Tactile senses and foraging in birds, with emphasis on kiwi

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Abstract

Probe-foraging birds must often rely on senses other than vision for prey-detection. One such sense is 'remote touch': the detection of vibration and pressure cues from prey within the substrate. Remote touch is mediated by the 'scolopacid-type bill-tip organ', which consists of a honeycomb of sensory pits in the bill-tip, containing clusters of mechanoreceptors. This organ was originally described in the neognathous shorebird family Scolopacidae, but was recently also discovered in paleognathous kiwi (Apterygidae): an example of convergent/parallel evolution. My aim was to discover how widespread this organ is among birds, compare its anatomy and function in foraging between kiwi and other probe-foraging birds and elucidate in detail the foraging behaviours and senses used by free-living kiwi. Within the thesis I compare the bill-tip organs of kiwi and shorebirds using material from the brown kiwi (Apterygidae: *Apteryx mantelli*) and bar-tailed godwit (Scolopacidae: *Limosa lapponica*). I provide the first description of the organ in a third family of birds, the ibises (Threskiornithidae), and give evidence that it may exist in simplified form in a fourth family, Rallidae. The Scolopacidae, Apterygidae, Threskiornithidae and Rallidae are widely separated on the avian phylogenetic tree. This suggests that the evolution of the scolopacid-type bill-tip organ and associated sense is favoured by a probe-foraging lifestyle. Foraging trials confirm the bill-tip organs of brown kiwi and Madagascar crested ibises (*Lophotibis cristata urschi*) are functional for remote touch. The ibises rely solely on remote touch to find buried prey, whereas brown kiwi use the sense in conjunction with olfaction. Free-living brown kiwi display no obviously visually-guided behaviours, instead using hearing (head-lifting in response to noises audible to the observer), olfaction (odour sensing behaviour, 'sniffing,' in the direction of these sounds) and touch. Kiwi tap ahead with their bill-tip when walking and move their facial bristles forward when foraging, forming a 'net' on the ground. The bristle follicles in kiwi (and some other insectivorous bird species) are innervated with Herbst corpuscles, suggesting tactile function. Female kiwi probe on average 30% deeper than males and juveniles, but there are no other differences in foraging behaviour between the sexes.

Acknowledgements

A PhD is the ‘long haul’ and sitting down to write the acknowledgements section for one is a daunting task. I have so very many people to thank that some of them will inevitably get left out. If that happens to be you: I apologise! I am deeply grateful for everyone’s input and if I accidentally forget to mention you through some horrible oversight, come and find me: I’ll buy you a beer instead (or a wine, if you’d prefer).

The fact that this PhD happened at all is the fault of Isabel Castro. I met Isa when I was 11 years old, after reading her article on face-to-face copulation in hihi in Forest & Bird. I wrote her a letter about her article and she invited me to help out with her post-doc on Mokoia Island, chasing hihi. I was so inspired by Isabel’s energy and passion for her subject that I decided I wanted to be a field biologist, too. When I was looking for a supervisor for post graduate studies, Isabel was the obvious choice. Isa: you have been more than a supervisor to me - you have been a friend and an inspiration for over half of my life. I’m not sure I can thank you enough!

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Thank you to the Ponui Research team – Izzie's Angels! Especially thank you Birgit Ziesemann for sharing so much time with me on the island, for the good times we had kiwi-chasing together and for the shared bottles of wine. Welcome Dawn & Sarah – I hope you have as fantastic a time on Ponui as we did. I'm glad that you will be looking after 'our' birds for us!

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I am kept going (and kept sane) by my wonderful friends and the people I tramp with (MUAC and the Stripy Brigade). Some of you I have known nearly forever, some of you are newcomers into my life. All of you have been great! Thank you for supporting me though this mad PhD lark. In no particular order, thanks to: Jenny Lynch, Jess Costall, Charlotte Minson, Natasha Petrove, Matt Randell, Matt Bennett, Jules Townsend, Catherine Layne, Katie Jones, Kurt McBeth, Nicole Schon, Alice Clarke, Hayden Short, Jackson Wong (thanks for fixing my knee), Rob Hunter, Kelly McDermott, Rob Dykes, Clare Robertson, John Appleby, Kay Dennis, Alice Wessel, Russell Steele, Pietre Allan, Mike Chalmers, Shaun Nielsen (did I spell it right?), Judith Stepper, Chris Thompson, Jeremy Corfield, Lee Shapiro (it's your fault I'm obsessed with kiwi), Matt Robertson (you warned me I'd become obsessed with kiwi), Karryn Baudet, Anna Gsell, Dave Scully, Amanda Scully, Isabel Castro, the Jobsis family, Therese McCrae, Rebecca O'Brien, Bridget Wrenn, Troy Makan, all of the Guest family (including Ed), all of the Hojem family (especially Carryn), the Claridges, Dave Sheerin, Emma Bean, Lorraine Cook, Gaylynne Carter, Geoff Walker, Sarah Pettigrew, Graham Bacon, Moreno Julli, Brian Crane, Josie Galbraith, Shane and Mia …. Oh my goodness, I've just realised what a terrible idea this list was – it's the one way to guarantee I've forgotten someone! And according to Facebook there are 212 of you! My dear friends you all know who you are: if you're not on this list here you're definitely on the one in my heart (awww!).

My family – you made me what I am (you might blame Rob Guest for the crazy bird thing, but you most certainly encouraged it!). Thank you for giving me everything, for believing in me, for supporting me through thick and thin. Mum – thank you for your faith in me and for being there always and getting me through the tough times. Dad, your pride in me drives me to achieve to the highest of my ability, thank you. Michele you're not only my favourite little big sister but also one of my best friends and a wonderful tramping companion! Alastair, you're the best big brother in the world, Agnes the most wonderful sister-in-law. Ken & Raylene, it's been a fantastic thing to know you're just down the road if ever I need you. Terry & Katya, thank you so much for your support and your love. All of you inspire me, I am so very lucky to have you as my family.

The years I have spent at university, especially the years doing my PhD, have been the best years of my life thus far. I will always hold my memories from this time very dear. Thank you all of you wonderful wonderful people (and all of the wonderful birds) who have made the last few years what they were!

Preface

This thesis contains six chapters and two appendices, plus a general Introduction and Discussion. Data from Chapter 1 are included in two separate publications, one already published, the other still in preparation. Chapters 2 – 6 and Appendix 2 each constitute a single published, or in press paper, Appendix 1 has been submitted to the New Zealand Journal of Ecology. Chapters are presented in the order in which they were submitted for publication, except for Chapter 1 which was completed last. All the data presented here are new, and each chapter builds upon those written before it. Therefore, there is extensive cross-referencing between chapters. Chapters have been reformatted for consistency within the thesis.

This is a thesis by papers, and each paper also stands alone. Therefore, there is inevitably some repetition of material between chapters, particularly in some of the topics covered in the introductions, and in the methods sections.

Authorship of papers arising from Chapters 1 – 6, Appendix 1

I am the first author in all published, in press or submitted papers arising from Chapters 1 – 6 and Appendix 1 of this thesis. In all cases my input into the paper was the greatest in terms of development of ideas, synthesis of experiment design, collection and analysis of data, and drafting of each manuscript. Below, I give the references for the publications arising from each chapter of the thesis and elucidate the roles of the co-authors on those publications. Isabel Castro was my chief PhD supervisor and Murray Potter and Maurice Alley co-supervised. Therefore, each of these people additionally supported the writing of each paper within their supervisory roles.

Chapter 1:

This chapter contains information on the bill-tip morphology of five kiwi species published in the *Journal of Anatomy*, where it appears in conjunction with data from my BSc (Hons). In this thesis I only present the data which I collected as part of my PhD,

after I completed my BSc (Hons). It also contains data on the kiwi and godwit bill-tip organs which will be submitted within a manuscript to *Brain, Behaviour and Evolution*, together with information on kiwi and godwit brains collected by Jeremy Corfield (data on the brains are not presented here).

Chapter references: Cunningham, S., Castro, I., and Alley, M. 2007. A new prey-detection mechanism for kiwi (*Apteryx* spp.) suggests convergent evolution between paleognathous and neognathous birds. Journal of Anatomy. 211 (4): 493-502.

Cunningham, S.J. and Corfield, J. Comparative anatomy of the trigeminal system in a kiwi and a shorebird *in prep.*

Contributions of co-authors: Isabel Castro was heavily involved in conception of ideas and planning for fieldwork and in discussions of kiwi bill-tip histology, for the *Journal of Anatomy* paper. She also commented on chapter drafts. Maurice Alley initially taught me to interpret histology slides during my BSc (Hons) and contributed to the interpretation of histological material presented in the *Journal of Anatomy* paper. He also kindly discussed with me the interpretation of the histology of kiwi Herbst corpuscles presented within this chapter (particularly the three-dimensional shape of the central axon, and the density of the outer zone), and commented on chapter drafts. Jeremy Corfield collected and analysed data on kiwi and godwit brain morphology for the *Brain, Behaviour and Evolution* paper, which is not presented in this chapter. He also acted as a liaison with the micro-CT technician at Auckland University when I sent kiwi and godwit beak samples up for scanning, and taught me how to construct three dimensional models from µCT scans using the image analysis software package AMIRA™.

Chapter 2:

This chapter appears exactly as published in *Animal Behaviour* (except for the replacement of ‘kiwis’ with the more correct plural, ‘kiwi’).

Chapter reference: Cunningham, S.J., Castro, I. & Potter, M.A. 2009. The relative importance of olfaction and remote touch in prey detection by North Island brown kiwis. Animal Behaviour 78: 899-905.

Contributions of co-authors: Isabel Castro contributed ideas on experimental design for this paper. She carried out behaviour trials with kiwi at San Diego Zoo while I was across town working with kiwi at San Diego Wild Animal Park. Unfortunately, husbandry management practices for kiwi at the Zoo and Park meant we were unable to control trial conditions sufficiently. The data from these trials are therefore not included in this chapter or in the paper. Isabel also spent considerable time helping to improve the wording of the results section. Murray Potter contributed ideas on experimental design for this paper. Both co-authors commented on manuscript drafts.

Chapter 3:

This chapter appears exactly as published in *The Auk.*

Chapter reference: Cunningham, S.J., Alley, M.R., Castro, I., Potter, M.A., Cunningham M.J., Pyne M.J. In press. Bill morphology of ibises suggests a remote-tactile sensory system for prey detection. Auk.

Contributions of co-authors: Maurice Alley was involved in sourcing an ibis specimen for histology via veterinarian Michael Pyne at Currumbin Wildlife Sanctuary, Australia. He also discussed interpretation of histology slides, and the trend in bill-morphology with habitat use with me. Isabel Castro and Murray Potter discussed the trend in bill-morphology with habitat use with me and provided advice on the inclusion of some of the ideas in the discussion section. Malcolm Cunningham discussed at length the physics of sound wave travel in various substrates with me, and contributed to ideas presented within the discussion section. Mic Pyne (veterinarian, Currumbin Wildife Sanctuary) euthanized the ibis used for histology, and undertook initial sample preparation (fixing the head and bill in formalin). He provided extensive help organising import and export permits to bring the ibis head to New Zealand. Maurice, Isabel, Murray and Malcolm all commented on paper drafts.

Chapter 4:

Chapter reference: Cunningham, S. J., Castro, I., Jensen, T. and Potter, M. A. Remote touch prey-detection by Madagascar Crested Ibises. *Submitted to the Journal of Avian Biology.*

Contributions of co-authors: Isabel Castro contributed ideas on experimental design for this paper. She assisted me with training ibises at San Diego Zoo and Wild Animal Park, and with carrying out the trials. Tom Jensen assisted me with training ibises at San Diego Zoo and Wild Animal Park, with carrying out the trials, and with sourcing equipment and organising permission to undertake work with these birds. Murray Potter contributed ideas on experimental design for this paper. All co-authors also commented on paper drafts.

Chapter 5:

Chapter reference: Cunningham, S. J. and Castro, I. The secret life of wild brown kiwi: direct foraging observations and other nocturnal behaviours. *Submitted to the New Zealand Journal of Ecology.*

Contributions of co-authors: Isabel Castro helped to collect some of the video footage used in this paper, provided advice on manuscript structure, and commented on drafts.

Chapter 6:

Chapter reference: Cunningham, S. J., Alley, M., and Castro, I. Facial bristle structure and function in insectivorous birds in New Zealand. *Submitted to the Journal of Morphology.*

Contributions of co-authors: Maurice Alley contributed to early discussions of ideas for this paper, provided advice on the writing of the results, and ideas on the functional significance of structural differences between Herbst corpuscles. Isabel Castro contributed to early discussions of ideas for this paper. Both co-authors commented on manuscript drafts.

Appendix 1:

Appendix reference: Cunningham, S.J. and Castro, I. Short term effects of leg-mounted radio-transmitters in brown kiwi. *Formatted for the New Zealand Journal of Ecology*.

Contributions of co-authors: Isabel Castro provided and analysed data on behaviour of kiwi on exiting burrows, and provided photographs of leg injuries and entanglement caused by transmitters. She also commented on manuscript drafts.

Data on injuries to kiwi caused by transmitters was collected by the whole kiwi research team on Ponui Island during successive catch weeks. These data were compiled by Isabel, and analysed by me for this paper.

Authorship of Appendix 2

I am the second author of the paper presented in Appendix 2. This paper arose from an ongoing study of olfaction in wild kiwi which is related to the topics of this thesis, and in which I continue to have a part. The majority of data analysis, synthesis of ideas, and drafting of the paper were carried out by the first author, Isabel Castro. Below I set out my contribution to this paper.

Appendix reference: Castro, I., Cunningham, S.J., Gsell, A.C., Jaffe, K., Cabrera, A., Liendo, C. *In press.* Olfaction in birds: A closer look at the Kiwi (Apterygidae). Journal of Avian Biology.

My contribution: I was involved in initial and ongoing discussions with co-authors (particularly Isabel Castro and Anna Gsell) regarding ideas for this point-of-view paper. I collected (but did not analyse) the majority of the field data used in the paper, during the course of video-recording for Chapter 5 and Appendix 1 of this thesis, and during training experiments to assess whether behaviour trials from Chapter 2 could also be run in the wild (we did not achieve this). The drawings for Figure 1 were done by me, using still frames from video-recordings I collected. I commented on the paper drafts. I was not involved in the laboratory work for this paper, nor in collecting or analysing papers for the extensive literature review. The ‘author contributions’ section at the end of Appendix 2 acknowledges the roles of the other authors on this paper.

Further acknowledgements

Many other people helped me during the writing of papers and throughout my PhD in various ways. Their input is recognized in the ‘acknowledgements’ sections at the end of each chapter, and in the general thesis acknowledgements.

Susan Cunningham, PhD candidate	Dr. Isabel Castro, chief supervisor

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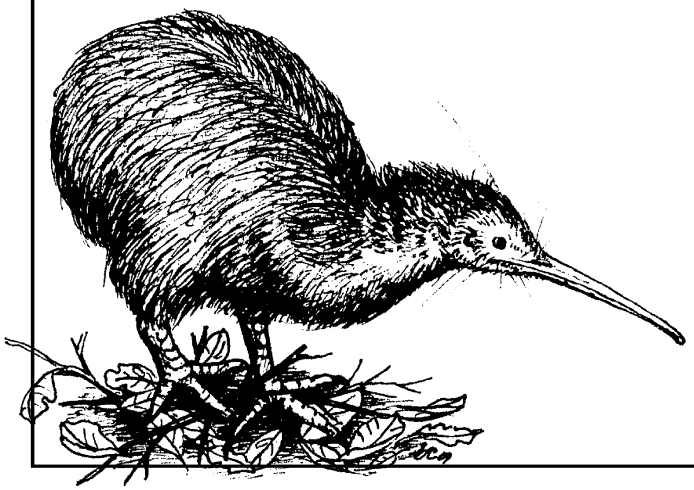
Introduction



This thesis is primarily about the sensory systems used in prey-detection by probe-foraging birds, with a particular focus on a tactile sense called ‘remote touch’, and the anatomy of the bill-tip organ associated with it. The bulk of the data presented here were collected from kiwi (Apterygidae), although Chapters 3 and 4 focus on ibises (Threskiornithidae). I also present some comparative data from a shorebird (Scolopacidae), plus a broad overview of the bill-tip anatomy of 31 species from 5 further avian families (Chapter 1), and the facial bristle feather histology of an additional four species of insectivorous New Zealand birds (Chapter 6).

Below, I provide a general introduction to the ecology and sensory systems of kiwi and briefly review the literature on the avian remote touch sense and associated brain and bill-tip specialisations, not including the information found during this study and contained in the other chapters of this thesis. I then set out my research approach and objectives, together with a short overview of the content of each chapter. Finally, I present a simplified phylogenetic tree of modern birds highlighting the positions of the main families discussed in this thesis. Literature reviews presented here are brief to avoid excessive repetition of material covered in the introductions to each chapter.

My main study species was the brown kiwi (*Apteryx mantelli*). This species is also known as the North Island brown kiwi and the northern brown kiwi. I use ‘brown kiwi’ and ‘North Island brown kiwi’ in the text. Throughout the thesis, I refer to the scolopacid-type bill-tip organ as either ‘scolopacid-type bill-tip organ’ or ‘shorebird-like bill-tip organ,’ depending on the date at which each chapter was submitted for publication.

Kiwi ecology and sensory systems

Kiwi (Apterygidae) are a family of five species of predominantly nocturnal, probe-foraging birds endemic to New Zealand (Burbidge et al. 2003). They live in forested and partially-forested habitats from the alpine areas to the coast and forage by probing into soil, leaf litter, rotting logs, damp sand and rotting seaweed (Colbourne & Powlesland 1988; Kleinpaste 1990). Kiwi diet consists predominantly of soil and leaf-litter invertebrates, generally taken in proportion to their availability (Kleinpaste 1990, Miles 1995). Kiwi are long-lived (up to 50 years: Holzapfel et al. 2008; although on average 12 years in the wild: McLennan et al. 1996) and possess a variety of breeding systems which range from complete monogamy (with and without male-only incubation, McLennan 1990), through to co-operative breeding with helpers at the nest, depending on species and population (Colbourne 1991, Taborsky and Taborsky 1999).

Kiwi possess a suite of sensory specialisations unique among birds. Their eyes, visual fields and visual centres within the brain are reduced compared with those of other birds of their size (Martin et al. 2007), despite the possession of a nocturnal-adapted retina (Corfield 2009). Instead of vision, kiwi have specialised tactile, olfactory and auditory senses. The tips of the kiwi premaxilla and mandible bones are honeycombed with sensory pits, each packed with Herbst corpuscles (vibration and pressure sensitive mechanoreceptors), suggesting a tactile specialisation of the bill-tip for remote touch (see below) (Cunningham et al. 2007). Kiwi possess an enlarged principal sensory trigeminal nucleus (PrV) and nucleus basorostralis (Bas) (Martin et al. 2007, Corfield 2009), regions of the brain responsible for the relay and processing of tactile information from the bill and tongue (Berkhoudt et al. 1981, see also below). This enlargement of the PrV and Bas suggests that tactile information from the bill is very important to kiwi (Martin et al. 2007, Corfield 2009). Kiwi further possess long facial bristle feathers (‘whiskers’) which may also have a tactile function.

Kiwi are famous for their olfactory system. Their nares are placed at the tip of the long bill, a position unique among birds. The olfactory chamber at the base of the kiwi bill is well developed and large, and the olfactory epithelium is extensive (Bang 1971, Corfield 2009). In addition, areas of the kiwi brain involved in processing olfactory

information are highly specialised. The olfactory bulb is uniquely modified into a cortical sheet which surrounds the frontal lobe of the brain and is very large relative to olfactory bulbs in other birds (Bang & Cobb 1968, Bang 1971, Corfield 2009). Kiwi are able to locate hidden artificial food using the sense of smell alone in aviary trials (Wenzel 1969, 1971), and may use olfaction for social purposes as well (Jenkins 2001).

Kiwi ear structure and areas of the brain responsible for auditory processing are specialised for hearing high frequencies (Corfield 2009). Barn owls similarly have specialised high frequency hearing, which they use to locate prey (Dooling et al. 2000). Kiwi react strongly to sounds of footsteps or other movement in leaf litter (pers. obs.), and may use hearing to detect large, leaf-litter dwelling invertebrate prey (Corfield 2009). It seems unlikely that kiwi hearing has coevolved with kiwi vocalisations, as a large proportion of both male and female calls fall below the frequencies where kiwi hearing is specialised (Corfield 2009).

The scolopacid-type bill-tip organ and remote touch

The scolopacid-type bill-tip organ

Like kiwi, probe-foraging shorebirds in the family Scolopacidae possess a specialised structure in their bill consisting of a distinctive honeycomb of sensory pits in the distal parts of the premaxilla and mandible bones. These sensory pits contain clusters of some tens of Herbst corpuscles per pit (Bolze 1968, Piersma et al. 1997). Terminal cell receptors (Grandry corpuscles) may also be present (e.g. Gottschaldt 1985, Piersma et al. 1997). There appears to be a continuum in the number of Herbst corpuscles/sensory pit which correlates with the extent to which each species relies upon probing as a hunting strategy (Bolze 1968, Zweers & Gerritsen 1997, Barbosa & Moreno 1999). This bill-tip organ has been known to exist in shorebirds for over a century (Leydig 1868; also Clara 1925).

Another type of bill-tip organ has been described from the bills of Anatidae and Psitticidae species. It is very different in structure from the bill-tip organ found in shorebirds and consists of rows of tactile papillae housed behind the nail of the bill-tip.

It was first described in parrots by Goujon (1869). To avoid confusion, I have named this bill-tip organ the "psitticid-type bill-tip organ" and the bill-tip organ found in the Scolopacidae the "scolopacid-type bill-tip organ". For a detailed account of the structure and function of the psitticid-type bill-tip organ, see Gottschaldt (1985).

The scolopacid-type bill-tip organ mediates a sense called 'remote touch'. Remote touch involves the detection, at some distance from the bill-tips, of (a) vibrations in the substrate caused by actively burrowing prey (e.g. in sanderlings *Calidris alba,* Gerritsen & Meiboom 1986), or (b) pressure patterns created by buried hard-shelled bivalves (e.g. in red knots *Calidris canutus,* Piersma et al. 1998). Foraging by remote touch allows an expanded area of substrate to be sampled with each peck or probe of the bill, and is therefore more efficient than foraging by direct touch (chance) alone. Remote touch may allow a probe-foraging bird to rapidly assess prey-density in an area and therefore the profitability of foraging there (Gerritsen & Meiboom 1986).

Herbst corpuscles and the remote touch sense

Remote-touch is thought to be mediated by the Herbst corpuscles within the bill-tip organ (Zweers & Gerritsen 1997, Piersma 1998, Nebel *et al.* 2005). These consist of a central sensory nerve axon surrounded by specialised Schwann cells, the nuclei of which are arranged in two rows along opposite sides of the axonal long axis. The bodies of the Schwann cells extend as "fingers", wrapping around the axon and interlocking with extensions of opposite Schwann cells to form a lamellated central core. This central core is encapsulated by a mucin fluid-filled outer zone of coiled collagen fibres interspersed with flattened fibrocytes and surrounded again by a distinct perineural capsule. The collagen fibrils of the outer zone fuse with the lamina of the inner core and also with the perineural capsule so that the inner core is suspended within the fluid-filled capsule (Gottschaldt 1985; Fig. 1).

Herbst corpuscles are rapidly-adapting mechanoreceptors, sensitive to the acceleration components of vibratory stimuli (Gottschaldt 1985). They can therefore provide information to a probing bird about pressure and vibration signals within the substrate (Zweers & Gerritsen 1997). Most Herbst corpuscles respond to vibration stimuli between 50–2000 Hz, with one-to-one discharge (single 'spike' per cycle) at 100-1000 Hz (Gottschaldt 1985). This range of sensitivity is ideal for detecting seismic waves

caused by burrowing invertebrates (e.g. 50-800 Hz for Scarabaeidae larvae in topsoil, Floyd and Woodland 1981; and 1000-7000 Hz for worms in soil, Heppner 1965).

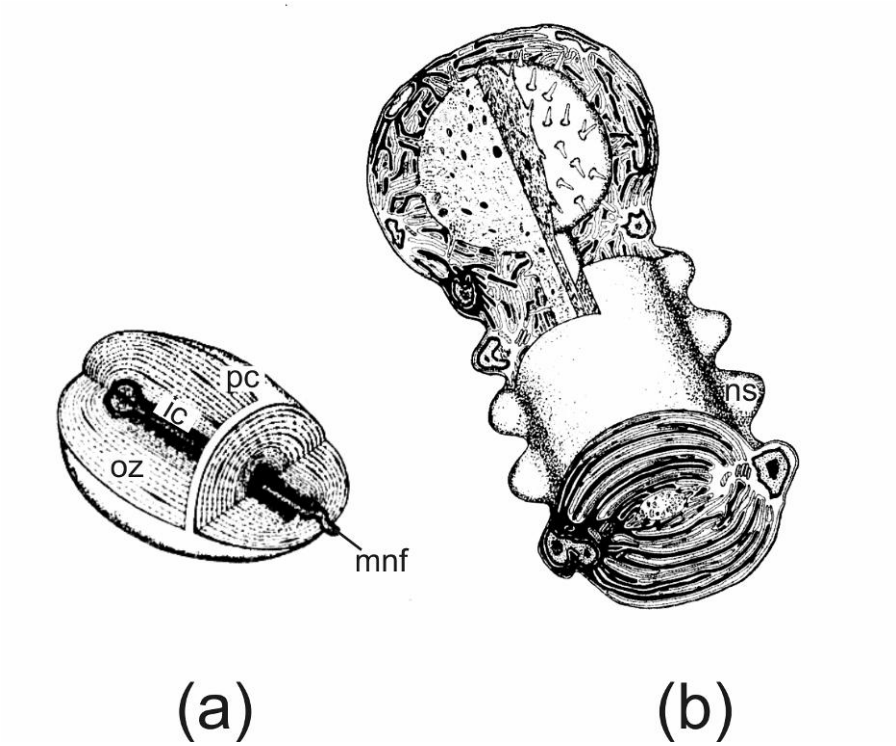


Figure 1: (a) Schematic diagram of an entire generalised Herbst corpuscle. ic = inner core, oz = outer zone, pc = perineural capsule, mnf = myelinated nerve fibre. (b) An enlargement of the terminal end of the inner core of a generalised Herbst corpuscle, shown from the outside. ns = nucleus of a Schwann cell. The inner core can be seen in cross section at the bottom of the drawing. This figure is modified from Gottschaldt (1985).

