likely from the researchers), invertebrates (including flies), eels (*Anquilla* spp.; possibly from tern gut

Table 2. Predator and other species' DNA detected in samples from black-fronted tern (*Chlidonias albostriatus*) colonies on the Wairau riverbed, 2007–2009.

Predator DNA	Sample type				
	Adult	Chick	Eggshell	Nest material	Total
Harrier (*Circus approximans*)	2	1	171	1	175
Cat (*Felis catus*)	9		6		15
Mouse (*Mus musculus*)			3		3
Norway rat (*Rattus norvegicus*)			1		1
Ship rat (*Rattus rattus*)			2		2
Stoat (*Mustela erminea*)	8	1	3		12
Oystercatcher (*Haematopus finschi*)		1	1		2
Plover (*Vanellus miles novaehollandiae*)			3		3
Pūkeko (*Porphyrio melanotus melanotus*)			1		1
Shag (*Phalacrocorax* spp.)			1		1
Subtotal (predators)	19	3	192	1	215
DNA – unknown or not predator	11	10	84	23	128
No DNA	4	4	40	47	95
Total	34	17	316	71	438

contents), rabbits (*Oryctolagus cuniculus*) and to a lesser extent chaffinch (*Fringilla coelebs;* 2), greenfinch (*Carduelis chloris;* 1), silvereye (*Zosterops lateralis lateralis;* 1), cow (*Bos primigenius;* 1) and hare (*Lepus* spp.; 1). Three of the DNA matches returned apparently spurious results: two for black-necked stilt (*Himantopus himantopus mexicanus*), a species not found in New Zealand; and one shearwater (*Puffinus* spp.), unlikely to be found on the Wairau. Because we cannot conclusively explain the presence of DNA from these two species at this locality we have ruled them out from further analysis. Some samples contained DNA from a combination of predators and non-predators (e.g. chaffinch + tern + harrier DNA).

Harrier DNA was obtained from 40% (175/438) of all samples and from 81% (175/215) of samples from which DNA of potential predators was identified (Table 2). Of the 192 potential predator DNA results from eggshell remains, 89% (171 samples) were identified as harrier DNA.

Cat DNA comprised 7% (15) and stoat DNA 6% (12) of the potential predator samples. Most cat and stoat DNA samples were obtained from adult carcasses (9 and 8, respectively). Only six cat and three stoat DNA samples were obtained from eggshell.

As part of the wider nest success study where 2275 tern nests at 80 colonies were monitored, 64% (683/1063) of depredated nests were found with no eggshell remains at the next nest-monitoring visit following predation. Nest material was swabbed and/or analysed from 71 of these empty nests but only one sample yielded predator DNA (harrier).

Video results

Eighty-five nests in 17 colonies were videoed in the course of this study, providing 559 days and 483 nights of footage. Predation was successfully videoed at 19 nests within 10 colonies. These colonies were widely distributed along the Wairau River (Fig. 1).

In total, 19 predation events were captured on video (Table 3). Predation of eggs was the only type of lethal event recorded; no predation of chicks or adults was videoed. The video footage showed harriers, black-backed gulls, hedgehogs, ship rats and pied oystercatchers consuming eggs at nests, as well as a stoat removing eggs from nests (Table 3). Nine out of the 10 colonies where predation was videoed were located on islands.

Harrier predations were most commonly recorded (nine events). These occurred at five colonies in 2008 and one colony in 2009. Black-backed gulls were videoed preying on eggs on three occasions at three different colonies. The two ship rat predations occurred within one colony. However, a ship rat was videoed visiting a tern nest at another colony at a time when all the surrounding nests were preyed on by an unknown predator. Two hedgehog predations (and one hedgehog scavenging event) were videoed from one mainland colony. Both pied-oystercatcher events occurred within one colony.

The only videoed stoat predation occurred at one tern nest in an island colony over an 8-h period. At mid-morning, a stoat carried away one of two eggs present in the nest. Eight hours later a stoat removed the second egg. It was a fine day, but the stoat in the latter footage was completely wet, suggesting that it had swum to the island colony immediately prior to removing the remaining egg.

Twelve of the 19 videoed predations resulted in 'empty nests' (i.e. nests that contained no eggshell at the next monitoring visit). Black-fronted terns removed all eggshell remains after predation at eight of the nests (and partially removed the shell from a ninth nest); a stoat (s) carried the tern eggs away from one nest in its mouth; and black-backed gulls swallowed eggs whole at three nests.