The Herbst corpuscle clusters within sensory pits of the scolopacid-type bill-tip organ are hypothesised to form functional units, made up of individual corpuscles that fire at different signal intensity thresholds, allowing the bird to measure the distance to a burrowing prey item (Zweers & Gerritsen 1997). Herbst corpuscle clusters within sensory pits are densely arranged around the bill-tips, allowing any differences in intensity of a signal from one side of the bill to the other to be detected, thus establishing the direction of the signal source (i.e. buried prey item) (Zweers & Gerritsen 1997).

The trigeminal system and representation of the bill-tip in the brain

The bill-tips of birds are innervated by the trigeminal nerve (cranial nerve V). This nerve has three branches, of which the ophthalmic and maxillary innervate the upper bill, and the mandibular innervates the lower bill. The ophthalmic and maxillary branches carry purely sensory information, while the mandibular branch has both sensory and motor functions (Barnikol 1953). The three branches of the trigeminal nerve enter the brain stem by way of the trigeminal ganglion. Afferent fibres of the nerve ascend in the ascending trigeminal tract, leading to the main sensory nucleus of the trigeminal nerve (PrV). Sensory information is relayed from the PrV, via the quintofrontal tract, to the nucleus basorostralis (Bas) of the telencephalon (Berkhoudt et al. 1981).

In birds possessing either a scolopacid-type, or a psitticid-type bill-tip organ (including kiwi, Scolopacidae shorebirds, parrots and waterfowl), and also in ibises, the PrV and Bas are enlarged (Stingelin 1965, Martin et al. 2007, Corfield 2009, Gutiérrez-Ibáñez et al. 2009). This expansion of the tactile sensory processing areas of the brain is particularly obvious in specialist probe-foragers such as the dunlin (Scolopacidae: *Calidris alpina*), in which the last few millimetres of the bill are overrepresented in the expanded telencephalon (Pettigrew & Frost 1985).

Independent evolution of the scolopacid-type bill-tip organ in kiwi and shorebirds

The scolopacid-type bill-tip organ was known only from the shorebird family Scolopacidae, until its discovery in kiwi by Cunningham et al. (2007). Scolopacid shorebirds belong to the superorder Neognathae, whilst kiwi are paleognathous, meaning their shared bill-tip structure is likely to be an example of convergent or parallel evolution. If the kiwi bill-tip organ is found to be functional for remote-touch, this would suggest that remote touch and the associated bill-tip structures are favoured by a probe-foraging lifestyle. We might then expect them to occur in further families of probe-foraging birds, e.g. ibises - in which an enlarged Bas has already been reported (Stingelin 1965).

Research approach

The aim of this thesis was to discover how widespread the scolopacid-type bill-tip organ is among birds, to compare its anatomy and function in foraging between kiwi and other families of birds possessing it, and to elucidate in detail the foraging behaviours of kiwi.

In order to address this aim, I had several research objectives:

(a) To make a detailed morphological and histological description of the bill-tip organ in kiwi (using brown kiwi, *Apteryx mantelli*) and Scolopacidae shorebirds (using the bar-tailed godwit, *Limosa lapponica baueri*); and to assess the degree of convergence between the two.

(b) To investigate whether the scolopacid-type bill-tip organ, having evolved independently in shorebirds and kiwi, is also present in any other families of birds; and to make a morphological and histological description of the bill-tip organ in any new family in which it might be discovered.

(c) To confirm that the bill-tip organ is functional for remote touch in kiwi and ibises, and discover the extent to which it is used in probe-foraging, in conjunction with which other senses.

(d) To observe and describe the behaviour of free-living brown kiwi, in particular to make inferences about the use of the senses by kiwi in the wild, and the degree of behavioural foraging niche partitioning which could be present between long-billed females and shorter-billed males and juveniles.

Objective (a) is addressed in Chapter 1, where I used traditional histology techniques (paraffin embedding and routine histological stains) to describe the histology of brown kiwi and bar-tailed godwit bill-tip organs, including the structure of the Herbst corpuscles therein. To describe in detail the morphology of the bill-tip organ I used micro-computed tomography to create three dimensional models of the tips of the premaxilla and mandible bones, and the keratin rhamphotheca. This technique allowed

me to trace the branching of channels for nerves and blood vessels supplying the bill-tip organ within the bone of the bill-tips, and to compare the overall structure of the organ between the two species.

Objective (b) is addressed in Chapters 1 and 3. In Chapter 1 I examined 128 museum skeletal specimens of 46 bird species in eight families within four orders (Figure 2). I discovered honeycombs of pits typical of the scolopacid-type bill-tip organ in ibises, and dense pitting that might constitute a simplified version of the scolopacid-type bill-tip organ in two long-billed rails (Rallidae). I followed this up in Chapter 3 with a morphological description of the bill-tip organ in ten ibis species from museum skeletal specimens, plus a histological description of the organ in the Australian White Ibis (*Threskiornis molucca*). While carrying out this work I observed a correlation between ibis bill-tip organ morphology and habitat use, and developed a habitat index based on data compiled by BirdLife International (2008) for each ibis species to test the significance of this correlation. I did not investigate the structure of the bill-tip organ in the rails any further, because one species (*Capellirallus karamu*) is extinct and known only from fossil bones (Tennyson & Martinson 2006) while the other is restricted to Madagascar (*Rallus madagascariensis*) and specimens for histology could not be obtained.

Objective (c) is addressed in Chapters 2 and 4, which present the results from foraging behaviour trials carried out with captive brown kiwi (Chapter 2) and Madagascar Crested Ibises (*Lophotibis cristata urschi*) (Chapter 4). The trials were initially designed for kiwi and were adapted for the ibises after my discovery of the ibis bill-tip organ. They aim to demonstrate (a) that these birds use remote sensing to detect prey and do not rely on direct touch (chance contact of the prey item by the probing bill-tip) alone and (b) that one of the remote senses used to locate prey is remote touch, mediated by the bill-tip organ.

Remote touch detection of an actively burrowing prey is reliant on the interception of seismic vibrations (vibrotactile cues) produced by that prey. Buried/burrowing prey items might also be remotely detected via substrate disturbances on the surface (visual cues), olfaction or taste (chemical cues) or sounds made by burrowing prey (auditory cues).

In order to discover which of these sensory cues were most important to probe-foraging birds for prey-detection, I could have used either of two possible methods. The first involved interfering with specific sensory capabilities of the birds by anesthetising or cutting sensory nerves to show unequivocally the loss of which sense had the most impact on foraging performance. Aside from the ethical implications of such actions, especially when performed on endangered species such as those used in this study, I felt that altering a bird's sensory capabilities in an invasive manner was likely to have unpredictable effects on the bird's overall behaviour, potentially confounding experimental results.

The second option (which I took) was instead to alter the environmental conditions in which birds probed for mealworm prey across a series of trials. I carried this out in such way that each non-visual sensory cue (chemical, vibrotactile or auditory) was isolated in a separate trial by confusing, overwhelming, or removing other cues (see Methods sections of Chapters 2 and 4 for further details of experiment design). Trials were video-recorded and aspects of foraging success under each treatment extracted from the recordings. I could then infer from birds' foraging success under different trial conditions which senses were most important.

In each trial, mealworm prey were presented to the birds buried in trays of a soil substrate. Visual cues were removed in all trials by smoothing the soil surface after burying the mealworms deeply in the tray. In the ibis trials, trays were presented to the birds before the worms had a chance to dig to the surface. In the kiwi trials, trays were placed in the kiwi enclosures at dusk prior to the birds emerging from their sleeping burrows. I attempted to slow the movement of mealworms towards the surface in kiwi trials by burying them below a layer of 1-ply tissue paper. The reduced visual fields of kiwi further make it unlikely they were able to use visual cues to detect prey (Martin et al. 2007).

Chemical cues produced by the mealworms were overwhelmed by mixing the soil substrate with a powder made of ground freeze-dried mealworms at a ratio of 1 part mealworm powder to 9 parts soil. At this soil: mealworm powder ratio the entire tray smelt strongly of mealworms while retaining similar visual and tactile properties to unmixed soil. The chemical composition of freeze-dried mealworms should have been

as similar as practically possible to that of live or freshly killed mealworms although I cannot rule out that the process of freeze-drying may have subtly altered the mealworms' chemical signature. The success of this technique in reducing kiwi prey capture efficiency suggests that it was effective in overwhelming or confusing chemical cues as perceived by the birds.

To mask auditory cues produced by the mealworms burrowing in the soil I first measured how loud these were by making a recording of the sound of mealworms burrowing in an experimental tray using an MP3 player and microphone set-up that had previously been used for recording bird calls. I analysed the amplitude of these sounds (~30dB) using audio-analysis software Raven™. To mask these sounds in the foraging trials I used a broad-band white noise hiss which contained all the frequencies produced by the mealworms as well as a broad range of frequencies above and below these. I also ran a trial in the lab to ensure white-noise playback did not alter mealworm behaviour (presented in Chapter 2).

Auditory masking thresholds have not been experimentally established for kiwi or ibises, so I played back the white noise at the highest level tolerated by the birds (50 – 55 dB). I cannot be certain that white noise at this level would have been sufficient to mask auditory cues produced by the prey. However, white noise masking has been used successfully in the past in similar foraging trials with Australian magpies *Gymnorhina tibicen* (Floyd & Woodland 1985). These and other species which forage for buried prey using auditory cues (e.g. American robins *Turdus migratorius*; Montgomerie & Weatherhead 1997) display distinctive 'head cocking' behaviour when foraging. This behaviour was not observed in kiwi or ibis trials with or without white noise playback.

Vibrotactile cues were removed by killing mealworms by freezing for ~12 hours. Mealworms were presented freshly dead before there was time for decomposition to change their odour signatures. Killed mealworms may have been easier for the birds to discover using direct touch as they would not display the escape behaviour I observed in the live mealworms. I tried to account for this in the kiwi trials by burying killed mealworms in the corners and sides of the plastic grid in the bottom of the foraging tray – locations to which the live mealworms were observed to rapidly move, and in which any left over live mealworms were inevitably found. The immediate presentation of

trays to the ibises reduced the time available for live mealworms to present escape behaviour in these trials.

Objective (d) is addressed in Chapter 5. To collect data for this objective I spent time video-recording free-living brown kiwi in a dense population on Ponui Island, in the Hauraki Gulf, New Zealand. I used handheld infrared cameras and lamps to record the kiwi performing their natural, nocturnal behaviours in the wild, and gathered over 6 hours of usable footage of 25 + different individuals. I typified kiwi behaviours from the footage and described many for the first time. I transcribed the videos frame by frame and assessed the behaviour patterns to make inferences about sensory systems being used by the birds. I compared measures of foraging behaviour, including probing depth and microhabitat use, to investigate whether foraging niche partitioning was occurring between sex and age classes within the population.

Chapter 6 and Appendix 1 represent side branches to the main study. While studying the behaviours of wild kiwi, it came to my attention that the facial bristle feathers of the birds might be important sensory apparatus. A literature search revealed that very little was known about the histology or function of facial bristles in birds generally. I therefore conducted a small histological study of facial bristles in five insectivorous New Zealand birds, including brown kiwi. My aim was to improve my understanding of the sensory behaviour of kiwi, and to add some data to the literature on this topic (Chapter 6). Because this study is related to kiwi sensory systems I have included it as a chapter, rather than an appendix.

I am interested not only in the links between behaviour and anatomy, but also in the conservation of species and the welfare of animals that take part in behaviour studies. On completing data collection for Chapter 5, I realised I had the information necessary to write a paper on the fine-scale behavioural and welfare impacts of radio-tagging on wild brown kiwi (Appendix 1). Appendix 1 also includes data on kiwi exiting burrows collected and analysed by Dr. Isabel Castro, and data on leg injuries caused by transmitters collected by the entire Ponui Island research team (see Preface).

In Appendix 2, I present a copy of a point-of-view paper, currently in press with the Journal of Avian Biology, on which I am second author. The paper is about the roles of

olfaction in birds generally, and kiwi particularly, and is therefore highly relevant to the topic of this thesis (sensory biology of probe-foraging birds, especially kiwi). It is the initial publication from an ongoing study of the uses of olfaction by brown kiwi, conducted by my chief supervisor Dr. Isabel Castro. This paper utilises the video-recordings I made for Chapter 5 and Appendix 1, and also videos I made near the beginning of my PhD when I was trying (unsuccessfully) to train wild kiwi to forage for food I presented to them, as a preliminary to attempting to run the behaviour trials described in Chapter 2 with free-living birds. For further detail on my role in producing this paper, see the Preface.

A simplified phylogenetic tree of modern birds

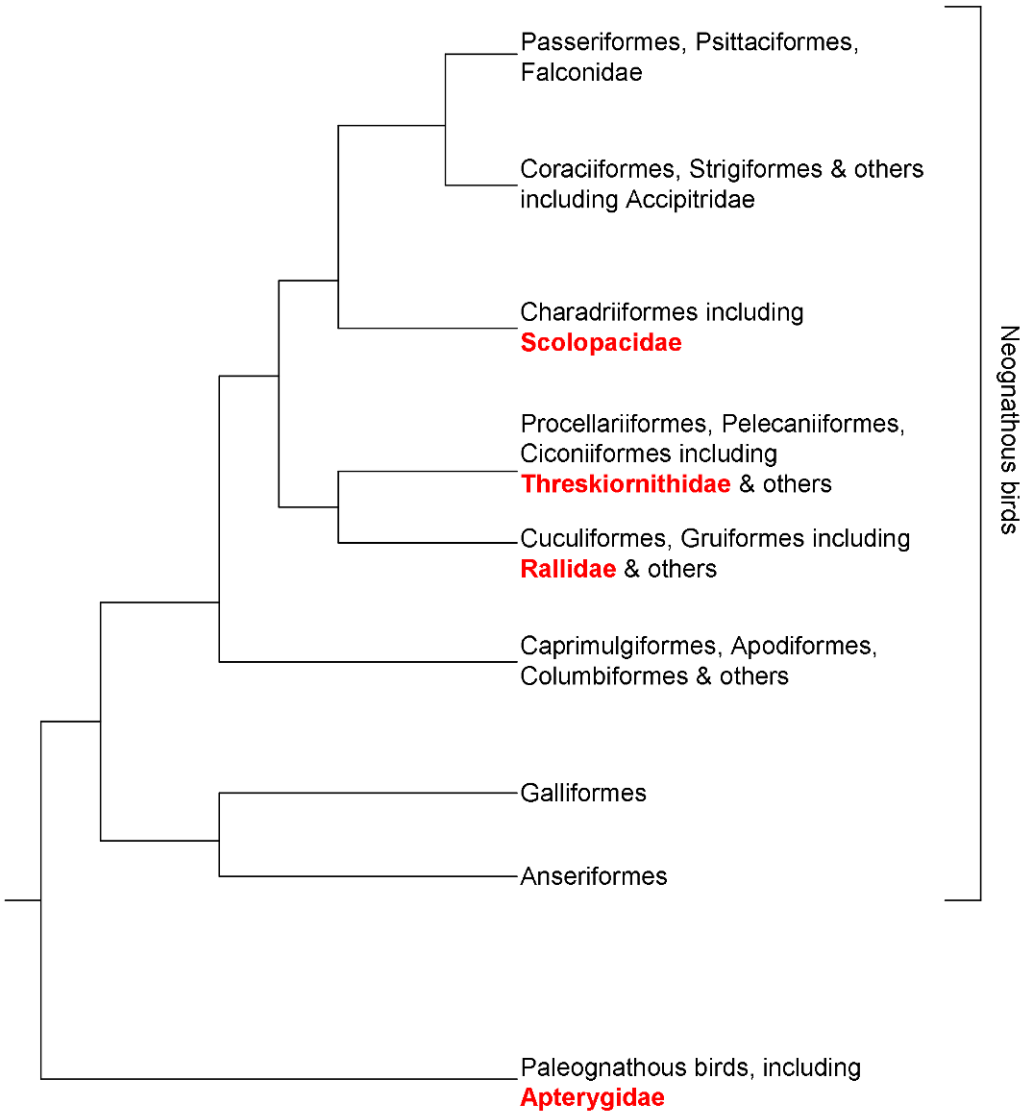


Figure 2: A highly simplified, schematic phylogenetic tree of modern birds showing the positions of the families studied in this thesis in bold, red type. Branch lengths do not reflect genetic distance, but are intended only to show relationships between orders and families. This figure is drawn following the phylogeny published by Hackett et al. (2008).

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Chapter 1

The scolopacid-type bill-tip organ in birds

Chapter references: Cunningham, S. J., Castro, I., and Alley, M. A. (2007) A new prey-detection mechanism for kiwi *(Apteryx* spp.*)* suggests convergent evolution between paleognathous and neognathous birds. *Journal of Anatomy* 211(4): 413 - 502

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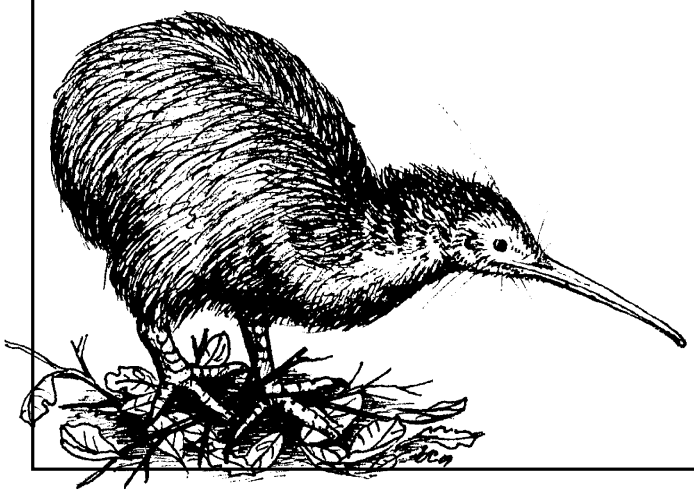

Abstract

Birds that forage by probing into turbid water and/or granular substrates must often rely on senses other than vision to locate food. One such sense is remote-touch, mediated by an organ (the 'scolopacid-type' bill-tip organ) consisting of a distinctive honeycomb of sensory pits in the tip of the premaxilla and mandible, packed with mechanoreceptive Herbst corpuscles. This organ was originally described in shorebirds from the family Scolopacidae. It has recently also been discovered in kiwi (Apterygidae) and ibises (Threskiornithdae), where it has evolved independently. I used museum skeletal collections to discover whether further families of birds might possess the scolopacid-type bill-tip organ, and if it was restricted to probe-foragers. I found honeycombs of sensory pits typical of this organ only in the Apterygidae, Scolopacidae, and Threskiornithidae; although I found dense pitting in bill-tips of two long-billed rails (Rallidae) which might represent a simplified version of the organ. I also carried out a detailed comparative study of the bill-tip organs of brown kiwi (Apterygidae; *Apteryx mantelli*) and bar-tailed godwit (Scolopacidae; *Limosa lapponica*), to investigate the degree of convergence in their bill-tip anatomy. Kiwi and godwit bill-tip organs shared the same basic elements (Herbst corpuscles arranged similarly within honeycombs of cone-shaped sensory pits), but varied significantly in structural detail, particularly of the premaxilla tip and of the Herbst corpuscles themselves. This variation may be due to the different phylogenies of the two species, other sensory specialisations in kiwi, and the differing demands of foraging in terrestrial (kiwi) versus intertidal (godwit) habitats.

Introduction

Avian bills vary in form between species, for the most part reflecting their role in foraging. All contain mechanoreceptive nerve endings, including Herbst and Grandry corpuscles, the location and numbers of which reflect species-specific feeding behaviours (Gottschaldt 1985). For example, seed-eating finches possess mechanoreceptors in parts of the bill involved in the opening of seeds (Krulis 1978); probe-foraging shorebirds (Scolopacidae) possess sensory pits in the bill-tip, packed with Herbst corpuscles, which detect prey movements in soft substrates (Bolze 1968, Gerritsen & Meiboom 1986, Peirsma et al. 1998); and the filter feeding behaviours of some waterfowl are controlled by large numbers of mechanoreceptors in the beak and tongue, especially Grandry corpuscles (Gottschaldt 1985).

Mechanoreceptors in bird bills are innervated by branches of the trigeminal nerve (Dubbeldam & Karten 1978). Tactile feeding specialists (e.g. waterfowl, parrots and Scolopacidae shorebirds) have an enlarged principal sensory nucleus of the trigeminal nerve (PrV) which is the first processing centre in the brain for mechanosensory information from beak, tongue and face (reviewed in Gutiérrez-Ibáñez et al. 2009). Waterfowl, shorebirds and parrots also share concentrations of mechanoreceptors within the bill-tips known collectively in the literature as ‘bill-tip organs’ (e.g. Gottschaldt 1985, Gerritsen & Meiboom 1986). Bill-tip organs are not all identical in structure or function and two distinct types have been so far been described.

The first of these types I have named the ‘psitticid-type’ bill-tip organ. It was first discovered in parrots by Goujon (1869), but also occurs in waterfowl (Gottschaldt 1985). The psitticid-type bill-tip organ consists of rows of ‘touch papillae’ which extend from the deep dermis to the surface behind the keratin tip of the bill in waterfowl (Gottschaldt 1985), and through small holes within the keratin rhamphotheca in the parrot (Goujon 1869). Each papilla is highly innervated with Herbst and Grandry corpuscles (Goujon 1869, Gottschaldt 1985). In addition to the bill-tip organ, waterfowl possess mechanoreceptor-containing pits in the distal parts of the beak bones, and mechanoreceptors within the beak dermis (Berkhoudt 1980). Parrots additionally possess touch papillae in the tongue (Zweers et al. 1994). It seems likely that the

psitticid-type bill-tip organ is used in manipulation of food items (Goujon 1869, Wild 1981), or control of filter feeding in waterfowl (Berkhouldt 1980). Further investigation may show differences between the parrot and waterfowl bill-tip organs that would warrant them being classified as separate structures.

The scolopacid-type bill-tip organ was described in detail in probe-foraging shorebirds (Scolopacidae) by Bolze (1968). It is made up of a honeycomb of sensory pits in the tips of the premaxilla and mandible bones, which become increasingly densely packed distally. Sensory pits may be oval or polygonal in cross section, and contain numerous Herbst corpuscles. Each pit also contains a central bundle of nerve fibres which communicate, though perforations in the base of the pits, with thick branches of the trigeminal nerve running within the beak bones (Bolze 1968, Piersma et al. 1998, Nebel et al. 2005). Recently, a bill-tip organ close to identical in structure has been discovered in two further groups of probe-foraging birds: kiwi (Apterygidae; Cunningham et al. 2007) and ibises (Threskiornithidae; Cunningham et al. in press: *Chapter 3*). Both of these groups have an expanded PrV, suggesting tactile specialisation (Stingelin 1965, Martin et al. 2007). Behavioural studies with shorebird (Gerritsen & Meiboom 1986, Piersma et al. 1998), kiwi (Cunningham et al. 2009: *Chapter 2*), and ibis (*Chapter 4*) species suggest that in all cases the scolopacid-type bill-tip organ is functional for 'remote-touch': the detection of vibrations or pressure disturbances caused by prey items buried within soft substrates.

Remote-touch is most likely to be mediated by the Herbst corpuscles within the bill-tip organ (Zweers & Gerritsen 1997). These consist of a central nerve axon surrounded by specialised Schwann cells which form a lamellated 'inner core'. This inner core is encapsulated by a mucin fluid-filled 'outer zone' containing coiled collagen fibres. The outer zone is surrounded again by a perineural capsule. The collagen fibrils of the outer zone fuse with the lamina of the inner core and also with the perineural capsule so that the inner core is suspended within the fluid-filled capsule (Gottschaldt 1985). Herbst corpuscles are rapidly-adapting mechanoreceptors, sensitive to the acceleration components of vibratory stimuli (Gottschaldt 1985). They can therefore provide information to a probing bird about pressure and vibration signals within the substrate (Zweers & Gerritsen 1997). It is thought that Herbst corpuscle clusters within the sensory pits of the scolopacid-type bill-tip organ may form functional units, made up of

individual corpuscles that fire at different signal intensity thresholds (Zweers & Gerritsen 1997). Herbst corpuscle clusters are densely arranged all around the bill-tips, potentially allowing the bird to measure the intensity difference of a signal from one side of the bill to the other, thus establishing the direction of the signal source (i.e. buried prey item) (Zweers & Gerritsen 1997). The more Herbst corpuscle clusters (i.e. sensory pits) that are present within a bill-tip organ, the greater the accuracy of that organ in pinpointing the direction of a signal (Zweers & Gerritsen 1997).

Shorebirds, kiwi and ibises are not closely related families of birds (Hackett et al. 2008). Therefore the scolopacid-type bill-tip organ and associated remote-touch sense has likely evolved independently in these groups. I used museum skeletal collections to discover whether other birds might also possess this organ, and whether it is restricted to probe-foraging birds. I also carried out a detailed comparative study of the morphology of the bill-tips of a kiwi (Apterygidae; *Apteryx mantelli*) and a shorebird (Scolopacidae; *Limosa lapponica baueri*), to investigate the degree of convergence between two independently evolved bill-tip organs.

Methods

Comparative bill morphology

I examined bills of 126 museum skeletal specimens representing 46 probe-foraging and non-probe-foraging species in eight families (Table 1) both macroscopically and under a light microscope to determine whether sensory pits in the form of a scolopacid-type bill-tip organ (i.e. a dense honeycomb of pits in the bill-tip) were present. I measured skull length (from the bill-tip to the furthest back point of the skull), bill length (upper bill; from the nasofrontal hinge to the tip of the pre-maxilla) and dorsal extent of pitting in the upper bill using Vernier callipers.

I photographed upper and lower bills of specimens dorsally, laterally (left and right) and ventrally, taking care to include all sensory pits visible. For practical purposes, I estimated the numbers of sensory pits present by counting all those visible within the photographs. I reduced double-counting of pits by careful comparison between images to avoid recounting pits that were visible in more than one photograph, however minor

inaccuracies were inevitable and numbers must be taken as best estimates. Specimens examined are held in the collections of Auckland War Memorial Museum, Auckland, New Zealand; Te Papa Tongarewa / Museum of New Zealand, Wellington, New Zealand; San Diego Museum of Natural History, San Diego, USA; the American Museum of Natural History, New York, USA and the British Natural History Museum, Tring, England.

Comparative anatomy of the bill-tip organ in brown kiwi and bar-tailed godwit
One adult female bar-tailed godwit and seven brown kiwi (four adult males, one adult female, and two juveniles – one male and one female) were used to describe and compare the anatomy of kiwi and godwit bill-tip organs. Bill-tips of the godwit and one adult male brown kiwi were scanned using micro-computed tomography (µCT) and also subsequently processed for histology. A second adult male and the adult female brown kiwi were µCT scanned only, and the remaining kiwi specimens (the third and fourth adult males and the two juveniles) were processed for histology only.

The brown kiwi and bar-tailed godwit are protected species under New Zealand law. Therefore, all material was sourced from specimens that had died for reasons unconnected to this project and that were brought to Massey University or Auckland University for necropsy and research purposes. Sample sizes are consequently the maximum that were available to me.

Micro-computed tomography: I trimmed the bill-tips of the godwit and three kiwi (2 adult males, 1 adult female, as described above) for µCT at 15 mm from the tip and wrapped them in cling film to keep the upper and lower bills in the correct (natural) position with respect to one another. The bills were mounted vertically in a SkyScan 1172 Micro-CT Scanner and scanned at 17.3 µm voxel resolution.

I reconstructed 3D models of the bill-tips of the kiwi and the godwit in AMIRA (v3.1; Zuse Institute, http://www.amiravis.com/), using the µCT images. I measured features of the bill-tips from raw µCT images. I used both the models and the raw images to describe the morphology of the premaxilla and mandible bones in detail. For kiwi, the description is based on all three specimens used for µCT, and measurements given are

averages of the three birds ± 1 SD. I use 'proximal' to describe regions of the bill closer to the head, 'distal' to describe regions closer to the bill-tip.

Histology – brown kiwi and bar-tailed godwit

I trimmed the first 14 mm of the bill-tips of the godwit and four brown kiwi (2 adults males, 2 juveniles), and then split the upper and lower bills longitudinally for sectioning in the saggital plane. I trimmed the bill-tip of a 5th kiwi (an adult male, previously µCT scanned) coronally at 3, 6 and 9 mm from the upper bill tip and 2 mm from the lower bill tip (corresponding to the 6 mm upper bill trimming), for sectioning in the coronal plane. I also attempted coronal sections from more proximal portions of the bar-tailed godwit bill but these were unsuccessful due to the fragile nature of the sample. The keratin rhamphotheca of each specimen was softened following Luna's (1968) method, and the trimmed pieces were decalcified using neutral EDTA (Bancroft and Stevens 1982), routinely processed, embedded in paraffin, and sectioned at 3 µm. The sections were stained with haematoxylin and eosin and Masson's trichrome (Luna 1968).

I measured width, length and numbers of Herbst corpuscles per sensory pit from digital photomicrographs saggital sections from each specimen. Measurements were made using the ImageJ (National Institutes of Health 2008) digital image analysis system. I made drawings from the coronal sections of the adult male brown kiwi bill-tip to show the positions of major nerves and blood vessels.

Statistical analyses

Most data were not normally distributed, and were analysed using non-parametric Spearman Rank correlations, Kruskal-Wallis H-tests and Mann-Whitney U-tests. Data on kiwi sensory pit counts from museum specimens were log-transformed to conform to a normal distribution and analysed using a one-way ANOVA.

Data are presented as means ± 1 SD, or means and ranges, unless stated otherwise.

Permits

Kiwi and godwit histology and µCT scanning was carried out under New Zealand Department of Conservation permits: NO-19321-DOA, TT-19804-DOA and WA-25982-RES.

Results

Bill morphology – museum specimens

I found a dense 'honeycomb' of sensory pits, typical of the scolopacid-type bill-tip organ, in 28 probe-foraging species from three families; Scolopacidae, Apterygidae and Threskiornithidae. I found dense pitting in the bills of two long-billed rails (Rallidae), the Madagascar Rail (*Rallus madagascariensis*), a probe-forager; and the extinct Snipe-rail from New Zealand (*Capellirallus karamu*), presumed to have foraged by probing. All other species I examined had either no pitting, or a row of pits running parallel to the cutting edges of the premaxilla and mandible, sometimes accompanied by a sparse scatter of other pits across the bill-tips. These latter species included both probe-foraging and non probe-foraging birds (Fig. 1; Table 1).

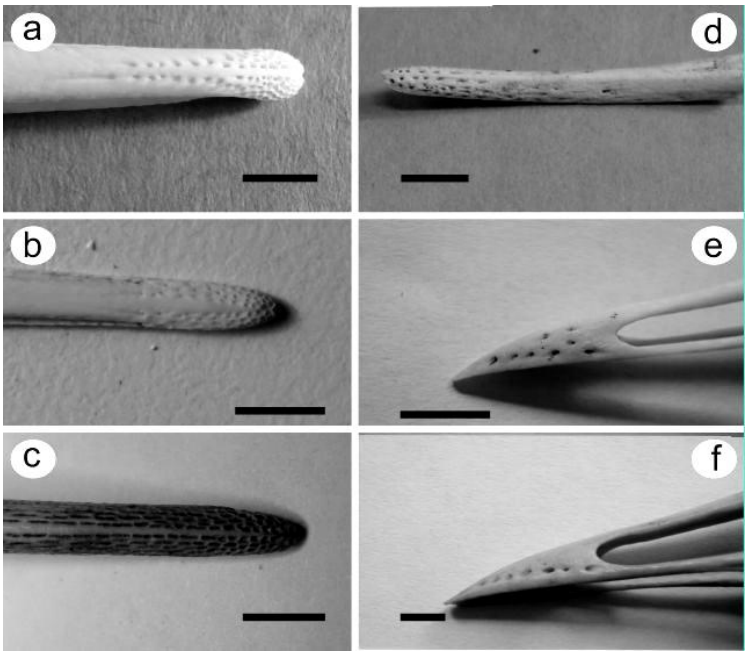

Figure 1: Different types of pitting of the bill-tip in six bird species from three families.
(a) – (c) A 'honeycomb' of sensory pits in the bill-tip, typical of the scolopacid-type bill-tip organ. (a) Apterygidae: Okarito rowi *Apteryx rowi*, premaxilla, dorsal view; (b) Scolopacidae: bar-tailed godwit *Limosa lapponica*, premaxilla, dorsal view; (c) Threskiornithidae: Madagascar crested ibis *Lophotibis cristata*, premaxilla, dorsal view.
(d) Regular, dense pitting at the bill-tip. Rallidae: New Zealand snipe-rail *Capellirallus karamu*, mandible, ventral view.
(e) Few, large pits in a row near the cutting edge of the bill, plus a sparse scatter of pits across the bill-tip. Rallidae: banded rail *Rallus philippensis*, premaxilla, lateral left view.
(f) Few, large pits in a row near the cutting edge of the bill without accompanying scatter of pits. Rallidae: weka *Gallirallus australis*, premaxilla, lateral left view.
Scale bars 5mm. *Photographs: S. Cunningham; © a, b, d, Te Papa Tongarewa The Museum of New Zealand; e, f, British Museum of Natural History, Tring.*

Table 1: Family, species and sample sizes of museum specimens and the number and distribution of pits in the bill-tip. "Row" refers to pits in a single row near the cutting edges of each bill; these species sometimes also had a few, scattered pits elsewhere. Species in bold are commonly or habitually probe-foragers (water or granular substrates). * = specimen/photograph quality too poor to count bony pits.

Family (N species)	Species (N individuals)	Average no. of bony pits (range)	Pit distribution
Apterygidae (5)	***Apteryx australis* (6)**	531.3 (454 – 584)	Honeycomb
	***A. mantelli* (21)**	433.9 (322 – 666)	Honeycomb
	***A. owenii* (8)**	394.7 (366 – 425)	Honeycomb
	***A. rowi* (2)**	645.0 (640 – 650)	Honeycomb
	***A. haastii* (6)**	467.6 (401 – 541)	Honeycomb
Charadriidae (4)	*Elseyornis melanops* (1)	0	None
	Charadrius veredus (1)	4.0	Row
	Vanellus novahollandiae (1)	27.0	Row
	Anarhynchus frontalis (1)	0	None
Scolopacidae (13)	***Calidris alba* (2)**	364.0 (353 – 375)	Honeycomb
	***C. alpina* (3)**	269.0 (206 – 312)	Honeycomb
	***C. canutus* (2)**	292.0 (235 – 349)	Honeycomb
	***C. tenuirostris* (1)**	307.0	Honeycomb
	***Coenocorypha aucklandica* (1)**	1728.0	Honeycomb
	***Coenocorypha pusilla* (1)**	1297.0	Honeycomb
	***Gallinago gallinago* (3)**	741.5 (667 – 816)	Honeycomb
	***Limnodromus griseus* (1)**	468.0	Honeycomb
	***L. scolopaceus* (1)**	257.0	Honeycomb
	***Limosa lapponica* (3)**	217.7 (156 – 263)	Honeycomb
	***Lymnocryptes minimus* (1)**	428	Honeycomb
	***Numenius madagascariensis* (1)**	300	Honeycomb
	***Tringa totanus* (1)**	153	Honeycomb
Recurvirostridae (1)	***Himantopus himantopus* (1)**	0	None
Haematopodidae (1)	***Haematopus ostralegus* (1)**	0	None
Rallidae (11)	†*Cabalus modestus* (11)[1]	14.3	Row
	†***Capellirallus karamu* (1)**	111.0 mandible only	Dense at tip
	†*Diaphorapteryx hawkinsii* (2)[1]	63.5	Row
	Atlantisia rogersi (1)	16.0	Row
	Gallirallus australis (2)	33.5 (10 – 57)	Row
	G. philippensis (4)	20.3 (8 – 33)	Row
	***Rallus aquaticus* (1)**	53.0	Row
	***R. elegans* (2)**	43.0 (37 – 49)	Row
	***R. limicola* (1)**	28.0	Row
	***R. longirostris* (2)**	50.0 (48 – 52)	Row
	***R. madagascariensis* (1)**	108.0	Dense at tip
Threskiornithidae (10)	***Bostrychia carunculata* (3)**	469.5 (417 – 522)	Honeycomb
	***Eudocimus albus* (4)**	1553.0 (1548 – 1558)	Honeycomb
	***E. ruber* (6)**	1317.5 (1211 – 1421)	Honeycomb
	***Lophotibis cristata* (1)**	733.0	Honeycomb
	***Plegadis chihi* (4)**	2402.3 (2137 – 2660)	Honeycomb
	***P. falcinellus* (2)**	2298.5 (1888 – 2709)	Honeycomb
	***Phimosus infuscatus* (1)**	*	Honeycomb
	***Theristicus caudatus* (3)**	394.2 (337 – 423)	Honeycomb
	***T. melanopis* (1)**	*	Honeycomb
	***Threskiornis molucca* (1)**	1009.0	Honeycomb
Upupidae (1)	***Upupa epops* (2)**	0	None

† extinct [1]Possibly probe-foraging

Species with honeycomb pit distribution had a significantly greater number of sensory pits in the bill-tip on average, than did species with other types of pit distributions (Mann-Whitney U-test, W = 689, $p < 0.001$). Within the family Apterygidae, in which sample sizes allowed further data exploration, species varied significantly in the number of pits in the bill-tip (ANOVA on log-transformed data: $F_{3, 31} = 5.835$, $p = 0.003$). A post-hoc Bonferroni test showed that *Apteryx australis* had significantly more sensory pits in the bill-tip than *A. mantelli* ($p = 0.014$) or *A. owenii* ($p = 0.003$), but not significantly more than *A. haastii* ($p = 0.754$). No other significant differences were found between species (Fig. 2). *A. rowi* was not included within the ANOVA because of the small sample size for this species and the fact that the two specimens were siblings (i.e. not independent). These two specimens had higher sensory pit counts than all other kiwi, except some *A .mantelli* individuals (Table 1; Fig. 2). A larger sample size is needed to determine whether these specimens are representative of *A. rowi* generally.

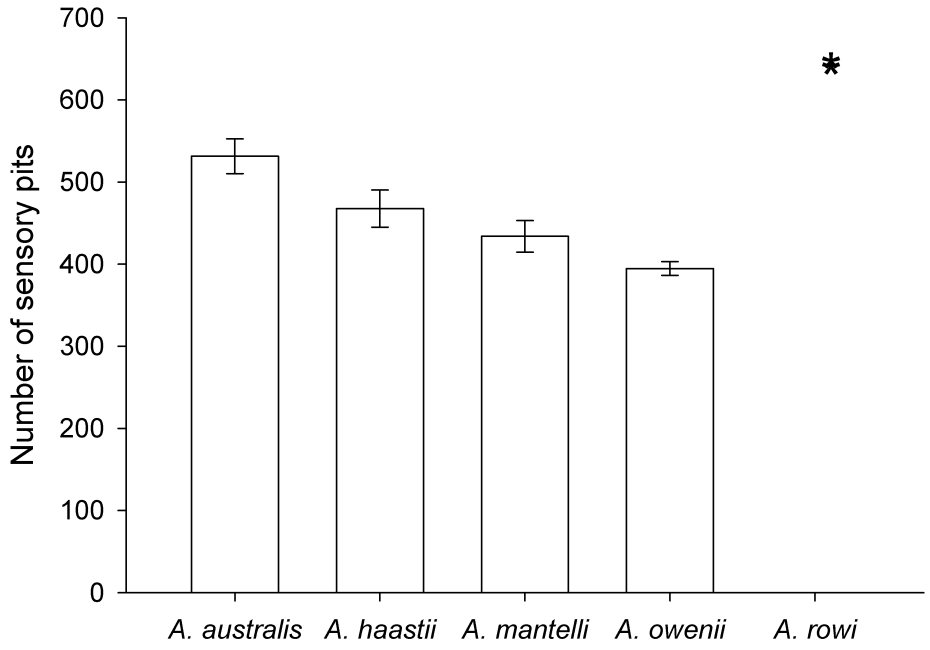

Figure 2: Average number of sensory pits in the bill-tips of four kiwi species (Apterygidae). *Apteryx australis* has significantly more sensory pits in the bill-tips than *A. mantelli* or *A. owenii*. *A. haastii* is not significantly different from *A. australis*, *A. mantelli* or *A. owenii*. There is no significant difference between *A. mantelli* and *A. owenii*. Data from the two *A. rowi* specimens are presented as two asterisks, for comparison. Columns represent the average number of sensory pits, error bars are ± 1 standard error. See Table 1 for sample sizes.

Bar tailed godwit bill-tip structure

The bar-tailed godwit bill-tip structure is described in detail below, from µCT scans in the coronal plane taken at 17.3 µm intervals throughout the bill-tip. In Figure 3 I present every 40th of these scans to illustrate the description. Images in the figure are therefore taken at 0.69 mm intervals.

External morphology & structure of the keratin coat: The upper bill of the godwit overlapped the lower by 2.27 mm. The keratin rhamphotheca of the upper bill extended 1.42 mm beyond the tip of the premaxilla bone; that of the lower bill extended 1.69 mm beyond the mandible bone. The rhamphotheca was rounded at the lateral edges of both upper and lower bills, and there was no lateral overlap of the lower bill by the rhamphotheca of the upper when the beak was closed (Fig. 3) (c.f. kiwi, below).

Morphology of the premaxilla: The godwit premaxillary bone was tripartite in the most proximal segment, extending back from 9 mm from its tip. It consisted of a convex dorsal ramus (2.3 mm wide x 0.5 mm deep at 13 mm from the premaxilla tip) and two ventral rami, left and right (each 1.2 mm wide x 0.4 mm deep at 13 mm from the premaxilla tip). The ventral rami were separated from one another by a 0.6 mm gap, and were positioned at 173º with respect to one another (i.e. almost horizontal) (Fig. 3p).

The outer edges of the dorsal ramus fused with the outer edges of the ventral rami 9.3 mm from the premaxilla tip. Distal from this point, the dorsal ramus developed two processes which extended ventrally, eventually fusing with the inner edges of the ventral rami, 8.9 mm from the tip of the premaxilla (Fig. 3n, o). This fusion created two large channels in the premaxilla, measuring 0.7 x 0.5 mm (left) and 0.6 x 0.5 mm (right). Sensory pits opened proximally (e.g. were angled towards the bill tip) into these channels from all surfaces of the premaxilla bone, except the ventral surface. Numbers of sensory pits in the bill increased towards the tip. At 1.3 mm from the tip of the premaxilla, the two channels began to separate into multiple sensory pits (Fig. 3c, d). At this point, they measured 0.4 x 0.2 mm (left) and 0.3 x 0.3 mm (right).

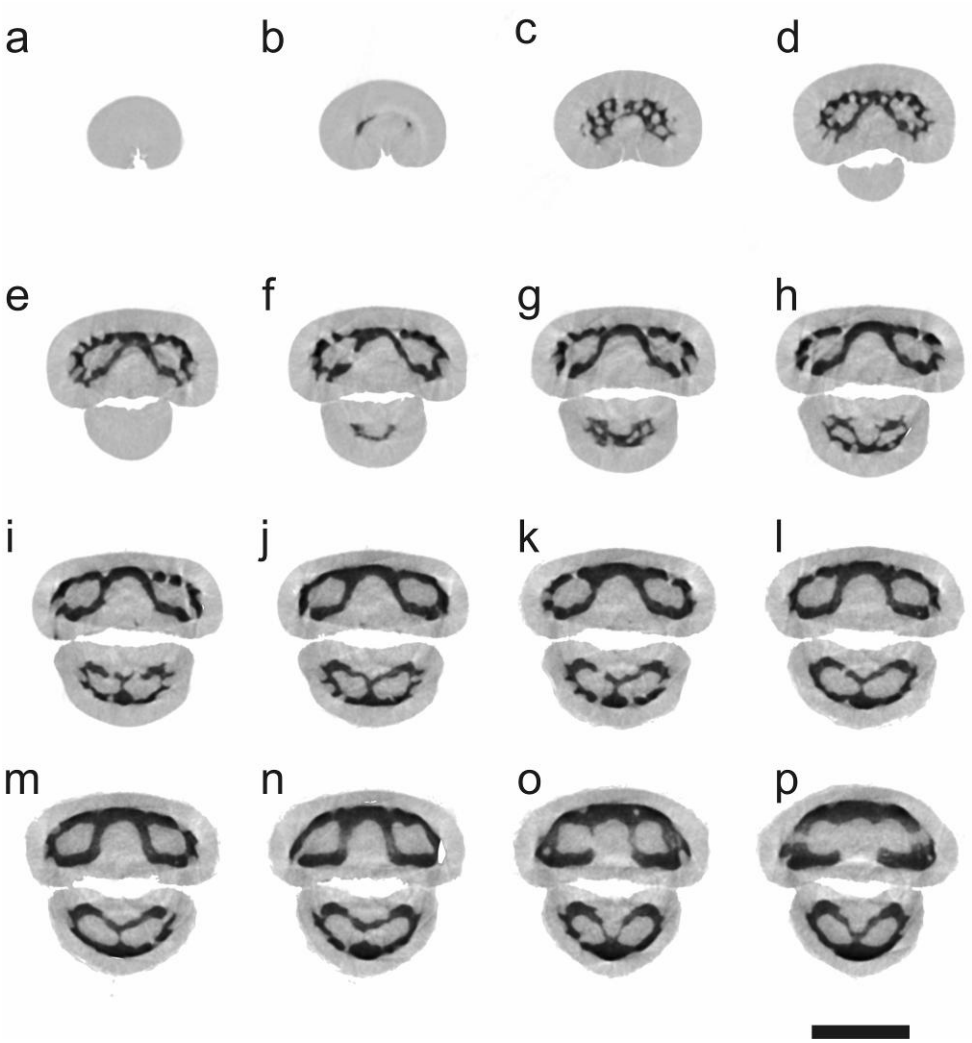

Figure 3: MicroCT coronal sections through upper and lower bill-tips of an adult female bar-tailed godwit, opposed as in life. Consecutive slices are 0.69 mm apart. Pictures read distal ((a) 0.81 mm from the keratin tip of the bill) to proximal ((p) 9.09 mm from the keratin tip of the bill). The keratin tip of the lower bill first becomes visible in (d); the mandible bone itself in (f). Mid-grey areas = keratin and soft tissue, dark-grey to black areas = bone. Scale bar = 2 mm.

Morphology of the mandible: At 10 mm from the mandible tip, the mandible bone was U-shaped and contained two, wide channels, each measuring 5 mm x 7 mm. The channels ran distally within the mandibular bone and sensory pits communicated with them from all sides, becoming more numerous towards the tip (Fig. 3h - p). The channels separated into multiple sensory pits 1.5 mm from the tip, and measured 0.2 x 0.2 mm (left) and 0.3 x 0.2 mm (right) at this point (Fig. 3g, h).

Brown kiwi bill-tip structure

Bill-tip structure was remarkably consistent between the three kiwi specimens scanned and there were no notable differences between the two males and the female. In Fig. 4, I present every 40th coronal µCT scan taken of the bill-tip of one of the adult male kiwi, to illustrate the description of brown kiwi bill-tip structure given below. Images within the figure are taken at 0.69 mm intervals through the kiwi bill-tip. Figures 5 and 6 are drawn from coronal histology sections of the same adult male brown kiwi bill-tip and show the positions of nerves and blood vessels.

External morphology and structure of the keratin coat: The tip of the upper bill was bullet-shaped and streamlined, overlapping (by 5.57 ± 0.74 mm) and protecting the lower bill, which was tucked seamlessly beneath. The keratin rhamphotheca of the upper bill extended 1.27 ± 0.36 mm beyond the tip of the premaxilla bone; that of the lower bill extended 0.62 ± 0.29 mm beyond the mandibular bone. The downward facing nares were shielded dorsally by curtains of keratin (Fig. 4g- k). The keratin of the upper bill extended ventrally at the lateral edges, overlapping a similar keratinaceous dorsal extension at the lateral edges of the lower bill, and creating a close seal between upper and lower bills when the beak was closed (Fig. 4h-p, Fig. 5a).

Morphology and histology of the premaxilla: The morphology of the most distal 13 mm of the kiwi premaxilla was more complex than that of the godwit. At the proximal, cut edge of the three kiwi bill-tips µCT-scanned (13.1 ± 1.2 mm from tip of the premaxilla), the premaxillary bone was in three parts: a dorsal ramus above the olfactory canals (convex, 3.6 ± 0.1 mm wide x 0.5 ± 0.1 mm deep); and ventral left and ventral right rami (below the olfactory canals, flat, 2 ± 0.0 mm wide x 0.9 ± 0.0 mm deep at the outside edges, tapering to 0.3 ± 0.0 mm deep at the inner edge). The ventral rami were positioned at 192.3 ± 6.7º to one another (e.g. close to horizontal) and were separated by a 0.2 ±0.1 mm gap (Fig. 4p). Histological coronal sections of a single male kiwi showed that two large nerve branches (presumably of the trigeminal nerve) and an artery travelled along the upper bill, between the ventral rami of the premaxilla, and a further bundle of three nerve branches accompanied by a vein also ran along the bill, dorsal to the outer edge of each of the ventral rami (Fig. 5a).

A double row of pits were visible in the µCT scans of all three kiwi beaks, running proximally along the upper bill from the bill-tip organ. These pits opened distally into two narrow, longitudinal channels ('dorsal channels') within the dorsal ramus of the premaxilla. The dorsal channels each measured 0.2 ± 0.0 mm in diameter, and did not extend proximally beyond the most proximal dorsal sensory pit. Many other, smaller channels also perforated all three rami of the premaxilla.