As well as predation events, video recorders also captured scavengers and nest desertions. Mice were videoed scavenging at nests after three predations. One hedgehog was identified scavenging eggs from a nest that had been preyed on by a harrier, and a banded dotterel *(Charadrius bicinctus)* appeared to move a fragment of tern eggshell after a hedgehog had preyed on the eggs.

Nine nest desertions were videoed. Seven of these were at colonies where harriers had been videoed preying on tern eggs within the same period as the nest desertions.

Table 3. Video footage of successful predations at black-fronted tern (*Chlidonias albostriatus*) nests on the Wairau riverbed and corresponding DNA results. P = present; NP = not present; Y = sample collected; N = sample not collected; N/A = not applicable.

Nest ID	Year	Egg remains	DNA sample	Predator ID (footage)	Scavenging (footage)	DNA Result
CL06	2008	P	Y	Harrier	None	Harrier/magpie
HL06	2008	P	Y	Harrier	None	Harrier
BR14	2009	P	Y	Harrier	Mouse	Harrier/rabbit
BR33	2009	P	Y	Harrier	Mouse	Harrier/mouse
NAL07	2008	P	Y	Harrier	Tern	Human
WM37	2008	P	Y	Hedgehog	None	Human
WM34	2008	P	Y	Harrier	Tern & hedgehog	No DNA
GFD06	2008	NP	Y	Harrier	Tern	Black-billed gull
WM50	2008	NP	Y	Hedgehog	Tern & dotterel	Human
MM35	2009	NP	N	Stoat	None	N/A
BR03	2009	NP	N	Black-backed gull	None	N/A
BR04	2009	NP	N	Ship rat	Tern	N/A
BR08	2009	NP	N	Ship rat	Tern	N/A
BR25	2009	NP	N	Harrier	Tern	N/A
BL06	2009	NP	N	Black-backed gull	None	N/A
PY10	2009	NP	N	Black-backed gull	None	N/A
P(C)18	2009	NP	N	Pied oystercatcher	Tern	N/A
P(C)01	2009	NP	N	Pied oystercatcher	Tern & mouse	N/A
NAL11	2008	NP	N	Harrier	None	N/A

See Table 2 for scientific names of most predators. Black-backed gull (*Larus d. dominicanus*), black-billed gull (*L. bulleri*), hedgehog (*Erinaceus europaeus occidentalis*), magpie (*Gymnorhina tibicen*), rabbit (*Oryctolagus cuniculus*).

DNA results at videoed nests

In 2008 we collected eggshell remains, nest swabs and nest material from seven of the eight nests where predation had been videoed. We did not collect a nest swab at the eighth nest because only one of the two eggs was missing and we did not want to disturb the adult that was incubating the remaining egg. In 2009, eggshell remains were collected for DNA analysis from two of the 11 nests at which predation had been videoed. Thus, overall we collected remains for DNA testing from nine videoed nests.

For four of these nine nests (44.4%), the DNA results corresponded to the videoed events, with harriers being identified as the predator by both methods (Table 3). At one of these nests, magpie DNA was also identified, but no magpie was seen on the video. The video recording failed soon after the harrier predation, and it is possible that a magpie visited the nest after the video stopped recording. At another of the nests, mouse DNA was identified along with harrier DNA, and this is consistent with the video, which shows harrier predation followed by a mouse scavenging at the nest. At another nest, rabbit and harrier DNA were obtained, whereas the video showed harrier predation and subsequent scavenging by a mouse. Rabbits and their sign are abundant on the Wairau riverbed and it is likely that the egg sample was contaminated with the background presence of rabbit DNA.

At the remaining five nests (55.6%), the DNA results did not correspond to the videoed results (Table 3). Samples from three of these nests contained only human DNA, whereas the video showed that these were preyed on by hedgehogs (two nests) and a harrier. One sample contained no DNA even though the video recorded harrier predation followed by hedgehog scavenging and the subsequent removal of eggshell fragments from the nest by a tern. The final nest was preyed on by a harrier, whereas the nest sample contained black-billed gull (*Larus bulleri*) DNA. No black-billed gull was recorded on video at the nest.

Hedgehog DNA was not identified from any of the three nests (two egg samples and one nest swab) where they were filmed consuming tern eggs.

Discussion

Video surveillance identified avian predators (harriers and black-backed gulls) as the main cause of mortality at black-fronted tern nests on the Wairau riverbed. Although only 19 predations were recorded, this conclusion is supported by the more extensive DNA evidence indicating harriers as a major predator of tern eggs and direct observations of black-backed gulls catching and swallowing three black-fronted tern chicks at one colony on the Wairau riverbed (P. Gaze, DOC, Nelson, New Zealand, pers. comm.). The number of chicks at this colony dropped in the subsequent few days from 80 chicks to 10. No chick remains were found. R. McClellan (pers. comm.) recorded a similar event at a black-billed gull colony in Southland where black-backed gulls were observed taking two black-billed gull chicks from a colony of more than one hundred chicks. The next time the colony was monitored the chicks had virtually vanished and very few carcasses remained (R. McClellan, pers. comm.). Guthrie-Smith (1936) observed black-backed gulls drowning black-billed gull chicks and then swallowing them whole on rivers in Southland. Studies on other species of terns have recorded tern chicks being preyed on by various gull (*Larus*) species (Donehower et al. 2007; O'Connell & Beck 2003; Whittam & Leonard 1999). Great black-backed gulls *(Larus marinus)* and herring gulls *(Larus argentatus)* were the main chick predators of three tern species in the province of Nova Scotia, where the number of gulls present within the tern colonies was low during laying and incubation, but increased during hatching and chick rearing (Whittam & Leonard 1999).

Pied oystercatchers have never been reported consuming eggs at nests of braided river birds before, although they have previously been videoed visiting nests (Sanders & Maloney 2002). The pied oystercatcher predations in our study were all at one black-fronted tern colony where a pair of oystercatchers (probably nesting in the vicinity) was regularly observed being harassed by terns so we suspect that the oystercatcher predations were localised and probably carried out by a single bird, or pair. Individual birds of other species have been observed as 'specialist' feeders of tern eggs and chicks. For example, until it was culled, one individual specialist predatory gull (*Larus* spp.) accounted for 85% of all successful common tern *(Sterna hirundo)* chick captures in a Canadian study (Guillemette & Brousseau 2001).

Our results contrast with those of a similar, but more intensive video study in the Upper Waitaki Basin, which found that cats, ferrets and hedgehogs were the main cause of mortality at nests of braided river birds (Sanders & Maloney 2002). In that study, stoats and avian predators accounted for very few predations, and rat predation was not recorded at all.

Our study demonstrates that, while the same suite of predators is likely to be present in all braided river systems, the relative impact of each predator species can vary greatly. The impact does not appear necessarily to be related to predator abundance because harriers are abundant in both the Upper Waitaki Basin (Sanders & Maloney 2002) and the Wairau catchment (KES pers. obs.), but had markedly different impacts in the two studies.

Although predation of chick and adult black-fronted terns was not videoed during this study, the DNA results from wound swabs indicated that stoats and cats were the major predators of adult terns on the Wairau River. A stoat (or stoats) had been strongly implicated in the demise of adult black-fronted terns within one colony where eight carcasses swabbed returned stoat DNA and all 57 nests within the colony were abandoned on the same night. Keedwell (2005) also observed that an individual predator could destroy an entire black-fronted tern colony in a short space of time. Stoat DNA was also identified from a dead tern chick at a colony where a stoat had been videoed taking tern eggs from a nest.

Cat DNA was found on nine adult tern bodies across six tern colonies in the mid- to lower Wairau River in 2007 indicating that this predator, like stoats, can readily kill adult terns. Cats were the only predator to take adult braided river birds in the Waitaki Basin study (Sanders & Maloney 2002), and a single cat was thought to have caused the demise of 76% of nests and 10% of breeding adults in a black-fronted tern colony on the Rangitata River (O'Donnell et al. 2010). Predation of breeding adult birds is potentially of greater concern than the loss of nests because population growth can be sensitive to adult survival rates (Keedwell 2002).

The stoat and ship rat predations recorded in this study occurred at tern colonies on islands within the braided riverbed, whereas the cat and hedgehog predations occurred at colonies on mainland sites at the time of predation. Although all the mammalian predators present on these rivers can swim (King 2005; KES pers. obs.), these results suggest that different predator species vary in their willingness to cross water, but more research is needed on this.

Ship rats have been videoed taking eggs from nests of small passerines in New Zealand (Brown et al. 1998) and were identified as predators of black-fronted tern eggs in this study. Norway rats have been videoed eating carcasses of black-fronted tern chicks in the Ohau River (Keedwell 2003) but not visiting the nests of braided river birds (Sanders & Maloney 2002). Although we videoed ship rat visits to tern nests (lethal and non-lethal), these were from only two small tern colonies within one season. These results suggest that rats were a minor predator at tern nests on the Wairau River, at least at the time of this study.