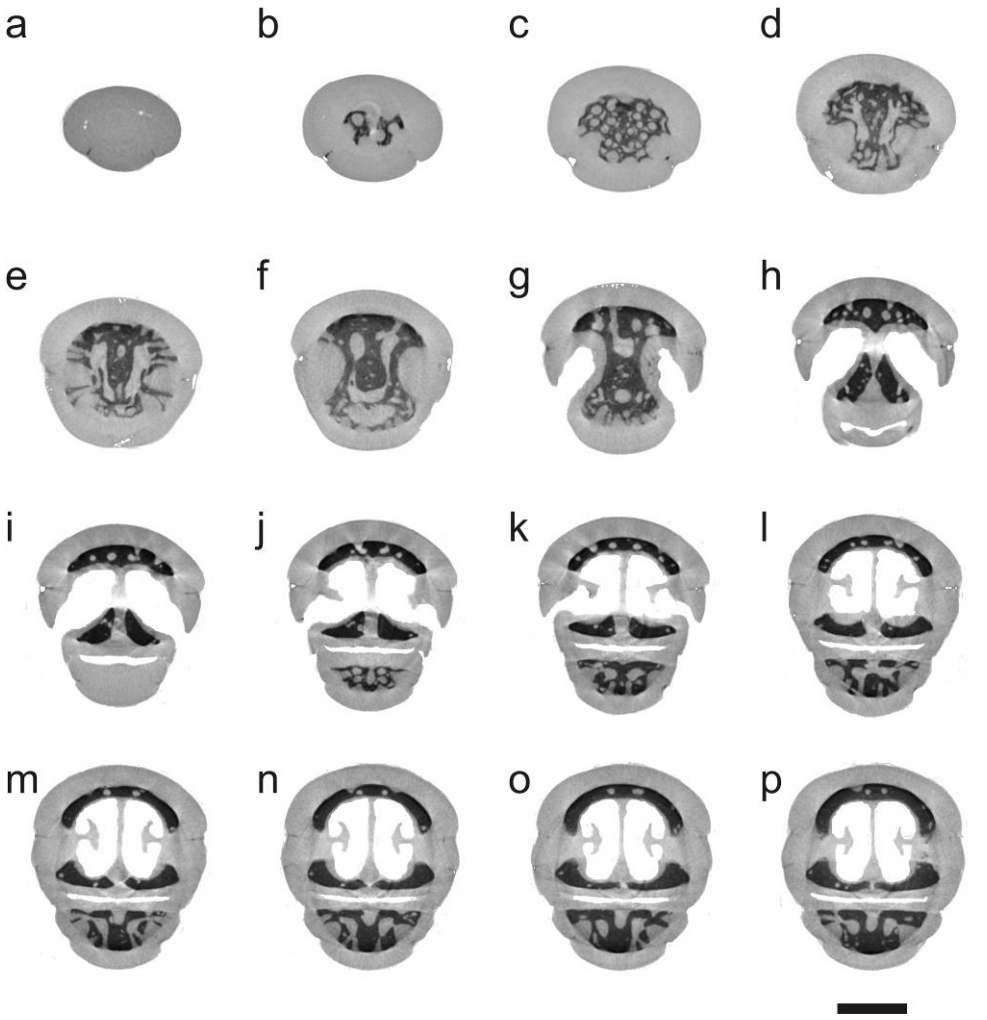

Figure 4 (a) - (p): MicroCT coronal sections through the upper and lower bill-tips of an adult male brown kiwi, opposed as in life. Consecutive slices are 0.69mm apart. Pictures read distal ((a) 0.22 mm from the keratin tip of the bill) to proximal ((p) 8.50 mm from the keratin tip of the bill). The keratin tip of the lower bill first becomes visible in (h). The mandible bone itself is first visible in (j). Cross sections of the nares are visible (g) – (k). The nasal canals are visible in all images proximal to these. The paired dorsal nerve channels are visible in the dorsal ramus of the premaxilla from (g) – (p). In (g), the circular ventral channel is clearly visible in the premaxillary bone. The central channel is present above this, and is connected by a narrow opening to the left dorsal channel and from there to a dorsal sensory pit. Note that the tip of the premaxilla bone (upper bill) is extensively honeycombed forward of the nares and that the tip of the lower bill sits directly below the narial openings. Mid-grey areas = keratin and soft tissue, dark-grey to black areas = bone. Scale bar = 2 mm.

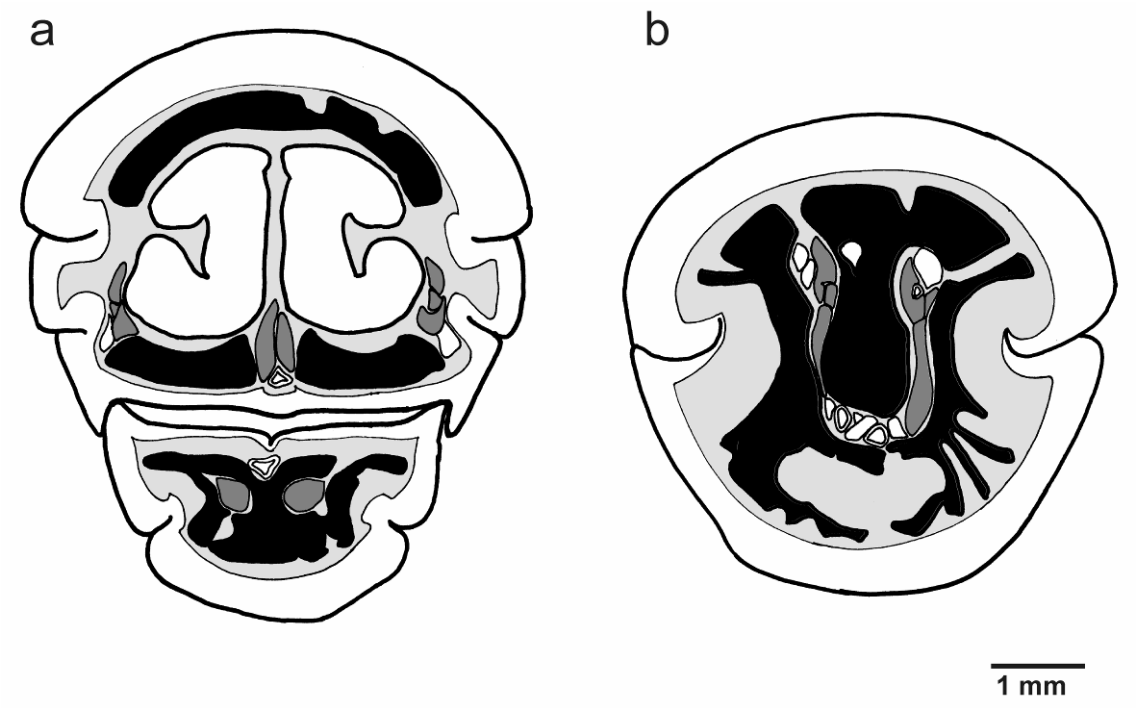

Figure 5: Diagram of two coronal sections through a brown kiwi beak, taken at: (a) 9 mm from the upper bill-tip (showing upper bill above, lower bill beneath); (b) 6 mm from the upper bill-tip (upper bill only, sectioned through the sensory pad area forward of the lower bill-tip). Bold lines indicate the outer surface of the keratin layer, finer lines indicate the junction between the dermal and keratin layers and the outlines of major blood vessels and nerves. Black areas represent the bones of the premaxilla and mandible. Dark grey shaded areas represent cross sections through the major nerves. Areas of soft tissue are shaded pale grey, the keratin and major blood vessels are left white. In (a), the upper bill is perforated by the two nasal passages, bordered with bold lines and coloured white.

The narial openings extended into the olfactory canals between 7.1 ± 0.4 mm and 4.3 ± 0.5 mm from the tip of the premaxilla bone. Through the nares, the ventral rami of the premaxilla were deep and narrow (0.8 ± 0.0 mm wide x 1.3 ± 0.2 mm deep) and angled up towards each other at 59.3 ± 5.0 º (Fig. 4h). The ventral rami eventually fused medially 4.7 ± 0.4 mm from the tip of the premaxilla. Towards the distal end of the nares, the dorsal ramus of the premaxilla developed a central process which extended ventrally to meet the fused ventral rami. Dorsal and ventral rami fused 4.2 ± 0.2 mm from the premaxilla tip. The fusion of the rami left a circular channel ('central channel') for nerve and blood vessel entry to the distal bill-tip organ (Fig. 4g). This channel measured 0.7 ± 0.1 mm in diameter. A second, ventral circular channel ('ventral channel') developed between the fused ventral rami, becoming completely enclosed in bone 4.2 ± 0.1 mm from the tip of the premaxilla. This channel measured 0.5 ± 0.1 mm in diameter (Fig. 4g). The two dorsal channels had widened to 0.3 ± 0.0 x 0.4 ± 0.1 mm in diameter each by the point at which they entered the symphysis of the premaxilla.

The number of sensory pits in the dorsal surface of the premaxilla increased steadily distal of 5.2 ± 1.1 mm from the tip of the premaxilla. Sensory pits became visible in the ventral surface (the 'sensory pad' of the bill-tip organ, Cunningham et al. 2007) distal of 4.2 ± 0.3 mm.

At 3.8 ± 0.2 mm proximal from the tip of the premaxilla, the central channel split into two branches, measuring 0.7 ± 0.2 x 0.4 ± 0.5 mm (left) and 0.7 ± 0.2 x 0.3 ± 0.5 mm (right). These two branches fused with the left and right dorsal channels, forming two long, narrow slots within the premaxilla symphysis. Distal from this point, the ventral channel broadened and became U-shaped, while the two branches of the dorso-central channel lengthened vertically. All three channels opened broadly into numerous sensory pits. At 3.2 ± 0.1 mm from the tip of the premaxilla, the two dorso-central channels fused with the broad ventral channel, creating a large U-shaped cavity within the premaxilla bone (Fig. 4f). Each of the arms of the 'U' contained large, laterally flattened nerve branches; the bottom of the 'U' contained numerous blood vessels (Fig. 5b). The club-shaped block of bone in the centre of the cavity lengthened distally to meet the bone of the sensory pad area at 3.0 ± 0.3 mm proximal to the tip of the premaxilla, dividing the cavity into two vertically elongated slots measuring 1.9 ± 0.1 x 0.3 ± 0.1 mm (left) and 1.9 ± 0.2 x 0.3 ± 0.1 mm (right) (Fig. 4 d, e). These two slots began to branch off into individual sensory pits at 2.5 ± 0.2 mm from the tip of the premaxilla, and completely finished this process by 1.6 ± 0.2 mm from the tip of the bone (Fig. 4c). At this point, longitudinal and transverse sections of 45 ± 1.2 sensory pits were visible.

Each sensory pit contained 1- 2 central nerve bundles and blood vessels, surrounded by Herbst corpuscles (Fig. 6). The soft tissue directly beneath the dermal layer on all sides of the upper bill-tip was abundantly supplied with small capillaries (Fig. 6).

Morphology and histology of the mandible: The mandibular bone contained two parallel, wide channels carrying long nerve branches (presumably of the mandibular ramus of the trigeminal nerve) (Fig. 4p, also Fig. 5a). These channels measured 0.6 ± 0.1 x 0.4 ± 0.0 mm (left) and 0.6 ± 0.1 x 0.4 ± 0.1 mm (right) at 6 mm proximal to the tip of the mandible, and tapered to 0.4 ± 0.0 x 0.3 ± 0.0 mm (left) and 0.4 ± 0.1 x 0.2 ± 0.1 mm (right) by 1.3 ± 0.2 mm proximal to the tip; after which they divided into multiple individual sensory pits. Rows of sensory pits communicated with the nerve channels

from the dorsal, lateral and ventral surfaces of the mandibular bone. The bone itself contained a deep, medial dorsal groove which carried an artery (Fig. 5a).

As in the upper bill, sensory pits contained 1- 2 central nerve bundles and blood vessels, surrounded by Herbst corpuscles.

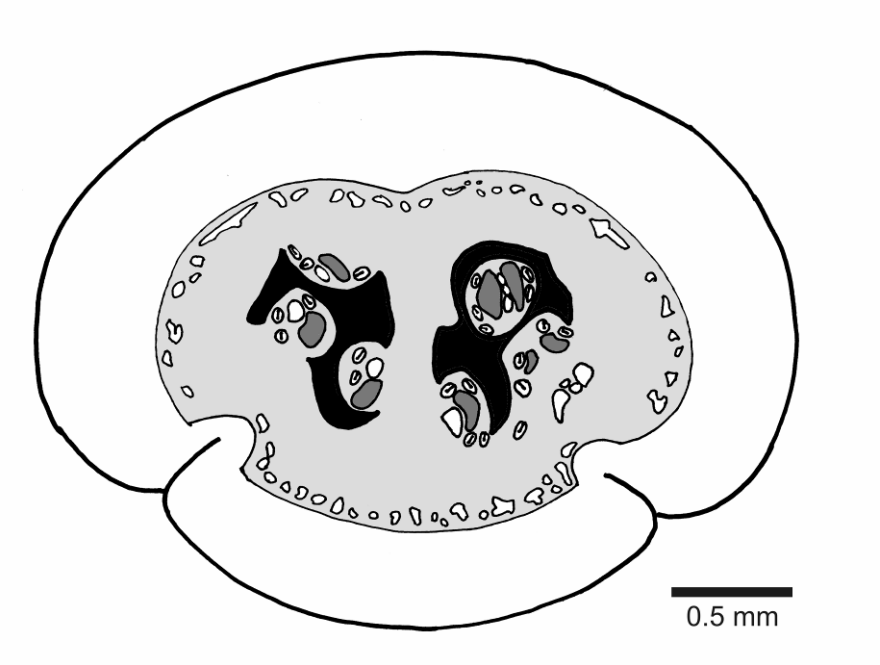

Figure 6: Diagram of a coronal section through a brown kiwi beak, 3 mm from the bill-tip. Bold lines indicate the outer surface of the keratin layer (white), finer lines indicate the junction between the dermal and keratin layers and the outlines of blood vessels (also white), nerves (dark grey) and Herbst corpuscles (white, with a central black line to represent the nerve axon). Black areas represent the premaxilla bone, areas of soft tissue are shaded pale grey. Note the large numbers of capillaries near the junction of the keratin and dermal layers.

Histology of sensory pits of bar-tailed godwit and brown kiwi

Herbst corpuscles were present within bill-tip sensory pits in the godwit and all five kiwi specimens sectioned for histology (Fig. 7). In both species, the Herbst corpuscles were aligned in various directions, and arranged along the sides of the pits. Nerve fibres and blood vessels were present in the centre of each pit (Fig. 7a & b). In saggital section, large nerves were also visible running down the godwit premaxilla and mandible, presumably within the bony channels described above. 10.27 ± 5.59 Herbst corpuscles were visible per bill-tip sensory pit in 3µm saggital sections of the godwit bill tip (n =

15 sensory pits). Significantly fewer Herbst corpuscles were visible per sensory pit in 3µm saggital sections of kiwi bills: 6.32 ± 2.38 Herbst corpuscles per pit, n = 38 pits, 4 kiwi (the 5th was sectioned coronally); Kruskal Wallis H = 5.91, df = 1, p = 0.015. There were no significant differences in the number of Herbst corpuscles visible per pit between individual kiwi (Mann-Whitney U-tests, all p > 0.05).

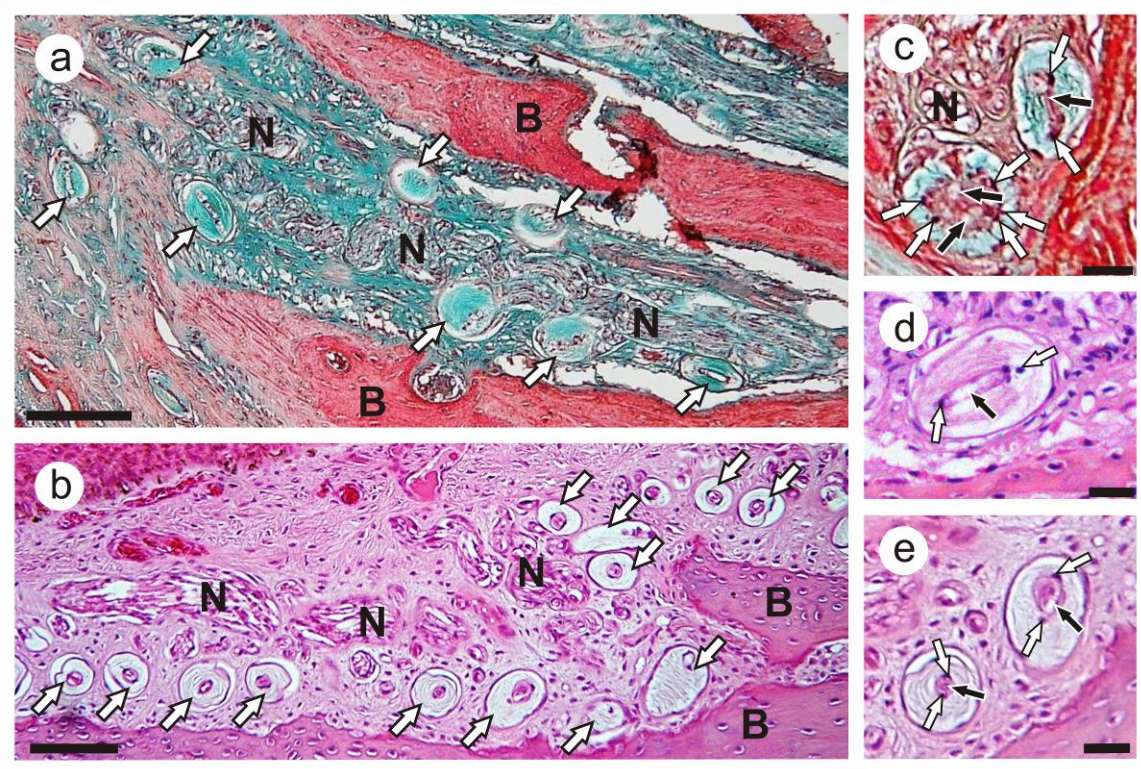

Figure 7 (a) and (b): Saggital sections through (a) a deep sensory pit in the brown kiwi premaxilla and (b) a shallow sensory pit in the bar-tailed godwit mandible. White arrows indicate Herbst corpuscles. B = bone, N = nerve tissue. Sections are stained with (a) Masson's trichrome and (b) haematoxylin and eosin. Scale bars = 100 µm.
(c): Two adjacent Herbst corpuscles in a sensory pit in the brown kiwi mandible, stained with Masson's trichrome. The lower corpuscle is sectioned longitudinally, showing the unusual disc shape of the axon, surrounded by the red-staining inner core. The upper corpuscle is in cross section. The outer zones of the two corpuscles are stained blue-green, indicating their composition of collagen fibrils. Black arrows indicate position of the central Herbst corpuscle axon; white arrows indicate Schwann cell nuclei of the inner core. Scale bar = 20 µm.
(d) and (e): Cross sections of Herbst corpuscles from (d) brown kiwi and (e) bar-tailed godwit bill-tip organs. Black arrows indicate position of the central Herbst corpuscle axon; white arrows indicate Schwann cell nuclei of the inner core. Sections are stained with haematoxylin and eosin. Scale bars = 20 µm.

Godwit: Herbst corpuscles in the godwit bill-tip organ were very similar in structure to those found in other birds. They consisted of a central axon which was circular to oval in cross-section and was surrounded by an inner core formed by Schwann cells.

Schwann cell nuclei lined up in two neat rows along opposite sides of the axon, so that generally one only was visible in cross section on either side (Fig. 7e). The whole was surrounded by a wide, pale-staining outer zone and enclosed in an outer capsule (Fig. 7e).

Kiwi: Herbst corpuscles in the kiwi bill differed from the godwit in that they had very broad, flattened axons, which formed elongate, flat-sided oblongs in cross section and circular discs in longitudinal section (Fig. 7c). Cross sections of kiwi Herbst corpuscles also showed that the Schwann cell nuclei of the inner core were aligned along the short sides of the axons, and more than one nucleus was often visible at either end of the axon (Fig. 7c, d). Because of the shape of the axon, the inner core reached through nearly to the outer capsule at the short sides. The distance between the inner core and the capsule was wider along the long sides of the axon (Fig. 7c, d).

The outer zones of Herbst corpuscles in the kiwi bill stained more strongly with haematoxylin and eosin than those in godwit bills (Fig. 7 d & e), suggesting that the kiwi Herbst corpuscles had expanded inner cores. When stained with Masson's trichrome, however, the outer zones of the kiwi Herbst corpuscles stained a deep blue-green, indicating a composition of collagen fibrils, typical of the outer zone of Herbst corpuscles generally (Fig. 7c). The inner core area around the axon stained red with Masson's trichrome (Fig. 7c). The collagen fibrils of the outer zone in kiwi may be denser than in the godwit, explaining the stronger staining.

Herbst corpuscles in the kiwi bill-tip organ were on average longer (kiwi 78.88 ± 27.65 µm, godwit 63.91 ± 15.92 µm) but not wider (kiwi 56.48 ± 23.73 µm; godwit 53.63 ± 11.52 µm), in cross section than Herbst corpuscles in the godwit bill-tip organ, Kruskal-Wallis test on length, $H = 8.23$, $df = 1$, $p = 0.004$; and width, $H = 0.11$, $df = 1$, $p = 0.738$.

There was significant inter-individual variation in both Herbst corpuscle length and width between the 4 kiwi specimens: length, Kruskal-Wallis $H = 75.03$, $df = 3$, $p < 0.001$; width, Kruskal-Wallis $H = 69.62$, $df = 3$, $p < 0.001$.

Discussion

Comparative morphology of museum specimens

I found honeycombs of sensory pits typical of the scolopacid-type bill-tip organ only in families from which it has previously been described: kiwi (Apterygidae), ibis (Threskiornithidae) and probe-foraging shorebirds (Scolopacidae) (see Bolze 1968, Peirsma et al. 1998, Nebel et al. 2005, Cunningham et al. 2007, Cunningham et al. in press: *Chapter 3*). In kiwi, there was significant variation between species in the number of pits within the bill-tip honeycomb, perhaps reflecting differences in ecology, or simply differences in overall body or bill size (*Apteryx australis* had higher numbers of pits than most other species, and has one of the longest bills of all kiwi: female bills reach over 14 cm, Robertson et al. 2003).

I found a dense arrangement of pits in the bill-tips in two long-billed probe-foraging rails (Rallidae: the Madagascar Rail *Rallus madagascariensis* and the extinct New Zealand Snipe-rail *Capellirallus karamu*), suggestive of a simplified version of the scolopacid-type bill-tip organ. The presence of sensory pits in the bill of *Capellirallus karamu* was first mentioned by Olson (1975), but no further description of these was given. Histological material was not available for these species, so I was unable to determine whether the pits in the bill-tips contained Herbst corpuscles and might be functional for remote-touch. Even if Herbst corpuscle clusters were present, the comparatively low number of sensory pits would likely mean that the rail bill-tip organ was less directionally accurate than a true scolopacid-type bill-tip organ (Zweers & Gerritsen 1997). However, sensory pitting of this type might still confer improved prey capture success over a bill-tip unable to gather any remote touch information at all. The dense pitting in the bills of these two rails may therefore represent an intermediate stage in the evolution of the scolopacid-type bill-tip organ, or a specific adaptation to the foraging strategies of these species.

In some other probe-foraging rails (e.g. *Rallus aquaticus*), and in a number of non probe-foraging birds, I found a row of a few, large pits positioned along the cutting edges of the premaxilla and mandible, sometimes accompanied by a sparse scatter of

other pits in the bill tips. These types of pits were described from non probe-foraging shorebirds (Charadraiidae) by Bolze (1968) who showed that they were channels for nerves and blood vessels. Herbst corpuscles do not form clusters within these pits (Bolze 1968), and the arrangement cannot therefore be called a scolopacid-type bill-tip organ. Such a bill-tip is unlikely to be functional for remote-touch, and suggests that other senses are predominantly used in foraging by these birds (e.g. vision or direct touch: Hulscher 1976; Martin & Piersma 2008).

We found no pits in the bill-tips of an oystercatcher (*Haematopus ostralegus*), stilt (*Himantopus himantopus*) and two hoopoes (*Upupa epops*). Specimen quality may be responsible for our inability to discover any scattered pits in the bill-tips of these birds. The honeycomb pitting of the scolopacid-type bill-tip organ is unmistakeable and is even visible through the keratin rhamphotheca in some museum specimens. Unless the bill-tip was lost, or the keratin thick or heavily pigmented (not the case in the specimens mentioned above), it is unlikely that I would have failed to detect the scolopacid-type bill-tip organ, were it present.

Comparative morphology of godwit and kiwi bill-tips

Kiwi and godwit bill-tip organs both consist of honeycombs of sensory pits, cone-shaped in long section, in the tips of both premaxillary and mandibular bones. In both species, the sensory pits contain clusters of Herbst corpuscles, which are arranged around the sides of each pit, surrounding central nerve bundles and blood vessels. The sensory pits communicate with channels running within the beak bones via broad perforations in their bases. These channels contain blood vessels and thick branches of what are presumably rami of the trigeminal nerve. Despite these broad similarities, there are many differences in bill-tip structure between the two species, as discussed below.

Bill-tip structure: The symphysis of the three rami of the premaxilla bone occurred closer to the bill-tip in kiwi (~4 mm proximal to the bill-tip, immediately distal of the nares) than in the godwit (~9 mm proximal to the bill tip). This difference may be due to the very distal position of the nares in kiwi, which appears to be the result of the extremely highly developed olfactory system in these unusual birds (e.g. see Wenzel

1971). The symphysis of the kiwi premaxilla contained a complex internal arrangement of channels which fused to form a large U-shaped cavity distal to the nares. The bill-tip sensory pits communicated with this cavity, which contained broad branches of the trigeminal nerve. In the godwit, the internal structure of the premaxilla symphysis was simpler, containing two parallel channels communicating with dorsal and lateral sensory pits. Unlike the kiwi, the godwit did not possess a honeycomb of sensory pits (a 'sensory pad' Cunningham et al. 2007) in the ventral tip of the premaxilla.

Kiwi use the sensory pad of upper bill-tip extensively to investigate the terrain ahead when walking in forested habitats and to investigate obstacles and novel objects in their environment (Castro et al. in press: *Appendix 2*; *Chapter 5*). Kiwi also use their bill-tip organ for remote touch prey-detection (Cunningham et al. 2009: *Chapter 2*). The kiwi bill-tip organ therefore seems to be of dual utility: not just used for detecting vibrations from prey but also functioning like a hand or an organ of touch – perhaps in the manner of a blind man's cane (Haeusler 1923).

When closed, the overlapping lateral extensions of the upper and lower keratin rhamphotheca in kiwi created a tight seal between the bills, with the tip of the lower bill tucked seamless beneath that of the upper. Kiwi probe with their bills closed (S. Cunningham pers. obs.). The bullet-shaped tip, close sealing of the upper and lower bills, and shielded nares are probably adaptations to probing in sometimes hard soil substrates: preventing soil from entering the mouth or nares, stopping the bill-tips from being forced open during probing, and protecting the potentially weaker lower mandible (as suggested by McLennan 1990). In the godwit, the rounded lateral edges of the keratin rhamphotheca mean the bill-tips are less streamlined than in kiwi, and the lower bill is protected to a lesser extent by the upper bill during probing. Godwits probe within soft sand and mud sediments in intertidal areas (Johnsgard 1981) and have a distally rhynchokinetic beak (Zusi 1984). Differences in the external morphology of kiwi and godwit bills probably reflect the godwit's need for greater flexibility in the bill-tips, and the lesser forces exerted on the godwit bill when probing in generally softer substrates than those encountered by kiwi.

Herbst corpuscle structure: Herbst corpuscle structure differed significantly between kiwi and the godwit. In the godwit, Herbst corpuscles were circular in cross section with

a cylindrical central nerve axon. Their outer zones consisted of collagen fibrils which stained palely under haematoxylin and eosin. In the kiwi, Herbst corpuscles tended to be ovoid in cross section, and were significantly longer (although not significantly wider) than those of the godwit. The ovoid shape of the kiwi Herbst corpuscles was probably due to their unusual broad, laterally flattened, disc-shaped central axon. Collagen fibrils of the outer zones in the kiwi Herbst corpuscles seemed denser than those of the godwit, as they stained more intensely with haematoxylin and eosin.

Species-specific differences in Herbst corpuscle structure are not unusual, and structural differences between Herbst corpuscles in different areas of the same birds' body have also been documented (Saxod 1978). Nonetheless, the godwit bill-tip Herbst corpuscles appeared more similar to 'typical' Herbst corpuscles in the bill-tip organs of other probe-foraging birds (e.g. red knot, Piersma et al. 1998; and Australian white ibis, Cunningham et al. in press: *Chapter 3*) and also more similar to Herbst corpuscles from facial bristle feather follicles in kiwi (*Chapter 6*), than did the kiwi bill-tip Herbst corpuscles.

The differences in structure between kiwi and godwit bill-tip Herbst corpuscles are likely to have functional implications. Pressure wave transmission is unpredictable in granular media such as soil (Liu & Nagel 1992), and vibration signals in soil substrates are therefore likely to attenuate more rapidly than those in water or saturated sand or mud. Perhaps the broadened axons and dense outer zones of kiwi bill-tip Herbst corpuscles improve the detection of vibrotactile cues under soil substrate conditions, whereas the cylindrical axons and looser outer zones of the bill-tip Herbst corpuscles of godwits, other shorebirds, and ibises, function better for detecting vibrations in water and wet sand. Kiwi use their bill-tip for exploring their surroundings (*Chapter 5*) as well as for remote touch, therefore the unusual structure of their Herbst corpuscles may also reflect this dual use.

Concluding remarks

The scolopacid-type bill-tip organ is present in three unrelated families of birds (Scolopacidae, Apterygidae and Threskiornithidae), and is possibly present in a simplified form in a fourth family (Rallidae). This suggests that the bill-tip organ and associated remote touch sense are strongly favoured by a probe-foraging life style. All

birds possess Herbst corpuscles within their bill-tips (Gottschaldt 1985) and many have at least some pits within the beak bones for the passage of nerves and blood vessels (Bolze 1968). In order to develop an organ sensitive to vibrotactile cues, a probe-foraging bird must group the bill-tip Herbst corpuscles into functional clusters and protect these within numerous bony pits in the bill-tip, a process described in detail for shorebirds by Zweers & Gerritsen (1997). My data suggest that this process occurs fairly readily in probe-foraging birds, resulting in independently evolved bill-tip organs (like those of the kiwi and godwit) that are remarkably similar in morphology, although they may differ in fine details of structure.

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Chapter 2

The relative importance of olfaction and remote touch in prey-detection by brown kiwi

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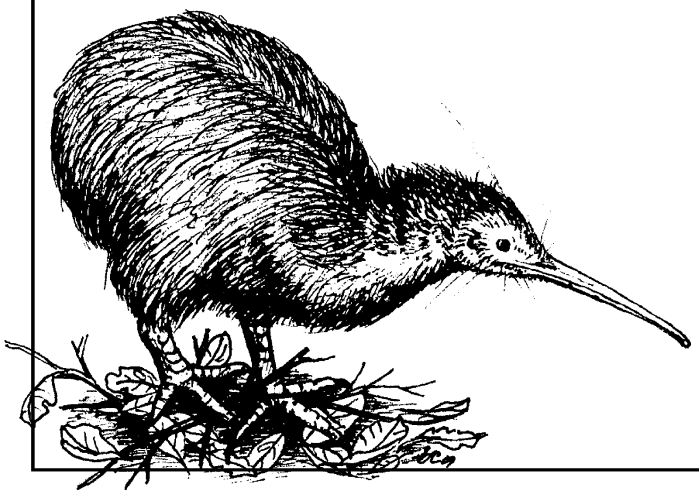

Abstract

Birds that forage by probing in sand, soil or mud substrates must often use senses other than vision to find their prey. Kiwi (Apterygidae) are nocturnal probing birds inhabiting forested areas in New Zealand. Their visual sense is reduced, but they have a highly developed sense of smell, a bill-tip organ similar to that found in Scolopacidae shorebirds, which may be used to detect vibrotactile cues produced by burrowing prey (remote touch), and prominent ear openings. We designed a foraging experiment presenting mealworm prey to eight captive North Island brown kiwi, *Apteryx mantelli*, under a variety of trial conditions to discover whether they were using hearing, olfaction, remote touch, or direct touch (chance alone), singly or together, to find prey. Kiwi were most efficient at finding prey using olfaction alone or in combination with other cues, but switched to locating prey with lower efficiency using remote touch, in the absence of olfactory cues. They did not appear to use auditory cues for foraging. The ability to switch between sensory modalities depending on the quality of the cues available has been documented before in other groups of birds (including thrushes (Turdidae) and sandpipers (Scolopacidae)) and would be of great advantage to wild kiwi foraging under a wide range of environmental conditions.

Introduction

Vision is generally considered to be highly important for birds, both in foraging and in other aspects of their lives (e.g. Sillman 1973). Even so, many bird species from a diverse range of families (e.g. Scolopacidae, Threskiornithidae, Apterygidae, Turdidae, Artamidae) forage for invertebrate prey hidden from view within sand, mud or soil substrates. While visual cues in the form of tracks, burrow entrances and substrate disturbance may indicate the location of some prey items, these birds must often rely on sensory systems other than vision to locate their food. The alternative senses that must be used by probe-foraging birds have therefore generated some interest among researchers.

Sandpipers (Charadriiformes; Scolopacidae) show specialisations for probe-foraging, and have been the focus of many studies investigating the sensory modalities used by birds to detect buried prey. Those in the genus *Calidris* use chemosensory systems such as taste and, perhaps, olfaction (Gerritsen et al. 1983; Van Heezik et al. 1983), chance location (directly touching prey while probing), and a specialized sensory system called 'remote touch' (Gerritsen & Meiboom 1986; Piersma et al. 1998). 'Remote touch' is mediated by an organ composed of numerous pits in the bone of the bill-tips packed with Herbst corpuscles and terminal cell receptors (Grandry corpuscles) (Bolze 1968; Piersma et al. 1998; Nebel et al. 2005). Of these two types of receptors, the physical properties of the Herbst corpuscles make them the more likely to be involved in remote touch (Zweers & Gerritsen 1997). Via these Herbst corpuscles, the bill-tip organ detects vibrotactile signals from invertebrates burrowing through the substrate, or pressure disturbances caused by sessile prey, at some distance from the bill-tip (Gerritsen & Meiboom 1986; Piersma et al. 1998). This allows the probing shorebird to locate prey more efficiently than by using direct touch alone, and in some cases may allow the bird to assess prey density in an area quickly and subsequently to assess the profitability of foraging there (Gerritsen & Meiboom 1986).

Birds that are not specialised for probe-foraging may also use senses other than vision to locate food. Studies on passerine species foraging for soil dwelling invertebrates (e.g. American Robins, *Turdus migratorius* (Montgomerie & Weatherhead 1997), and Australian Magpies, *Gymnorhina tibicen* (Floyd & Woodland 1981)), show that these birds are reliant on auditory cues when visual cues are not available. Procellariiform seabirds are able to locate prey patches over large oceanic distances using olfaction (Nevitt & Bonadonna 2005) and other species including blue tits, *Cyanistes caeruleus,* lories, *Lorius garrulous flavopalliatas,* and kakapo, *Strigops habroptilus,* can be conditioned to locate food using odour cues (Roper 2003; Hagelin 2004; Mennerat et al. 2005). Some bird species may be able to use mechanoreceptors in the feet to detect vibration cues from prey buried in soil (e.g. Floyd & Woodland 1981) although this has not yet been confirmed.

Kiwi (Apterygidae) are a family of five species of nocturnal, flightless birds endemic to New Zealand (Burbidge et al. 2003). They are found in forested and semiforested habitats from the coastline to subalpine zones, and possess a long, slightly curved beak which they use to extract hidden invertebrate prey including earthworms and beetle larvae from soil, leaf litter, rotting wood, damp sand, and rotting seaweed (Colbourne & Powlesland 1988; Kleinpaste 1990). Kiwi eyes, visual fields, and visual centres in the brain are highly reduced compared to those of other flightless birds of their size (Martin et al. 2007). The bill-tip of kiwi falls outside their visual field, and they are therefore unlikely to guide their beak using visual cues while foraging (Martin et al. 2007). Kiwi nostrils are placed at the tip of the long bill, a position unique among birds, and the olfactory chamber and olfactory bulb of kiwi are extensively developed and large (Bang 1971). A complex bill-tip organ very similar in structure to that of the Scolopacidae shorebirds has recently been described in all five kiwi species (Cunningham et al. 2007: *Chapter 1*), and enlarged centres in the brain for the relay and processing of olfactory and tactile information from the bill suggest that these senses are highly important to kiwi (Martin et al. 2007).

Earlier studies of sensory systems used in foraging by kiwi focused solely on olfaction, with mixed results. For example, Benham (1906) and Wenzel (1968, 1971) reported that

kiwi are very successful at finding food using only their sense of smell; however, Strong (1911), Jenkins (2001) and R. Flinn (unpublished data) failed to replicate these results, finding that kiwi often probe areas containing no prey, or else are unable to detect the presence of prey before an area has been probed. The recent discovery of a shorebird-like bill-tip organ in kiwi suggests they also use remote touch while foraging, and both auditory and direct touch cues are presumably also available. No study to date has addressed the kiwi's use of these alternative senses in foraging, although Jenkins (2001) suggested auditory (hearing) or vibrotactile (remote touch) cues might be important to kiwi, and Hauesler (1923) specifically noted the sensitivity of the kiwi bill-tip to touch.

We designed an experiment using captive North Island brown kiwi, *Apteryx mantelli,* (1) to confirm whether kiwi use any kind of remote sensing to detect prey, or simply find prey using direct touch (chance) and (2) to discover which sense or combination of senses is predominantly used in foraging.

Methods

Study species, individuals and captive facilities

We used eight North Island brown kiwi, four males and four females, all of which were held permanently in captivity as part of the conservation programme for this species. The birds were housed at two separate captive breeding facilities in the North Island of New Zealand: Rainbow Springs/Kiwi Encounter in Rotorua (hereafter Rotorua) and Westshore Wildlife Reserve in Napier (hereafter Napier). Table 1 summarises the captive facilities, sex and age of the birds used.

Experimental trials were carried out *in situ* in the large outdoor pens the birds were normally housed in. The pens measured on average approximately 66 m^2 in Napier and 93 m^2 in Rotorua, except for M1's pen at 179 m^2. Singly housed (rather than paired) birds were chosen to avoid interference between individuals during trials. M1 normally shared his large pen with a female, but the pair was separated during the course of our experiment for husbandry reasons unrelated to this study. Ambient

background noise was mostly traffic noise from nearby roads and approached 40 dB at dusk at both sites.

Table 1: Kiwi used in the experiments: their age, sex and the captive facility in which they were housed

Facility	Bird ID	Sex	Age
Napier	F1 Tika	Female	25 years
Rotorua	F2 Koru	Female	4 years
Rotorua	F3 Little Mo	Female	? (>3 years)
Napier	F4 Becs	Female*	8 months
Rotorua	M1 Tahi	Male	21 years
Napier	M2 Lee	Male	4 years
Rotorua	M3 Koanga	Male	2 years
Napier	M4 Mahaki	Male*	11 months

*juveniles

Experimental design

The experimental design consisted of six different treatments in which kiwi were presented with mealworms, *Tenebrio molitor*, buried in trays of soil substrate, and their foraging success on those mealworms was recorded (Table 2). Each experimental treatment was designed to isolate one or two of the senses of smell, remote touch, hearing, and direct touch, and involved combinations of two levels (in parentheses) of the following factors:

(1) prey (12 live mealworms; 12 mealworms killed by freezing overnight to remove vibration cues);

(2) substrate (plain soil; nine parts soil evenly mixed with one part powdered freeze dried mealworms (hereafter "mealworm-mixed soil") to overwhelm smell cues produced by prey);

(3) background noise (ambient noise conditions; broadband white noise playback at 48 - 50 dB to drown out the ca. 30 dB sounds produced by burrowing mealworms).

Table 2: Treatment details and sensory cues available to the birds for each experimental treatment

Treatment	Prey	Substrate	Background noise	Sensory cues available
1 (positive control)	Live	plain	ambient	all
2	Live	mealworm-mixed	ambient	auditory and vibrotactile
3	Live	plain	white noise	olfactory and vibrotactile
4	Live	mealworm-mixed	white noise	vibrotactile
5	Killed	plain	ambient	olfactory
6 (negative control)	Killed	mealworm-mixed	ambient	direct touch only (chance detection only)

Freeze-dried mealworms were obtained from Fluker Farms (Port Allen, LA, U.S.A.) and were ground to a coarse powder using a Magic Bullet Blender (Homeland Housewares, Los Angeles, CA, U.S.A.) prior to mixing with soil for treatments 2, 4 and 6 (see Table 2). White noise playback for treatments 3 and 4 (Table 2) was produced using a DSE 1GB MP3 player (Dick Smith Electronics, Palmerston North, N.Z.) and Sony™ SRSA5S or Phillips™ SBP1100/97 portable speakers. Sound level was checked at the centre of the foraging tray using a Mini Sound Level meter (Jaycar Electronics, Palmerston North, N.Z.) and standardized to 48-50 dB by setting the speakers to maximum and adjusting the volume on the MP3 player. Soil used in all trials was organic natural topsoil, sourced from Coastal Landscape Suppliers, Waikanae, New Zealand.

Each bird was exposed to 4 training nights to accustom it to the experimental set-up and to foraging in the presence of white noise playback. The birds were then offered two replicate trials of each experimental treatment, presented in randomized order, resulting in each bird being exposed to 16 nights of trials (four training trials + 12 experimental trials). Trials were video recorded using Sony Digital Handicams (DCR-HC40E, DCR-HC42E, DCR-HC45E or DCR-HC96E) on Nightshot mode in combination with infrared spot lamps (IRLamp6, Bat Conservation and Management Inc., Carlisle, PA, U.S.A.).

Trial set-up

We designed the trial set-up around the birds' usual feeding routine, to reduce both stress to the birds and training time before experiments could be run. Consequently, there were slight differences in set-up between the two captive facilities.

At both facilities, mealworms were presented in square wooden experimental trays measuring 25 cm wide and 8 cm deep. These were compartmentalized into 25 square open-topped Perspex cells measuring 5 cm wide and 4 cm high, to stop mealworms clumping together during each trial. The 12 mealworms were buried in a random distribution within the tray, as follows: 2 cm of topsoil substrate was put into the tray and the tray gently shaken to distribute and settle the topsoil evenly. Mealworms were placed on top of this soil centrally in their allocated grid squares (live mealworms) or along the edges or corners of the grid squares (killed mealworms) to mimic the locations to which the live mealworms were observed to move. Soil was added reaching to the top of the plastic grid, and a sheet of one-ply tissue paper was laid across all the grid squares to slow movement of the mealworms towards the surface of the tray. The tray was then filled to the brim with soil and smoothed over to eliminate the unlikely possibility that kiwi might use visual cues to locate prey. New soil was used for each trial to avoid build up of prey odours.

Foraging trays were placed in the kiwi enclosures shortly before dusk while the birds were still inside their sleeping boxes. Kiwi had access to the foraging trial as soon as they emerged in the evening, and most birds went immediately to the trial set-up. Kiwi were exposed to one trial per night, and the normal artificial diet supplied by the captive facility was withheld until immediately after completion of the trial (but for no longer than 3 h after dusk), to maximize the birds' motivation to forage for the mealworms. Birds had access to water ad libitum at all times, and all other aspects of their care remained the responsibility of the captive institution.

Trials at Rotorua took place within feeding boxes similar to those normally used at this facility to present artificial food to captive kiwi. The boxes measured 80 x 50 cm and 40 cm high and had a clear Perspex roof, through which the trials were videoed from a tripod placed directly behind the box. Speakers were fixed into the lower back corners

of the box and angled to project sound across the experimental tray. Entry to the boxes was through an archway protected by rubber flaps and the birds were free to enter and leave as they chose. Experimental foraging trays were dug into the ground in the centre of the boxes so the lip of the tray was near-flush with the ground. Soil in the pens at Rotorua was very soft, so rubber matting was placed over the soil inside the boxes to prevent the birds from probing outside the trays.

Kiwi in Napier were normally presented with artificial food under small wooden tables to keep rain off. The foraging trays were placed under these tables, and speakers were fixed to the legs of the tables directly beneath the table top and angled to project sound down across the foraging tray. Trays were dug shallowly or not at all into the ground. Rubber matting was not used here because soil in the pens at this facility was hard packed in the feeding areas. Tripods, cameras and lamps were placed 1 - 5 m from the experimental set-up and the area was videoed from a low angle to achieve visibility under the table tops.

Trials were considered completed when the birds had captured all 12 available mealworms, or had lost interest in the trial and left the foraging area completely for more than 5 consecutive min. Trials in which birds foraged in the tray for less than 60 s, failing to capture any prey, then left the foraging area for more than 5 min were considered ‘incomplete’ because of lack of motivation to forage, and were not included in data analyses.

Data used in the analyses were averages of the two replicates of each treatment for each individual where both were completed, or else were obtained from a single replicate where only one was completed. Table 3 summarizes the number of replicates of each treatment successfully completed by each kiwi.

Table 3: Number of replicates of each experimental treatment completed by each bird

Bird ID	Treatment						Total trials completed
	1	**2**	**3**	**4**	**5**	**6**	
F1	2	2	2	2	2	2	12
F2	2	0	2	2	2	2	10
F3	1	1	2	0	2	0	6
F4	2	1	1	1	2	2	9
M1	2	0	2	0	2	1	7
M2	2	2	2	2	2	2	12
M3	2	2	2	2	2	2	12
M4	2	2	2	2	2	2	12
Total trials completed	15	10	15	11	16	13	80

Video analysis and data recorded

We extracted data on kiwi probing rate (the number of probes/min) and two measures of foraging success, the percentage of all probes resulting in a prey capture (percentage of successful probes) and the total number of prey caught, from all video recordings using the freeware package VirtualDub (Lee 2008). The lower camera angles used in Napier allowed us also to measure the amount of time the birds spent standing in the foraging tray as opposed to beside it for these four individuals. These data were used to calculate prey captures/min to check whether the feet might play a role in prey detection.

Kiwi showed two behaviours in relation to foraging: tapping at the soil surface, which was linked to environmental exploration and/or prey detection, and probing which was associated with prey capture or a capture attempt. Probes generally involved insertion of the bill into the substrate, followed by levering of the bill within the probe hole created. Taps, on the other hand, generally involved only a single motion. Taps sometimes resulted in the insertion of the bill into the substrate a short distance, owing to the soft nature of the soil we used. This occasionally made taps and probes hard to distinguish. We therefore used the criterion that a probe must involve more than a single movement of the head. We felt this was justified as prey captures only ever occurred during probes fitting this description.

Mealworm response to white noise playback

We designed a small experiment looking at mealworm movement rates at room temperature to ensure that white noise playback at the levels we used was not affecting prey behaviour. We drew a 1 x 1 cm grid on the base of a white cardboard box (25 x 25 cm and 12 cm high). We placed one mealworm at a time on the central square of the grid and allowed it to move freely within the box for 1 min under ambient noise conditions (ca. 38 dB background traffic noise and electronics hum in the laboratory), while counting the 1 cm squares it moved across. At the end of 1 min, the same mealworm was replaced in the central grid square, and required to repeat the experiment under 48-50 dB background broadband white noise played from the same speaker and MP3 player set-up used in the kiwi trials. This experiment was repeated with 45 mealworms, alternating whether each mealworm ran the trial first under ambient or white noise playback conditions.

Statistical analyses

All foraging success data (number of prey caught, percentage of successful probes), data on number of prey left at the end of each trial and data on probing rate (probes/min) were normally distributed (Kolmogorov-Smirnov tests: all $P > 0.05$). Data for each measure of foraging success and probing rate were analysed using a mixed-model ANOVA with bird identity included as a random factor and prey type (live/killed), substrate type (plain/mealworm-mixed soil), noise (ambient/white noise) and the interaction terms prey type*substrate type and substrate type*noise included as fixed factors.

Data on number of prey left at the end of each trial were organised into four 'treatment groups' based on prey and substrate types (live prey in plain soil; killed prey in plain soil; live prey in mealworm-mixed soil; killed prey in mealworm-mixed soil). Differences between these groups were analysed using a mixed-model ANOVA with bird identity included as a random factor and treatment group as a fixed factor; further investigation of differences between groups was carried out using post hoc Bonferroni testing.

We used paired T-tests to analyse the difference in prey capture rates/min when kiwi were standing in the foraging trays versus on the ground outside and a Wilcoxon signed-ranks test to compare movement rates between mealworms exposed to white noise playback versus ambient noise conditions because these latter data were not normally distributed (Kolmogorov-Smirnov test: $P < 0.05$). Mixed-model ANOVA tests were carried out in SPSS version 15.0.0 (SPSS Inc., Chicago, IL, U.S.A.). All other analyses were carried out in MiniTab 15 (MiniTab Inc. State College, PA, U.S.A.).

Data in the text are presented as means ± 1 SD. All statistical tests are two-tailed.

Ethical note

North Island brown kiwi are a protected species, listed as 'Endangered' by the IUCN (IUCN 2008) and under intensive conservation management both in captivity and in the wild in New Zealand. The number of birds we were able to use was therefore limited, and we had little control over the age or sex of the individuals available to us. Taking the status of kiwi into account, we designed our experiment to have the very least possible impact on the birds used. All birds were returned to the sole care of their captive institution at the end of the experiment. The experiment was carried out under New Zealand Department of Conservation permits ECHB-23965-RES and BP-22390-RES, and with approval from the Massey University Animal Ethics Committee (protocol 06/91).

Results

Number of prey (mealworms) caught

Kiwi caught significantly more live prey (vibrotactile cues available) than killed prey (no vibrotactile cues) per trial on average (ANOVA: $F_{1, 31.709} = 10.884$, $P = 0.002$) and significantly more prey in plain (olfactory cues available) than in mealworm-mixed (no olfactory cues) substrates (ANOVA: $F_{1, 31.868} = 51.409$, $P < 0.001$), before leaving the experimental trays in each trial ('giving up'). There was a significant interaction between prey type and substrate type on the number of prey caught before the birds 'gave up' foraging (ANOVA: $F_{1,31.444} = 7.593$, $P = 0.01$), with number of live and killed prey captured in plain substrates being similar, but more live than killed prey captured in mealworm-mixed substrates (Fig. 1). There was no effect of white noise on the number of prey captured (ANOVA: $F_{1, 31.329} = 1.281$, $P = 0.266$).