Although mouse DNA was found in three eggshell samples it is unlikely (but not impossible) that mice could break open and consume tern eggs. Both our study and that of Sanders and Maloney (2002) videoed mice visiting, but not preying upon nests of braided river birds.

Although DNA evidence can be useful in detecting otherwise cryptic predators, some clear limitations need to be considered when interpreting DNA results. First, it is clear that predator DNA is not consistently obtained from nest remains. For example, hedgehogs were videoed preying on two tern nests and scavenging at a third deserted nest but were not detected from the DNA samples. This was in spite of hedgehogs spending considerable time consuming eggs, which might be expected to result in them leaving a lot of saliva.

Second, unlike video recording, DNA analysis does not discriminate between predators and scavengers when a returned result indicates that two potential predators visited the nest. For example, one swab contained harrier and magpie DNA, and it would not have been possible to definitively identify the predator (harrier) without the video evidence. However, we recorded few scavenging events on video, suggesting that, in this environment at least, DNA obtained from samples collected within 4 days of the predation is more likely to indicate a predator rather than a scavenger.

Third, a large portion (64%) of nests contained no eggshell remains at the next monitoring visit after a predation event, and analysis of the nest material at these empty nests did not yield predator DNA (except for one sample). Nests frequently contained no shell remains because these were removed by adult terns. In addition, in this study we videoed black-backed gulls swallowing tern eggs whole and a stoat carrying eggs away from the nest. This behaviour of stoats was also recorded in a video study in the Mackenzie Basin (Sanders & Maloney 2002).

The tendency of black-fronted terns to remove eggshell from their nests after predation of eggs meant that the videoed ship rat predations and oystercatcher predations in this study would have been missed if DNA analysis alone was used. Of the 19 videoed predations, DNA analysis identified predators from only four nests and all four of these were harrier predations. Without validation from video work it would not be possible to know the cause of egg mortality from samples that return 'no DNA' and/or from empty nests. The incidence of samples containing 'no DNA' was reduced in the final year of the study by improving collection methods, as described in the Methods. Gleeson et al. (2010) found that DNA quality declined if samples were left in the field for several nights before being collected and that daily checks resulted in the best quality DNA in samples. Daily checks may not always be practicable when monitoring birds, and disturbance from more frequent visits may increase the risk of nest desertion by river birds. We therefore recommend that, when using this method for river bird studies, samples should be collected as frequently as practicable, and that account should also be taken of the potentially adverse effects of disturbance.

In agreement with other researchers we consider that video recordings are a useful tool for predator research and management (Brown et al. 1998; Sanders & Maloney 2002;

Williams & Wood 2002; Stake et al. 2004). Digital video equipment is easier to use in the field than VCR equipment (Parker et al. 2008; KES pers. obs.). However, it should be noted that the digital video recorders we used in our study failed on occasion in the riverbed environment. The recorders were housed in waterproof plastic grey or black boxes and it is likely that the intense heat on exposed riverbed gravels and cobbles on sunny days sometimes caused the recorders to overheat and stop recording. When planning similar studies, we recommend that equipment is designed to minimise these problems.

The digital video technique is labour-intensive and expensive and the loss of equipment to floods is a constant risk when used in a braided river environment. In comparison, the DNA samples were simple and cheap to collect and process and entailed no risk to expensive field equipment.

This study identified avian and mammalian predator species that negatively affect the breeding success of black-fronted terns on the Wairau riverbed. The relative importance of these predator species should be considered when implementing effective predator control. However, the relative importance of various predator species could change through time and/or in response to predator control, and it may be useful to monitor changes in predator impacts, for example with further DNA sampling and video monitoring.

In summary, we present data from a new forensic technique that greatly improves our ability to infer the identity of nest predators, but that also needs to be interpreted in the light of its limitations. The video work suggests that, when DNA is detected in samples, it has usually been left by a predator, rather than a scavenger. However, our observations, and other studies, also suggest that the probability of detecting DNA in samples from prey items is likely to vary among predator species. For example, black-backed gulls, which tend to swallow eggs whole, and stoats, which tend to remove entire eggs, appear to be less likely to leave DNA at the nest. Thus, a major limitation of DNA sampling is that the samples yield an incomplete and biased picture of the relative impacts of different predator species. Further research on this 'detectability bias' would improve the utility of DNA analysis in wildlife ecology.

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