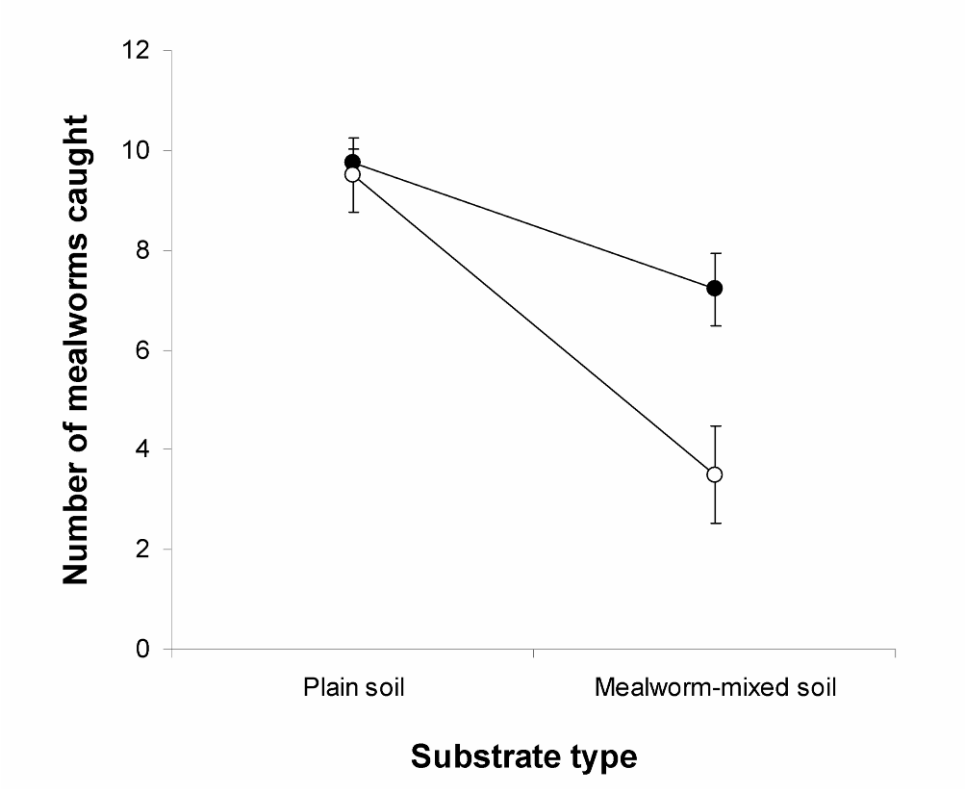

Figure 1. Mean number of live and killed mealworms captured by kiwi foraging in plain soil and mealworm-mixed soil. ●: live mealworms, ○: killed mealworms. Data are means ± SE. $N = 8$ kiwi.

Number of prey (mealworms) left

The mean number of prey left after kiwi 'gave up' foraging in each trial was higher for killed prey in mealworm-mixed substrates (where both olfactory and vibrotactile cues were effectively removed) than for any other substrate/prey combination (8.50 ± 2.58 prey left; post hoc Bonferroni tests: all $P < 0.01$). The mean number of prey left was lowest in plain substrate, where olfactory cues were available, regardless of whether the prey were live (2.25 ± 1.06) or killed (2.5 ± 2.07); no significant difference was found between these two groups (Bonferroni test: $P > 0.05$). There were significantly more live prey left in mealworm-mixed substrate (4.79 ± 2.53), where vibrotactile but not olfactory cues were available, than live or killed prey in plain substrate where olfactory cues were available. There were significantly fewer live prey (vibrotactile cues present) left in mealworm-mixed substrate than killed prey in the same substrate type (post hoc Bonferroni tests: all $P < 0.05$).

These results suggest that the motivation of kiwi to forage in the trays was dependent on the effort required to locate the left-over mealworms. There could be other reasons for this loss of motivation including interaction with environmental factors.

Percentage of successful probes

Kiwi experienced significantly higher probing success (a higher percentage of probes resulted in prey captures) in plain than in mealworm-mixed substrate (ANOVA: $F_{1,31.246} = 81.456$, $P < 0.001$), and significantly higher probing success under white noise than under ambient noise conditions (ANOVA: $F_{1,30.518} = 5.881$, $P = 0.021$). Prey type (live/killed) made no measurable difference to overall probing success (ANOVA: $F_{1,31.014} = 0.606$, $P = 0.442$), but there was a significant interaction between prey type and substrate type (ANOVA: $F_{1,30.653} = 11.113$, $P = 0.002$). The percentage of successful probes was similar for live and killed prey in plain substrate where olfactory cues were available for both prey types, but higher for live than killed prey in mealworm-mixed substrate, where olfactory cues were unavailable, but vibrotactile cues were available from live prey (Fig. 2a). This significant interaction remained after the effect of white noise had been accounted for by adjusting the percentage of successful probes under white noise conditions to match the percentage of successful

probes achieved under the same prey and substrate conditions with ambient background noise (ANOVA: $F_{1,30.556}$ = 9.788, P = 0.004; Fig. 2b).

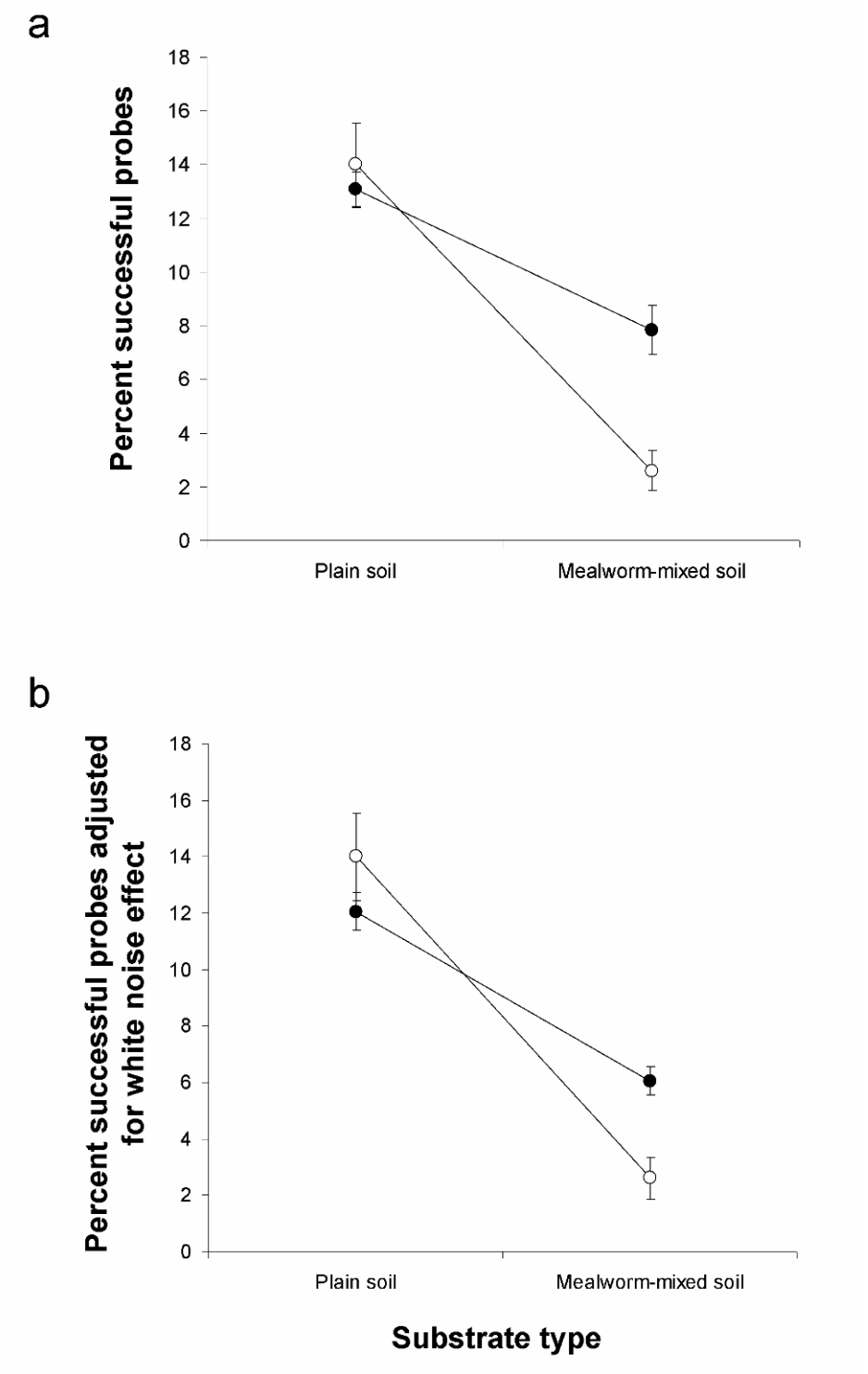

Figure 2. (a) Mean percentage of probes resulting in the capture of a live or killed mealworm by kiwi foraging in plain soil and in mealworm-mixed soil. (**b)** Data from Fig. 2a with the effect of white noise removed. ●: live mealworms, ○: killed mealworms. Data presented are means ± SE. N = 8 kiwi.

Foraging success per experimental treatment

Data on kiwi foraging success in terms of number of prey captured and percentage of successful probes for each experimental treatment reflects the results of the ANOVA tests. Foraging success was highest in treatments where olfactory cues alone or in combination with vibrotactile cues were available (1,3,5), lowest where neither olfactory nor vibrotactile cues were available (6) and intermediate where vibrotactile, but not olfactory, cues were available (2, 4). Fig. 3 shows the percentage successful probes by treatment.

Figure 3. Mean percentage of probes resulting in a prey capture for each of the six treatments (see Table 2). Data labels include treatment number and the remote sensory cues available to the birds under that treatment. Numbers above columns are the number of birds that completed each treatment. Data presented are means ± 1 SE.

Foraging rate (probes/min)

Kiwi probed slightly but significantly fewer times/min in mealworm-mixed than plain substrate (ANOVA: $F_{1,32.126} = 6.662$, $P = 0.015$), and more times/min under white noise than ambient noise conditions (ANOVA: $F_{1,31.332} = 4.252$, $P = 0.048$). No other factors affected probing rate (all $P > 0.05$).

Role of feet in prey detection (Napier birds only)

No significant difference was found between the number of mealworms caught/min when the feet were in the tray versus outside the tray either across all treatments (two-tailed paired *t* test: $t_3 = 0.53$, $P = 0.631$) or for treatments involving only live prey (two-tailed paired *t* test: $t_3 = -0.33$, $P = 0.766$). Vibrotactile cues used in foraging by kiwi must therefore be detected via the bill-tip organ.

Response of mealworms to white noise playback

White noise playback at 48-50 dB made no detectable difference to mealworm movement rates, with mealworms moving at an average rate of 8.47 ± 7.52 cm/min under ambient noise conditions, and 8.29 ± 7.25 cm/min under 48-50 dB white noise playback (Wilcoxon signed-ranks test: $T = 313.5$, $N = 45$, $N(\text{test}) = 35$, $P = 0.987$).

Discussion

This study confirms that kiwi use remote sensing to find prey and provides the first evidence that kiwi are able to use both olfaction and remote touch, but not hearing, to detect soil-dwelling invertebrates. Remote touch in birds was first described in the shorebird family Scolopacidae (Gerritsen & Meiboom 1986) and to the best of our knowledge our study is the first documented instance of the use of the system outside of the shorebirds. Palaeognathous kiwi and neognathous shorebirds are very distantly related groups, and the kiwi's possession of a shorebird-like bill-tip organ (Cunningham et al. 2007), combined with this new evidence of their use of remote touch, mediated by the bill-tip organ (not the feet), suggests convergent evolution of this complex sensory system in response to a similar foraging problem.

The number of kiwi (eight) involved in this study was small, because availability of kiwi was limited. Individual idiosyncrasies of the birds further meant that sample size varied between experimental treatments. Eight birds, however, was a sample size in keeping with similar studies of this nature (e.g. Montgomerie & Weatherhead 1997: four American robins; Gerritsen & Meiboom 1986: two sanderlings, *Calidris alba*).

Our results suggest that the sense used predominantly in foraging by captive kiwi is olfaction, as kiwi found live and killed prey equally well in plain soil substrates, capturing similar numbers of prey with similar efficiency, even though olfactory, auditory and vibrotactile cues were associated with live prey, whereas only olfactory cues were associated with killed prey. Under conditions where olfactory cues were uninformative or confused (represented by mealworm-mixed substrate trials in this experiment), the birds switched to using vibrotactile cues (remote touch). This is evidenced by their significantly greater foraging success both in terms of the number of prey caught and probing efficiency (percentage of successful probes) on live than killed prey under these circumstances, regardless of whether or not auditory cues were available.

The ability of kiwi to use more than one sensory modality while foraging, and switch between dominant modalities depending on the quality of sensory cues, is not unique. American robins, for example, use visual cues alone to find prey when these are available, and to switch to auditory cues where they are not (Heppner 1965; Montgomerie & Weatherhead 1997). Sanderlings are able to assess the quality of visual versus vibrotactile cues when hunting for burrowing polychaete worms, and to reject visual cues in favour of vibrotactile ones, where visual cues are found to be unreliable (Gerritsen & Meiboom 1986). Wild kiwi are exposed to a large range of environmental conditions while foraging, from leaf litter and loose soil in which olfaction would be most useful, to damp, even sand and mud substrates in which vibration cues would propagate well. The ability to switch between olfactory and vibrotactile cues depending on foraging conditions would be of huge advantage to them.

Auditory cues seemed unimportant to captive kiwi in foraging, as playing broadband white noise to mask prey sounds made no difference to the number of prey caught. In fact, kiwi probing rate was higher, and kiwi experienced higher success rates per probe, under white noise than under ambient noise conditions. White noise playback would have masked not only mealworm sounds but all other quiet background noises, which kiwi may use to gather information about their environment. The birds' tendency to 'speed up' the foraging process in the presence of white noise could therefore be explained in two ways. Kiwi may be uncomfortable spending time in an area where they cannot hear background noises (which may give information about the presence of predators or conspecifics) and therefore increased their foraging efficiency and probing rate to complete the trial faster, or, blocking these potentially distracting noises helped the birds to focus more exclusively on foraging, boosting their probing success. Either explanation highlights the importance of auditory cues in areas of the kiwi's life other than foraging. Further evidence of the importance of auditory cues to kiwi is given by the prominence of their ear openings, their loud vocalisations and the immediate 'head lift' reaction of wild kiwi to the sound of approaching footsteps on leaf litter (personal observation, see also *Chapter 5*).

The dominance of olfaction over remote touch in captive kiwi might be explained in a number of ways. Olfaction may be an inherently more rapid way to gain information about prey location than remote touch, or simply better developed than remote touch in kiwi. The loose and slightly uneven soil substrate we provided may not have been an ideal medium for propagating the seismic waves upon which remote touch is reliant, or else the confined sides of the foraging tray and Perspex grid within may have reflected vibrations in a manner confusing to the birds. Perhaps also, the captive kiwi we tested may have been habituated to using olfactory over remote touch senses because of their life-long exposure to a strong-smelling artificial diet and lack of experience of foraging using remote touch, relative to wild kiwi. It is documented that other bird species can become conditioned to rely on olfaction as a foraging cue (e.g Roper 2003; Mennerat et al. 2005). We ran this experiment in captivity because of the difficulties of using wild

kiwi in such a study. To investigate these possibilities further, however, similar trials need be run in the wild, and with alternative substrate types.

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Chapter 3

Bill morphology of ibises suggests a remote tactile sensory system for prey-detection

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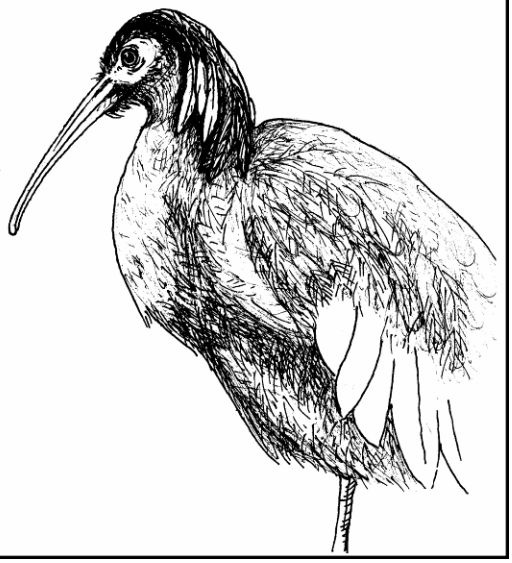

Abstract

Birds that forage by probing must often use senses other than vision to find their prey. Remote touch is a sense based on the interception of vibrations produced by moving prey in the substrate, or on the evaluation of pressure patterns produced by hard-shelled sessile prey. In probing birds, this system is mediated by an organ made up of clusters of mechanoreceptors housed within pits in the bone of the bill-tips. This bill-tip organ was first described in probing shorebirds (Scolopacidae), and more recently in kiwi (Apterygidae). Here we describe this bill-tip organ in a third family of probing birds, the ibises (Threskiornithidae). We examined the bill morphology of 11 species of ibis from 8 genera. We found bill-tip organs in species in a wide range of habitat types, from predominantly terrestrial to predominantly aquatic, which suggests that ibises may use remote touch when foraging both in water and in granular substrates. Our data imply a link between bill-tip morphology and habitat use – a pattern that we believe warrants further investigation.

Introduction

Birds that forage by probing exist in many habitats and in a diversity of families (e.g. Scolopacidae, Threskiornithidae, and Apterygidae). Most possess long, sometimes down-curved bills that facilitate probe-foraging. Although tracks, burrows and substrate disturbance may visually indicate the location of some prey items, probing birds must often rely on non-visual sensory systems to locate their food.

Sandpipers in the genus *Calidris* (Charadriiformes: Scolopacidae) locate their prey by using chemosensory systems such as taste and perhaps olfaction (Gerritsen et al. 1983, Van Heezik et al. 1983), through chance location (directly touching prey), and by using a specialised sensory system called "remote touch" (Gerritsen and Meiboom 1986, Piersma et al. 1998). Remote touch is mediated by an organ composed of numerous pits in the bone of the bill-tips that are packed with two types of mechanoreceptors, Herbst corpuscles and terminal cell receptors (Bolze 1968, Zweers and Gerritsen 1997, Piersma et al. 1998, Nebel et al. 2005). The physical properties of the Herbst corpuscles make them the more likely of the two to be involved in remote touch (Zweers and Gerritsen 1997). Herbst corpuscles detect seismic signals from invertebrates burrowing through the substrate and pressure disturbances caused by sessile prey at some distance from the bill-tip (Gerritsen and Meiboom 1986, Piersma et al. 1998) and thus allow a probing shorebird to locate prey more efficiently than by using direct touch. In some cases remote touch may allow the bird to quickly assess prey density in an area and therefore the profitability of foraging there (Gerritsen and Meiboom 1986). A shorebird-like bill-tip organ has recently been described in kiwi (Apterygiformes: Apterygidae), a family of nocturnal probing birds from forest habitats in New Zealand (Cunningham et al. 2007), which suggests the faculty of remote touch may be shared by other groups of probing birds.

Ibises (Ciconiiformes: Threskiornithidae, subfamily Threskiornithinae) are a cosmopolitan family of probing birds. Ibis species use many habitats, ranging from lakes and wetlands through bogs, fens, and marshes to forests, dry shrub and grasslands (Matheu and del Hoyo 1992). Their long, down-curved bill enables them to forage for invertebrates and small vertebrates by probing and sweeping in lagoons and estuarine

mud, in grasslands, in leaf litter and soil, and cracks in dry ground (e.g. Skead 1951, Keith et al. 1974, Kushlan 1978, Dzerzhinsky 1998). Ibises are usually described as "tactile" hunters (e.g. Kushlan 1978). Therefore, some foraging studies have assumed that ibises detect prey only by direct contact with the bill-tips, and that the time involved in chasing prey once it has been discovered is negligible (e.g. Kushlan 1979).

Spoonbills (Ciconiiformes: Threskiornithidae, subfamily Plataleanae), the closest relatives of the ibis group, are distinguished by their dorso-ventrally flattened, spatulate bill. They are exclusively aquatic feeders that forage by sweeping the bill from side to side in shallow water (Matheu and del Hoyo 1992). The bill of the black-faced spoonbill (*Platalea minor*) was studied in detail by Swennen and Yu (2004) who discovered that both the upper and the lower jaws were densely covered with pits similar to, though arranged differently from, those found in the bills of Scolopacidae species. Although histological evidence is lacking, they suggested that the pits would likely house Herbst corpuscles as found in shorebird bills (Swennen and Yu 2004). Swennen and Yu (2005) observed that the black-faced spoonbill kept its bill spoon in the water during pursuit of prey, and that sudden pecks were sometimes made at prey beyond the bill-tip. These observations suggest that the bill-tip organ of the black-faced spoonbill is functional for remote touch (Swennen and Yu 2005). Dzerzhinksy (1998) mentioned the presence of sensory pits in the bill of the white ibis (*Eudocimus albus*) but presented no histological examination of the bill for mechanoreceptors.

We examined the bill-tip morphology of ibises in eight genera for bony pits that might indicate the presence of a bill-tip organ similar to that found in shorebirds (see Table 1 for scientific names of species examined). The bill-tip of the Australian white ibis was examined histologically to verify whether the pits contained mechanoreceptors, and to confirm ibis species possess such a bill-tip organ. We investigated species with habitat types ranging from predominantly terrestrial to mainly aquatic. For exploratory purposes, we examined the data on ibis bill morphology in relation to habitat use and included data from four species of kiwi, a terrestrial probing bird, for comparison. We discuss the implications of a remote touch sense in the Threskiornithidae, and hypothesize there may be links between habitat use and bill morphology in this group.

TABLE 1. Summary of ibis species sampled including sample sizes, habitat index, average bill and leg measurements, and sensory-pit counts. Figures presented are averages where $n > 1$.

Species	*n* (total)	*n* (used to count sensory pits)	Habitat index	Bill length (mm)	Bill-tip width (mm)	Bill-tip depth (mm)	Femur length (mm)	Upper jaw			Lower jaw			% bill length pitted	Pit density (pits/mm)	Total number of pits
								No. pits outer surface	No. pits inner surface	Total	No. pits outer surface	No. pits inner surface	Total			
Wattled Ibis (*Bostrychia carunculata)*	3	2	1.33	130.91	2.76	3.09	71.35	134.0	72.0	206.0	222.5	41.0	263.5	9.84	27.7	469.5
White Ibis (*Eudocimus albus)*	4	2	3.80	141.61	4.60	4.75	61.98	774.0	46.5	820.5	658.5	74.0	732.5	34.11	29.7	1553.0
Scarlet Ibis (*Eudocimus ruber)*	6	2	3.80	146.72	4.74	4.51	58.53	635.0	17.5	652.5	639.5	25.5	665.0	28.84	30.1	1317.5
Northern bald Ibis (*Geronticus eremita)*	2	0	1.00	131.15	2.80	3.68	62.60	rhamphotheca present						-	-	-
Madagascar Crested Ibis (*Lophotibis cristata*)	1	1	1.63	128.88	3.07	3.25	71.52	398.0	31.0	429.0	270.0	34.0	304.0	19.56	26.5	733.0
Bare-faced Ibis (*Phimosus infuscatus)*	1	0	3.5	109.96	2.36	2.28	50.34	-	-	-	-	-	-	12.64	-	-
White-faced Ibis (*Plegadis chihi*)	4	3	3.5	114.21	4.49	4.44	57.99	1157.0	161.3	1318.3	916.3	167.7	1084.0	36.04	50.4	2402.3
Glossy Ibis (*Plegadis falcinellus*)	2	2	4.33	116.80	4.41	4.61	57.83	1026.5	167.0	1193.5	928.5	176.5	1105.0	37.19	45.0	2298.5
Buff-necked Ibis (*Theristicus caudatus)*	3	3	1.75	147.71	3.10	3.43	67.35	161.7	77.7	239.3	122.3	32.5	154.8	8.39	22.9	394.2
Black-faced Ibis (*Theristicus melanopis*)	1	1	1.00	153.63	2.55	2.11	71.71	129.0	81.0	210.0	lower jaw missing			9.50	8.8	-
Australian White Ibis (*Threskiornis molucca*)	1	1	2.60	151.82	4.10	3.60	73.62	528.0	80.0	608.0	286.0	115.0	401.0	22.85	23.5	1009.0
AVERAGE/TOTAL *n*	28	17	2.57	133.94	3.54	3.61	64.07	549.2	81.6	630.8	505.5	83.3	588.7	21.90	29.4	1272.1

Methods

Morphology

We examined 28 ibis skeletal specimens representing 11 species in 8 genera (*Threskiornis, Geronticus, Plegadis, Lophotibis, Eudocimus, Phimosus, Theristicus* and *Bostrychia*) at Te Papa Tongarewa/the Museum of New Zealand, Auckland War Memorial Museum, San Diego Natural History Museum, and the American Natural History Museum (New York). We noted the presence of sensory pits in the bill, and, using Kincrome Vernier callipers, measured bill length (upper bill, measured from the naso-frontal hinge), skull length (measured from the bill-tip to the back of the skull), the dorsal and lateral extent of pitting in the upper bill, and the lateral and ventral extent of pitting in the lower bill of each specimen. We photographed the upper and lower bills of each specimen from dorsal, ventral, lateral left and lateral right views, taking care to include the entire area of sensory pitting in each photograph. For practical reasons, and where photograph and specimen quality allowed, the number of sensory pits in the bill-tip was estimated by counting those visible in the photographs. Femur length (maximum) and tarsometatarsus width (minimum) were also measured as a gauge of the size of each individual.

Bill-tip organ measurements

We measured four aspects of the bill-tip organ:
(1) the absolute length of the bill-tip organ (mm), calculated by averaging the extent of pitting in mm on the dorsal, ventral, and lateral sides of the bill (measured from the bill-tip to the most caudal sensory pit on each side); (2) the percentage of bill length occupied by the bill-tip organ (calculated by dividing bill-tip organ length, as measured above, by the total length of the bill x 100); (3) the total number of sensory pits present on all surfaces (dorsal, lateral and ventral) of both the upper and lower bills; and the number of sensory pits on the outside surfaces of the bill only (dorsal and lateral surface of the upper bill, ventral and lateral surfaces of the lower bill); and (4) the average density of pits per millimetre of bill-tip organ length, calculated by dividing the number of sensory pits on the outside surfaces of the bill by the absolute length of the bill-tip organ.

Histology

Fresh tissues were obtained from a single juvenile Australian white ibis (permit WT2008-2289, Australian Government Department of the Environment, Water, Heritage and the Arts), that was euthanized for reasons unrelated to this project. The head and bill were fixed immediately after death in 10% buffered formalin. The bill-tip was then trimmed into six pieces: the first 14 mm of both the upper and lower bill-tips were split medially for sectioning on the saggital plane, and a 2-mm thick trimming was made from the cut ends of both the upper and lower bill for sectioning coronally. The keratin rhamphotheca was softened following Luna's (1968) method, and trimmed sections were decalcified using neutral EDTA (Bancroft and Stevens 1982), routinely processed, embedded in paraffin, sectioned at 3 µm, and stained with haematoxylin and eosin (Luna 1968). A single 5-µm section from the saggital plane and another from the coronal plane were stained with a silver stain (Sevier and Munger 1965) to target nervous tissue.

We measured width, depth and angle with respect to the bill-tip and number of sensory pits, and width, length, area and numbers of Herbst corpuscles from digital photomicrographs of silver-stained sections. All measurements were made using ImageJ (National Institute of Health 2008) digital image analysis system and are given as means ± SD below.

Habitat data

BirdLife International Data Zone online species datasheets (BirdLife International, 2008) summarize information on the types and relative importance of habitats used by avian species. We used this information to create an index of habitat use for each of our 11 ibis species, to facilitate exploratory comparisons between habitat use and bill morphology. The index was calculated by combining information on habitat type (H) and the relative importance of each habitat (U) for all species. Habitat types were ranked from "wettest" to "driest" and then assigned a score from 1 to 5 according to their rank (higher scores indicated more "aquatic" habitats). Habitat rankings were decided on the basis of subjective assumptions about the presence of surface water and the relative dampness of the ground in different habitat types (e.g. lagoons were ranked as "wetter" habitats than pastureland). Where it was not obvious that one habitat should

be ranked as more or less aquatic than another, (e.g. grasslands and pasturelands), habitats were assigned identical scores. The scale is a rough estimate for purposes of data exploration rather than an exact measurement of water saturation across habitats. Habitats are categorized in the BirdLife International Data Zone datasheets as being of critical, major, minor, insignificant, or unknown importance to each species. We assigned a weighting value to each category: 4 for critical habitats through to 1 for insignificant and unknown habitats. A weighting value of 1 was also assigned where the importance of a habitat was not stated ("unset"), to ensure that the habitat was represented within the species' final score while being conservative about its importance. Data on habitat types used by each species, weighted by their importance, were converted to a habitat use index (HI) using the following formula:

$$HI = \sum(HU) / \sum(U)$$

where increasing values of HI are equivalent to increasingly aquatic habitat use.

Data on percent of the bill pitted and number of pits in the bill-tip organs of four kiwi species - North Island brown kiwi (*Apteryx mantelli*),tokoeka (*A. australis*), great spotted kiwi (*A. haastii*),and little spotted kiwi (*A. owenii*) were obtained from a paper on kiwi bill morphology (Cunningham et al. 2007: *Chapter 1*). The HI was calculated for these species using the method described above. These data were added to the ibis data to provide a comparison with unrelated, terrestrial probe-foraging species.

Morphological variables for ibises and kiwi were plotted against the HI to explore the possibility of differences in morphology related to habitat use. The strength of potential relationships between HI and bill morphology for ibises alone and together with kiwi was investigated using Spearman rank correlations.

Results

Bill morphology

Sensory pits were found in the bill-tips of all ibis species investigated, except for northern bald ibis. We were unable to examine the bills of the two specimens of the

latter species because the keratin rhamphotheca was still present. The oval-to-polygonal sensory pits were found on all bill surfaces, including the inside of the tips. Pits on the outer bill surfaces were concentrated towards the tips, forming a "honey-comb" of closely-packed pits similar to that seen in the Apterygidae and in many species of Scolopacidae. There were two distinct types of sensory-pit distribution on the inner surfaces of the bill. In the majority of species, pits were found around the outside edges of both jaws, separated from a deep central groove by an area of transverse ribbing of the bone. In the genera *Theristicus* and *Bostrychia*, pits were more evenly distributed at the inner tip of the upper jaw, and were not separated by a central groove until several millimetres caudal to the tips (Fig. 1). We counted sensory pits in 17 of 28 individuals (9 of 11 species). In all 9 species, the highest numbers of sensory pits were on the outer surfaces of the bill (Table 1). In most species, the ventral surface of the lower jaw was divided longitudinally by a deep groove (Fig. 1, central row of pictures), whereas the upper jaw carried a longitudinal, lateral groove on either side.

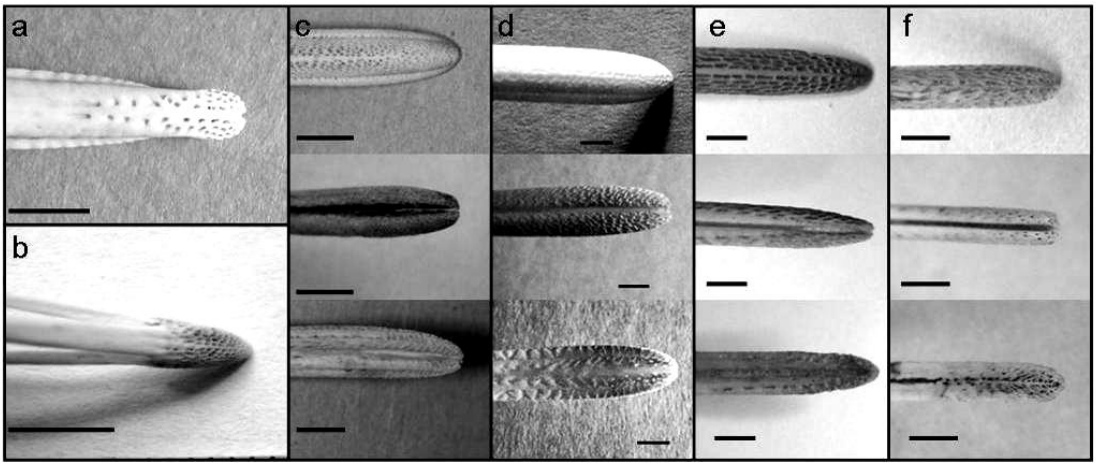

FIG. 1. Sensory pits in the bills of four ibis species (c-f) (top to bottom: dorsal tip of premaxilla, ventral tip of mandible, ventral tip of premaxilla), with dorsal views of (a) North Island brown kiwi (Apterygidae) and (b) sanderling (Scolopacidae: *Calidris alba*) bills for comparison. (c) white-faced ibis, (d) Australian white ibis, (e) Madagascar crested ibis (f) buff-necked ibis. Scale bars = ~5mm. (Photographs by S. Cunningham and T. Jensen.)

Histology: Australian white ibis

Coronal and saggital sections of both upper and lower bill tips of the Australian white ibis showed sensory pits packed with Herbst corpuscles. Corpuscles were sectioned on various angles, and ranged in size from 44 x 33 µm to 312 x 134 µm (average: 118 ± 43 x 78 ± 29 µm, n = 417 corpuscles). Silver stains confirmed the presence of nerve axons

within the centres of each Herbst corpuscle, nerve bundles within the centres of the sensory pits, and thick nerve branches running the length of both upper and lower bills.

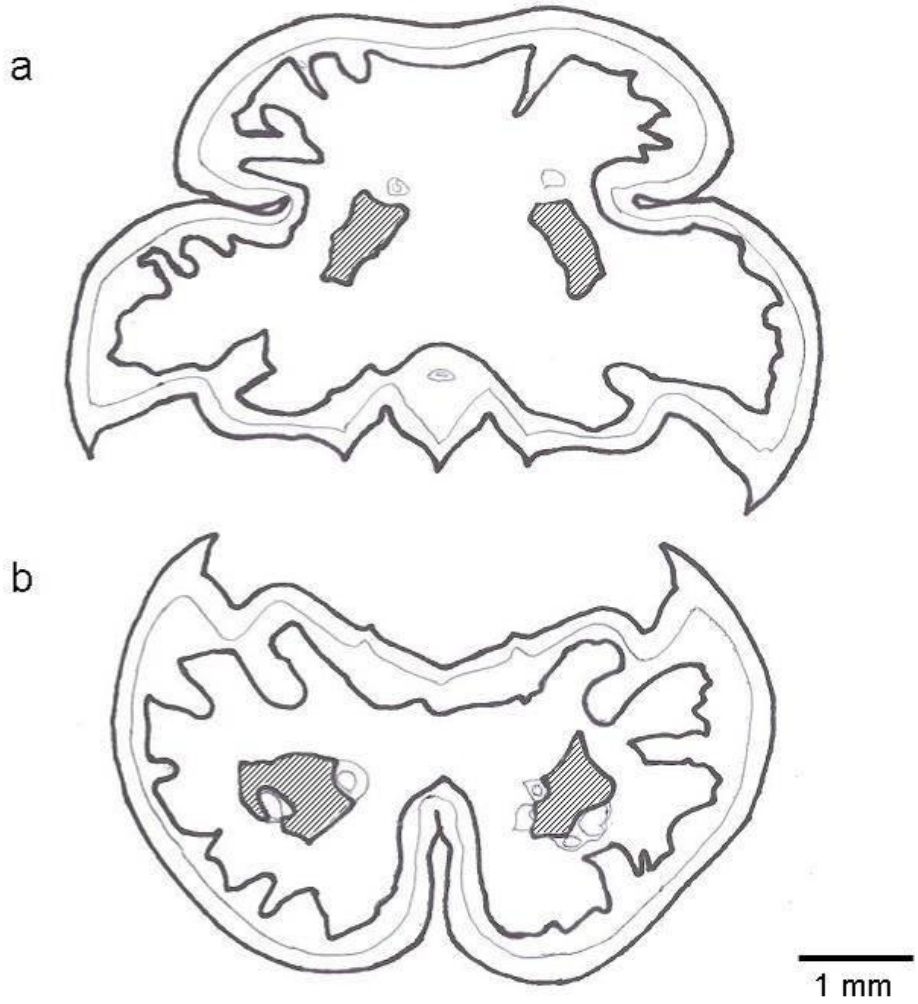

FIG. 2. Diagram of coronal sections through (a) the upper and (b) the lower jaws of the Australian white ibis, ~14 mm from the bill-tip. Bold lines indicate the outer surface of the keratin layer and the outlines of the premaxilliary and mandibular bones (which appear ragged because of the presence of numerous sensory pits). Fine lines indicate the junction between the dermal and keratin layers and the outlines of larger blood vessels. Hatched areas represent cross-sections through the major nerves which are present in both upper and lower bills.

Coronal section, upper bill

The upper bill measured 4.04 mm deep x 6.76 mm wide 14 mm caudal from the bill-tip. It carried deep (1.31 mm) medial lateral grooves on each side. Two large nerve bundles, presumably branches of the trigeminal nerve, were encased within the bone of the

premaxilla, adjacent to the groove on either side. Nerve bundles measured approximately 914 x 393 µm in diameter on the left side, and 824 x 399 µm on the right side. Several sensory pits were visible on the dorsal and lateral sides of the bill in cross section. The ventral surface of the upper bill was corrugated with ridges and grooves. A single sensory pit was visible in the ventral surface of the premaxillary bone on either side. The sensory pits measured on average 376 ± 212 µm deep and 335 ± 56 µm wide. Between 1 and 15 Herbst corpuscles were visible within each pit in cross section (average 3.6 ± 3.6, n = 14 pits) (Fig. 2a).

Coronal section, lower bill

The lower bill measured 3.44 mm deep x 5.83 mm wide 14 mm caudal from the bill-tip. It carried a deep (1.67 mm), medial longitudinal groove in the lower surface. Two large nerve bundles, presumably parts of the mandibular branch of the trigeminal nerve, were encased within the centre of the bone on each side of this groove and flanked by large blood vessels. Nerve bundles measured approximately 674 x 620 µm in cross section on the left side, and 779 x 709 µm on the right side at 14 mm caudal from the bill-tip. Several sensory pits were visible on the ventral and lateral sides of the bill. A single sensory pit was visible at the extreme outer edge of the dorsal surface of the mandible on each side, beneath a deep groove in the keratin layer. Pits measured on average 481 ± 181 µm deep from the surface of the bone to the base of the pit, and 280 ± 61 µm wide at the bone surface (n = 11 pits). Between 1 and 12 Herbst corpuscles were visible within each pit in cross section (average 5.3 ± 4.0, n = 11 pits) (Fig. 2b).

Saggital section, lower bill

Numerous sensory pits were visible in a medial saggital section of the first 14 mm of the lower bill-tip. Nine pits opened to the ventral surface of the mandibular bone, and partial sections of three more were visible beneath these. Sensory pits were angled towards the tip of the bill at an average of 130.8 ± 26.1º in relation to the outer ventral surface of the beak. Pits were widest at the bone surface, narrowing with depth. Some appeared to open into horizontal chambers beneath the bone surface (e.g. Fig. 3a). Pit depth increased with proximity to the bill-tip (r_s = 0.62, P = 0.033, n = 12), and the number of Herbst corpuscles visible per pit increased with pit depth (r_s = 0.74, P = 0.006, n = 12), resulting in an increasing number of Herbst corpuscles per pit towards

the tip of the bill. (Average sensory pit width at bone surface: 1,074 ± 340 µm, depth: 1,152 ± 946 µm; average number of Herbst corpuscles visible per pit: 18 ± 12).

The majority of Herbst corpuscles in the lower jaw were embedded within sensory pits, though a few small corpuscles were visible in the dermal layer between the bone and keratin of the bill, and a row of Herbst corpuscles was visible associated with the ventral side of the large nerve dorsal to the main body of the mandible bone. A large, distinct cluster of 14 Herbst corpuscles was also present in the dermis at the extreme tip of the bill, not associated with any sensory pits (Fig. 3b). Herbst corpuscles within the sensory pits measured on average 121 ± 43 µm long x 81 ± 26 µm wide (area: 8,500 ± 5,478 μm^2), whereas those along the dorsal nerve measured 114 ± 25 µm x 62 ± 8 µm (area: 5,259 ± 1,586 μm^2), and those in the bill-tip cluster measured 77 ± 17 µm x 41 ± 6 µm (area: 2,589 ± 795 μm^2).

Saggital section, upper bill

Ten sensory pits were visible along the dorsal side of the premaxilliary bone, in a medial saggital section of the first 14 mm of the upper jaw (Figs. 3c, d). Two of these pits branched near the base, forming a "double" pit (Fig. 3d). These sensory pits, like those in the lower jaw, were angled forward towards the bill-tip at an average of 138 ± 7º in relation to the outer dorsal surface of the beak. Two narrow, deep (1,666 ± 39 µm deep x 836 ± 54 µm wide) sensory pits were visible at the apex of the bill-tip, and a partial section of a third apical sensory pit was present proximal to these. Five sensory pits were present in the ventral surface of the premaxilliary bone, also opening forward towards the bill-tip with an average angle of 129 ± 24º. As in the lower jaw, sensory pits were widest at the opening and narrowed towards the base but no increase in depth of sensory pits towards the tip of the upper bill was observed, apart from the very deep pits in the tip of the beak (r_s = -0.09, P = 0.803, n = 10). Sensory pits on the dorsal side of the bill were generally deeper and narrower than those on the ventral side (dorsal pits: 1,015 ± 406 µm deep x 946 ± 256 µm wide, ventral pits: 859 ± 733 µm deep x 1,297 ± 788 µm wide).

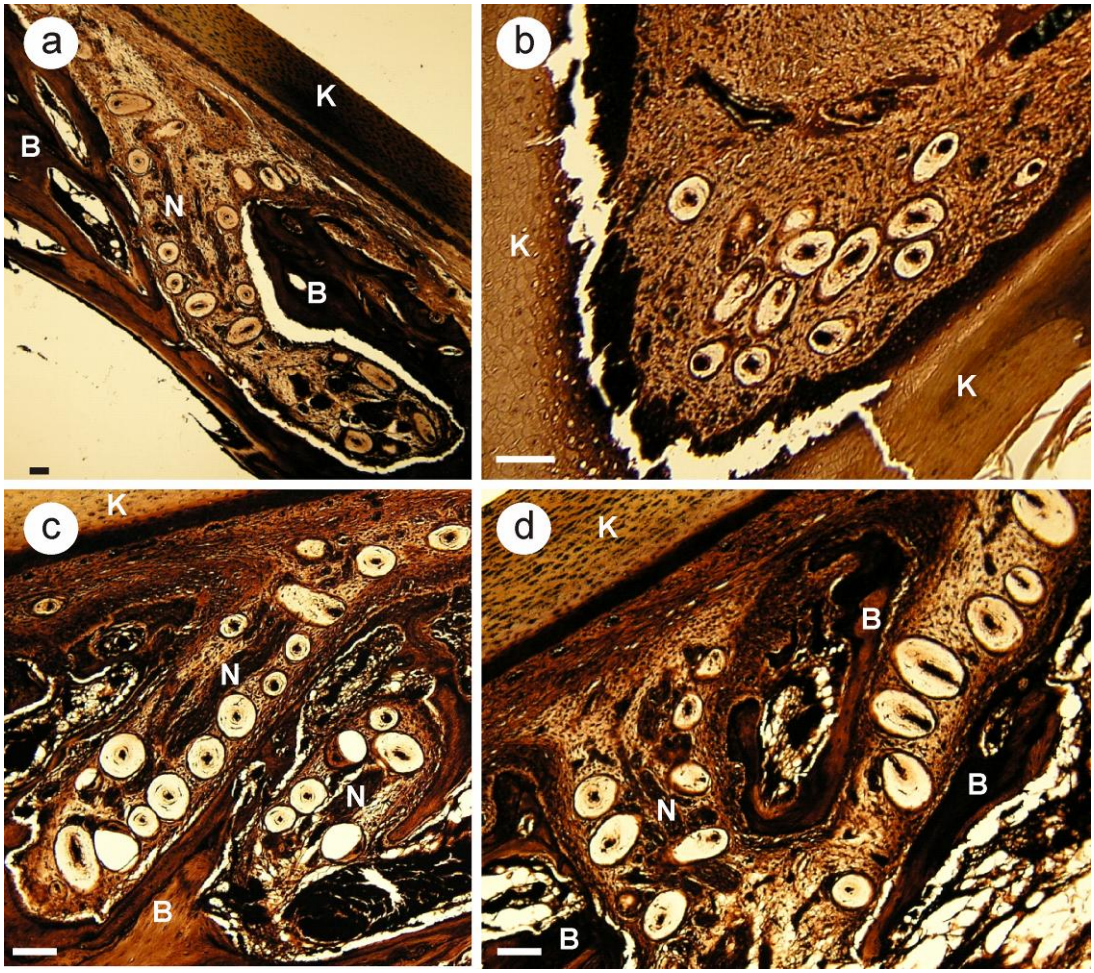

FIG. 3. Location of Herbst corpuscles and types of sensory pits within the Australian white ibis bill. (a) A sloping sensory pit in the mandible, containing numerous pale, ovoid Herbst corpuscles of varying sizes with agyrrophilic central axons, opens to a horizontal chamber under the bone surface. (b) A cluster of irregularly shaped Herbst corpuscles within the dermis at the very tip of the mandible - not associated with a sensory pit. (c) Two adjacent sensory pits in the premaxilla, showing numerous Herbst corpuscles along the sides of the pits, and strands of nervous tissue in the center. (d) Two adjacent sensory pits in the premaxilla joined at the base to form a "double" pit. Large, pale ovoid Herbst corpuscles are obvious within both pits. Slides are silver-stained. K = outer keratin layer, B = bone, N = nervous tissue. Scale bars ~ 100 µm.

The majority of Herbst corpuscles in the upper jaw were embedded within sensory pits, though a few corpuscles were visible in the dermal layer between the bone and keratin of the bill on the dorsal side. No cluster of small Herbst corpuscles was present at the bill-tip. Herbst corpuscles in the upper jaw measured, on average, 118 ± 44 x 79 ± 31 µm (area 8,181 ± 6765 μm^2), and the average number visible per pit was 11 ± 8.

Morphology and habitat use

All measures of the bill-tip organ in ibises increased significantly with increasingly aquatic habitat use (increasing values of HI; Table 2). This trend was supported by the addition of data on the percent of bill length pitted and total number of sensory pits for terrestrial foraging kiwi (Fig. 4 and Table 2). There was no trend in ibis bill length with habitat use, but both tarsus width and femur length declined with increasing HI values (Table 2).

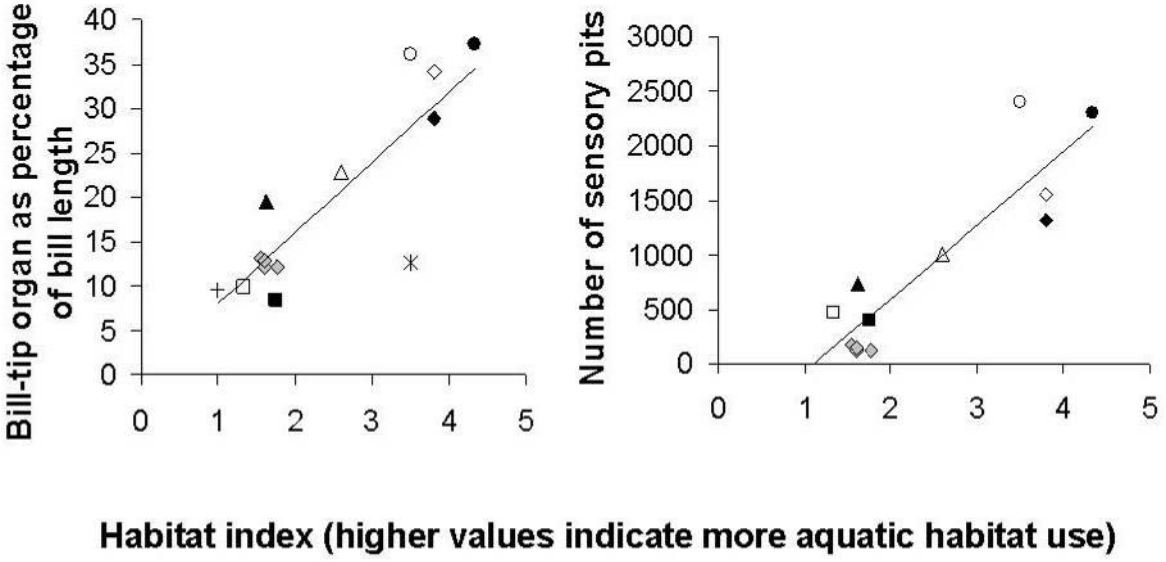

FIG. 4. Extent of the bill-tip organ as a percentage of the total bill length and number of sensory pits in the bill-tip organ, in relation to habitat index. Ibises: open circles = white-faced ibis, closed circles = glossy ibis, open diamonds = white ibis, closed diamonds = scarlet ibis, open triangles = Australian white ibis, closed triangles = Madagascar crested ibis, open squares = wattled ibis, closed squares buff-necked ibis, plus symbol = black-faced ibis, and asterisk = bare-faced ibis. Four kiwi species (North Island brown kiwi, tokoeka, great spotted kiwi, little spotted kiwi) are added for comparison (shaded diamonds).

TABLE 2. Spearman Rank Order correlations between morphological measurements and habitat index (HI). Significant results are presented in bold.

Morphological measurement		***n* (species)**	**r_s**	***P***
Ibises only:				
Bill	Length (mm)	10	0.42	0.233
Bill-tip organ	Absolute length (mm)	10	0.76	**0.010**
	Extent as percent of bill length	10	0.81	**0.004**
	Number of sensory pits (outer surfaces of bill)	9	0.85	**0.004**
	Number of sensory pits (total)	8	0.78	**0.023**
	Density of sensory pits (pits mm^{-1})	9	0.75	**0.021**
Leg	Femur length (mm)	11	-0.67	**0.023**
	Tarsus width (mm)	11	-0.73	**0.011**
Ibises and kiwi:				
Bill-tip organ	Extent as percent of bill length	14	0.73	**0.003**
	Number of sensory pits (total)	12	0.73	**0.007**

Discussion

Our morphological and histological results from the Australian white ibis and morphological data from nine other ibis species provide the first detailed evidence that many ibis species across a number of genera possess a bill-tip organ similar in structure to that found in the families Scolopacidae and Apterygidae. The ibis bill-tip organ is present in species that use habitats ranging from terrestrial grassland and forest (e.g. *Theristicus*, *Bostrychia* and *Lophotibis*) to the open water of lakes and lagoons (e.g. *Plegadis* and *Eudocimus*). More aquatic ibises appear to have more extensive and densely pitted bill-tip organs than terrestrial ibises. These birds may therefore be able to locate prey hidden both underground and within the water column, using a remote touch sensory mechanism like that used by shorebirds (Gerritsen and Meiboom 1986, Piersma et al. 1998) and kiwi (Cunningham et al. 2009: *Chapter 2*).

Implications of remote touch in ibises

Finding a shorebird-like bill-tip organ in ibises increases the number of families with this sensory organ to three: Apterygidae, Scolopacidae and Threskiornithidae. Each family occurs within a different order of birds, and while ibises and shorebirds belong to the super order Neognathae, kiwi are paleognathous. Therefore, remote touch sensory systems may have evolved multiple times within these groups of long-billed, probing birds. Common phylogenetic inheritance of a bill-tip organ capable of remote touch cannot be ruled out between ibises and shorebirds, given the controversy about the relationship of the Ciconiiformes to the Charadriiformes (reviewed by Parkes 1978). However, these groups are clearly separated in the recent phylogeny published by Hackett et al. (2008). The occurrence of a bill-tip organ in the bills of probing birds in a variety of families suggests that the development of this organ is favoured by a probe-foraging lifestyle and that we might expect to find it in other groups of probing birds. The presence of sensory pits has been reported in the bill of an extinct long-billed rail from New Zealand (*Capellirallus karamu*, Olson 1975), although inspection of museum specimens shows that these pits are not as dense in *Capellirallus* as in the Scolopacidae, Apterygidae or Threskiornithidae, and that they are generally not present in other rail species (S. J. Cunningham unpubl. data: *Chapter 1*).

In the past it has been assumed that probe-foraging ibises detect prey only when it touches the bill-tips (e.g. Kushlan 1979), that pursuit time between the detection and capture of prey is therefore almost non-existent, and that the birds can assess prey characteristics only after capture (Kushlan 1979). If ibises instead use remote touch, prey items can be sensed before they come in contact with the bill, and a "pursuit" phase between detection of prey and its capture (or miss) would follows. The bill-tip organ is sensitive to pressure waves in the substrate produced by prey, which may allow ibises to collect some information about prey characteristics (e.g., size) and, thus, select which prey to pursue. These possibilities should be taken into account in future studies of ibis foraging.

Morphological trends and habitat use

The habitat index that we calculated to rank ibises in terms of habitat use is supported by published field observations of several of the species that we included in our analysis. Ogden and Thomas (1985) and Frederick and Bildstein (1992) assessed the foraging habitats used by several of the species we examined, together with Green Ibis

(*Mesembrinibus cayennensis)* and Sharp-tailed Ibis (*Cercibus oxycerca)* for which we had no specimens. The two studies ranked these species from least to most aquatic as follows: Buff-necked Ibis (dry-land forager)*;* Green Ibis, Sharp-tailed Ibis, and Bare-faced Ibis (forage in moist soil, at water's edge, and occasionally in standing water)*;* and White Ibis, Scarlet Ibis and Glossy Ibis (forage almost exclusively in standing water). This rank ordering of species exactly matches the order in our habitat classification, although Bare-faced Ibis appears to be a more terrestrial forager than our index suggests, given that Frederick and Bildstein (1992) rarely found them foraging in water. Shifting the Bare-faced Ibis towards a lower HI value would improve the trend in Figure 4, so this discrepancy between our HI and field observations supports our hypothesis that ibis bill morphology is linked to habitat use. On the basis of our data and these published accounts, we predict that Green Ibis and Sharp-tailed Ibis will possess bill-tip organs similar in morphology to that of the Bare-faced Ibis.

The correlations that we found between habitat use and bill-tip organ morphology in ibises must be interpreted cautiously. We were able to sample only a small number of individuals per species, which potentially introduced bias. The tendency for more aquatic ibises to have more extensive bill-tip organs may also be attributable to underlying phylogenetic relatedness between ibis genera that use aquatic or terrestrial habitats. The internal phylogeny of the Threskiornithidae is not well resolved (Matheu and del Hoyo 1992); therefore, a role for phylogeny in causing this pattern can be neither confirmed, nor ruled out.

However, the evidence suggests that the positive association between number of sensory pits and aquatic habitat use extends beyond the ibises, which increases the likelihood that a relationship between habitat use and bill-tip organ morphology may be selectively advantageous. For example, kiwi (terrestrial foragers) exhibit relatively low numbers of pits in the bill-tip organ (~300) with pitting extending to ~12.5% of the bill length (Cunningham et al. 2007), whereas in spoonbills (exclusively aquatic foragers) pitting extends to >50% of the bill length (Swennen and Yu 2004). Adding data for kiwi species from Cunningham et al. (2007) to the scatterplots that relate ibis bill-tip morphology to habitat use supports the observed trend, and correlations between morphological variables and HI remain strong and highly significant. We therefore believe that the trend warrants further investigation, particularly given that this may lead

to a greater understanding of the function of bill-tip organs in birds in different substrate types.

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Chapter 4

Remote touch prey-detection by Madagascar crested ibises

Chapter reference: Cunningham, S. J., Castro, I., Jensen, T. and Potter, M. A. Remote touch prey-detection by Madagascar Crested Ibises. *Submitted to the Journal of Avian Biology.*

Abstract

Birds which forage by probing must often rely on sensory systems other than vision to detect their buried prey. Such senses may include hearing (e.g. Australian magpies (Atramidae), American robins (Turdidae)) or chemical senses/olfaction (e.g. kiwi (Apterygidae) and some shorebirds (Scolopacidae)). Probe foraging kiwi and shorebirds are also able to use vibrotactile cues to locate prey buried in the substrate at some distance from their bill-tips ('remote touch'). These birds possess an organ consisting of a honey-comb of sensory pits in bone of the bill-tips, packed with mechanoreceptive nerve ending (Herbst corpuscles). Such a bill-tip organ has recently also been described in ibises (Threskiornithinae), but its function not elucidated. We designed a foraging experiment presenting mealworm prey to three captive Madagascar Crested Ibises (*Lophotibis cristata urschi*) under a variety of trial conditions to discover whether they were using remote touch, mediated by their bill-tip organ; chemosense/olfaction; or hearing to locate buried prey. The ibises were reliant on remote touch for prey detection – the first time this sensory system has been demonstrated for this group of birds. They did not appear to use hearing or chemical senses/olfaction to aid in prey detection.

Introduction

Birds are generally thought to rely heavily on vision for most aspects of their lives (e.g. Silman 1973), but vision can be of little use when foraging for prey hidden in sand, soil, mud, or turbid water. While tracks, burrows and substrate disturbance may visually indicate the location of some prey items, probe-foraging birds must often rely on non-visual sensory systems to locate their food.

Bird species from a wide range of families (e.g. Scolopacidae; Apterygidae; Turdidae; and Atramidae) use varying sensory systems to locate buried prey. For example, American Robins (*Turdus migratorius)* and Australian Magpies (*Gymnorhina tibicen),* use auditory cues to find prey when visual cues are not available (Floyd & Woodland 1981; Montgomerie & Weatherhead 1997). Probe-foraging sandpipers use chemosensory systems such as taste and, perhaps, olfaction (Van Heezik et al. 1983; Gerritsen et al. 1983), chance location (directly touching prey while probing), and a sensory system called 'remote touch' to locate prey items. Remote touch is based on the interception of vibrotactile signals from burrowing prey, or pressure disturbances caused by sessile prey (Gerritsen & Meiboom 1986; Piersma et al. 1998). Nocturnal, probe-foraging kiwi (*Apteryx* spp.) use both olfaction (e.g. Wenzel 1968, 1971) and remote touch (Cunningham et al. 2009: *Chapter 2*) to find food. In both sandpipers and kiwi, the remote touch sense is mediated by an organ composed of numerous pits in the bone of the bill-tips packed with vibration-sensitive mechanoreceptors (Herbst corpuscles) (Bolze 1968; Piersma et al. 1998; Nebel et al. 2005; Cunningham et al. 2007).

Ibises (Ciconiiformes: Threskiornithinae) are usually described as 'tactile' hunters because of their method of foraging by probing into sediments and probing and sweeping in water (e.g. Kushlan 1978). Some studies of ibis foraging have assumed 'tactile' means that prey can only be detected by direct contact with the bill-tips, and that the time involved in chasing prey once discovered is therefore negligible (e.g. Kushlan 1979). Ibises, however, possess a bill-tip organ similar in structure to that found in the Scolopacidae and Apterygidae (Cunningham et al. in press: *Chapter 3*) suggesting they may also detect prey using remote touch, although this has not been tested for any ibis species.

The Madagascar Crested Ibis (*Lophotibis cristata*) is a forest ibis endemic to Madagascar (Mathieu & del Hoyo 1992). It possesses a bill-tip organ made up of polygonal sensory pits, densest at the bill-tip and extending back to almost 20% of the bill length (Cunningham et al. in press: *Chapter 3*). This ibis forages for invertebrates and small vertebrates in leaf litter and soil on the forest floor of both humid eastern and dry western forest types in Madagascar (Keith et al. 1974; Hino 2002).

We designed an experiment using captive Madagascar Crested Ibises to discover (1) whether ibises use any kind of remote sensing to detect prey, or simply find prey by using direct touch (chance) and (2) whether direct touch, remote touch, a chemical sense (taste/smell) or hearing is predominantly used in foraging.

Methods

Study species and captive facilities

We carried out this study at San Diego Zoo and San Diego Zoo's Wild Animal Park. Together, these two institutions house eight Madagascar Crested Ibises (subspecies *L. c. urschi*) of which five were available to us. We were ultimately able to collect data from only three individuals, as one ibis pair commenced courtship and had to be dropped from the study. The three birds included one male and one female, housed with other African birds in the African Aviary exhibit at San Diego Zoo's Wild Animal Park (hereafter male 1, female 2), and one female, housed with the courting pair of Madagascar Crested Ibises and other African birds in the Scripps Aviary at San Diego Zoo (hereafter female 1).

Experimental trials were carried out *in situ* in the aviaries in which the birds were normally housed. Trials were run between 0700 and 0800 hours, prior to the birds being fed and before the aviaries were opened to the public, to ensure that the ibises were motivated to forage. Ambient background noise was ~40 dB, sometimes approaching 65 dB due to loud bird calls in the aviaries.

Experiment design

Ibises were presented with mealworms (*Tenebrio molitor*) buried in tubs of soil (described below). Their prey capture rate (number of mealworms captured / minute foraging) was recorded under six different treatments: four experimental and two controls. All sensory cues were available in the positive control, and all sensory cues were masked, with prey detection possible only via chance contact with the bill-tip, in the negative control (Table 1). Each of the four experimental treatments was designed to isolate one or two of the senses of smell and/or taste, remote touch, or hearing (Table 1). Control and experimental treatments involved combinations of two levels (in parentheses) of the following factors:

1) Prey (6 live mealworms; 6 mealworms killed by freezing overnight to remove vibration cues);

2) Substrate (plain soil; 9 parts soil evenly mixed with 1 part powdered freeze-dried mealworms (hereafter "mealworm-mixed soil") to overwhelm chemical (smell/taste) cues produced by prey);

3) Background noise at the level of the foraging tray (ambient noise conditions; broadband white-noise playback at 50-55 dB to mask the ~30 dB sounds produced by burrowing mealworms).

Freeze-dried mealworms, obtained from Fluker Farms (Port Allen, LA, U.S.A.), were ground to a coarse powder using a Magic Bullet Blender (Homeland Housewares, Los Angeles, CA, U.S.A.) prior to mixing with soil for treatments 2, 4 and 6 (Table 1). White-noise playback for treatments 3 and 4 was produced using a DSE 1GB MP3 player (Dick Smith Electronics, Palmerston North, N.Z.) and Sony™ SRSA5S portable speakers (Table 1). Sound level was checked at the centre of the foraging tray using a Mini Sound Level meter (Jaycar Electronics, Palmerston North, N.Z.) and standardised to 50-55 dB by setting the speakers to maximum and adjusting the volume on the MP3 player. Soil used in all trials was organic landscaping soil, sourced from One Stop Equipment Rental, 254 Pine Street, Ramona, CA 92065.

Table 1 Treatment details and sensory cues available to the birds for each control or experimental treatment

Treatment	Prey	Substrate	Background Noise	Sensory cues excluded	Sensory cues available
1 *positive control*	Live	plain	Ambient	none	all
2	Live	mealworm-mixed	Ambient	chemical	auditory & vibrotactile
3	Live	plain	white noise	auditory	chemical & vibrotactile
4	Live	mealworm-mixed	white noise	Chemical, auditory	vibrotactile
5	Killed	plain	Ambient	Vibrotactile, auditory	chemical
6 *negative control*	Killed	mealworm-mixed	Ambient	All (except direct touch cues)	chance detection by direct touch only

Each bird was exposed to between one and four training trials to accustom them to the experimental set-up and to foraging in the presence of white-noise playback. They were then offered two (female 1) or three (female 2, male 1) replicate trials of each experimental treatment, presented in randomized order, with up to three treatments presented consecutively on a given morning, depending on the motivation of the birds to forage. Trials were video recorded using Sony Digital Handycams (DCR-HC40E, DCR-HC42E, DCR-HC45E).

Trial set-up

Mealworms were presented in 5 L opaque plastic tubs which were filled to 8-9 cm deep with soil. Mealworms were buried 6–8 cm below the surface at random locations within each tub immediately before beginning each trial, to ensure there was no time for the live mealworms to move upwards to the surface of the soil. After burying, the soil was carefully smoothed over to remove any visual cues as to prey location. New soil was used for each trial to avoid build up of prey odours.

At the beginning of each trial, one tub per bird was placed on concrete paths within the aviaries, and cameras were set up. Tubs were covered with plastic lids until the cameras were turned on, then uncovered to allow the birds access to the food.

Trials were considered completed when the birds had captured all six available mealworms or had lost interest in the trial and left the foraging area completely.

Video analysis and data collected

Data on prey capture rates (captures/min foraging) were extracted from the videos using the freeware video editing package VirtualDub (Lee 2008).

Statistical analyses

We carried out a non-parametric Friedman's test on prey capture rate in each treatment, blocked by individual ibis. We followed this with a non-parametric Conover's post-hoc test. Data used were averages of the two (female 1) or three (male 1, female 2) replicate trials of each treatment carried out by each individual bird (i.e. one data point per treatment per bird). Data are presented as mean ± SD.

Ethical note

This study was carried out under animal ethics approval from the San Diego Zoological Society, NIH IACUC assurance #A3675-01.

Results

We found a significant effect of treatment on prey capture rate, blocked by individual ibis (Friedman's test: $\chi^2 = 11.95$, df = 5, $p = 0.035$).

Significant differences in prey capture rate were found when the treatments in which vibrotactile cues were available (positive control, treatments 2, 3 and 4: 9.58 ± 1.76; 6.34 ± 2.23; 9.16 ± 2.72 and 6.88 ± 4.18 captures/min, respectively), were compared with the treatments in which vibrotactile cues were not available (negative control and treatment 5: 2.57 ± 0.30 and 2.44 ± 0.25 captures/min, respectively) (Conover's post hoc tests, all $p < 0.05$) (Figure 1).

No significant differences were found between any treatments in which vibrotactile cues were available (positive control, treatment 2, 3, and 4), or between the two treatments in which vibrotactile cues were not available (treatment 5, negative control) (Conover's post hoc tests, all $p > 0.05$). This suggests that chemical and auditory cues were unimportant to probe-foraging ibises in prey detection.

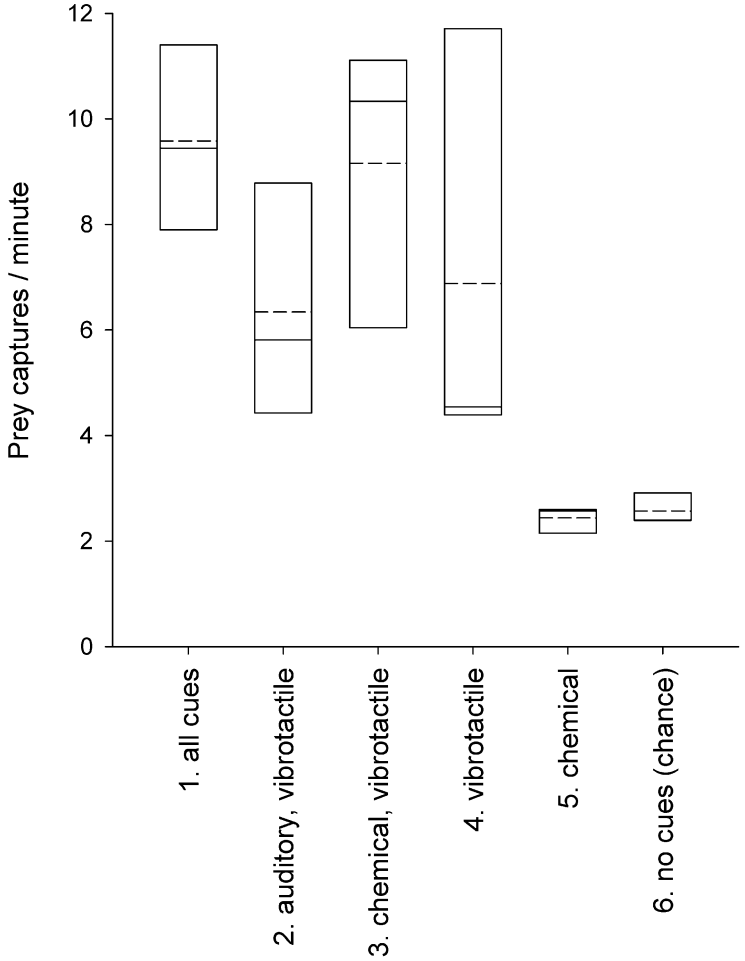

Figure 1 Box plots of prey capture rate in each of the two control and four experimental treatments (positive control far left, negative control far right). X-axis labels list treatment numbers and remote sensory cues available under each treatment (see Table 1). Solid lines represent range and median, dashed lines represent mean of data. Treatments 1-4 are significantly different from treatments 5 and 6 (post hoc Conover's tests all $p < 0.05$). N = 3 ibises.

Removing vibrotactile cues by killing mealworm prey (treatment 5 and the negative control) unavoidably resulted in a loss of auditory cues, also. However, treatments 3 and 4 show that masking mealworm sounds while leaving vibrotactile cues available does not result in a drop in prey capture rates when compared to the positive control. We can therefore infer that the observed reduction in prey capture rate in treatment 5 and the negative control was due to the absence of vibrotactile, rather than auditory, cues.

Discussion

Here we demonstrate for the first time that ibises are able to forage using remote touch, a system formerly known among birds only from the shorebird family Scolopacidae (Gerritsen & Meiboom 1986; Piersma et al. 1998), and more recently, from kiwi (Apterygidae) (Cunningham et al. 2009: *Chapter 2*). The birds we tested apparently used only remote touch and direct touch (prey detection by chance contact) to find prey. Overwhelming auditory and chemical cues had no measurable effect on their prey capture rates. The Threskiornithidae, Scolopacidae and Apterygidae are widely separated taxonomically (Hackett et. al 2008) suggesting remote touch has evolved several times in unrelated groups of birds in response to the challenges of probe-foraging.

The number of ibises (three) involved in this study was unavoidably small. However, three birds is a sample size in keeping with similar studies on prey-detection by birds (e.g. Montgomerie & Weatherhead (1997) four robins; Gerritsen & Meiboom (1986) two sanderlings). Despite the small sample size, the reduction in prey capture rate between treatments with and without vibrotactile cues is clear and significant. We are therefore confident Madagascar Crested Ibises in general will share the ability of our three study individuals to detect prey using remote touch.

At least nine other ibis species possess sensory pits in the bill-tip (Cunningham et al. in press: *Chapter 3*) so there is the very real possibility that these species are also able to forage using remote touch, although further behaviour experiments are needed to confirm this. Studies assuming that ibises detect prey by direct touch only (e.g. White Ibis, Kushlan 1979) may therefore underestimate the time spent ‘chasing’ prey by ibises probing in water or sediments. Remote touch may also allow ibises to gain some information about a prey item prior to capture, which may influence their decisions about which prey to pursue. Ignoring remote touch may therefore have implications for conclusions drawn from foraging studies on these birds.

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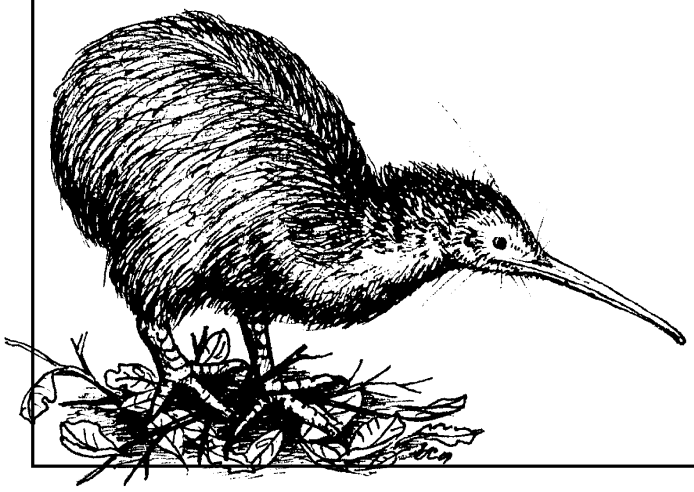
Chapter 5

The secret life of wild brown kiwi: direct foraging observations and other nocturnal behaviours

Chapter reference: Cunningham, S. J. and Castro, I. The secret life of wild brown kiwi: direct foraging observations and other nocturnal behaviours. *Submitted to the New Zealand Journal of Ecology.*



Abstract

Kiwi possess many unusual features that make them interesting subjects for behavioural study. However, their nocturnal, cryptic nature has meant that studies to date rely on data collected indirectly using radio-telemetry. We present the first study of wild brown kiwi (*Apteryx mantelli*) behaviour by direct observation. We used handheld infrared video cameras to obtain ~6 hours of video-footage of kiwi over 19 months. Kiwi used native forest and exotic pasture habitats while active at night and spent most of their time foraging (75%). Prey capture rates were significantly higher in pasture than forest. The remaining 25% of time was spent walking, vigilant, engaged in comfort behaviours, escaping disturbance, and investigating obstacles. Direct social and courtship interactions were observed rarely. The senses of hearing, olfaction and touch seemed most important to kiwi. Touch was used for investigating terrain and negotiating obstacles. Hearing was used in response to sounds made by observers and conspecifics. Olfactory search behaviours (OSBs) were used in the direction of these sounds, and olfaction was also apparently used to assess odours on the ground. Kiwi never used obviously visually guided behaviours. Behavioural repertoire size and diversity increased in winter, due to increases in OSBs towards conspecifics, and rarely observed behaviours. Prey capture rates also increased near-significantly in winter and microhabitat use was more diverse. Female kiwi at our study site had 30% longer bills than males, and probed into soil substrates on average 30% deeper. No other behaviours that might reduce competition between kiwi sexes were observed.

Introduction

Kiwi (Apterygidae) are an unusual family of five species of ground dwelling birds (Burbidge et al. 2003), endemic to New Zealand. They display many features that make them ideal model organisms for studying a range of aspects of behavioural and sensory ecology. For example, kiwi appear to have foregone vision as a major sense (Martin et al. 2007), and instead possess a suite of sensory specialisations that appear finely attuned to nocturnal life, but which are highly unusual among birds (Wenzel 1968; Cunningham et al. 2007; Martin et al. 2007; Corfield 2009). Kiwi also exhibit reversed sexual size dimorphism, particularly relating to bill length (Robertson et al. 2003) that may have evolved under intense competitive pressure. Evidence for this includes reports of high kiwi population densities from the 1800s (e.g. Buller 1877), current high densities of kiwi in some protected areas (e.g. Miles & Castro 2000), and the fact that kiwi were originally part of a suite of ground foraging insectivorous birds, many of which are now endangered or extinct (Wilson 2004). Kiwi further possess a breeding system where females lay large rich eggs which are incubated under a variety of social breeding arrangements including male alone incubation (McLennan 1990) and a mating system that appears to range from complete monogamy, through to co-operative breeding with helpers at the nest, depending on species and population (Colbourne 1991, Taborsky and Taborsky 1999; Ziesemann et al. in prep).

Kiwi are generally nocturnal and often live at sparse densities in thickly vegetated habitats, making them incredibly cryptic. For this reason the majority of studies of wild kiwi behaviour have been carried out indirectly using radio-telemetry (McLennan et al. 1987: Potter 1990; Miles 1995; Grant 2003; Taborsky & Taborsky 1995, 1999 and others) and analysis of sign (including faecal matter in diet studies, e.g. Kleinpaste 1990, Miles 1995, Shapiro 2005). Rarely have kiwi researchers been able to spend time directly observing kiwi apart from brief encounters (e.g. Colbourne & Kleinpaste 1983; Taborsky & Taborsky 1995). This has meant that, while valuable data on territoriality, spacing, habitat use and diet has been gathered, information on detailed aspects of wild kiwi behaviour and microhabitat use is lacking.

We used infrared cameras to directly observe wild brown kiwi (*Apteryx mantelli*) behaviour in open forest and pasture habitats over a period of 19 months. Here we describe nocturnal behaviours and foraging microhabitat use of wild kiwi in detail for the first time. We use these data to make inferences about the sensory systems used by kiwi in day-to-day life, and what these senses are used for. We also investigate whether there is any evidence for behavioural foraging niche partitioning between long-billed adult female kiwi, and shorter billed males and juveniles. Finally, we examine differences in kiwi foraging success between exotic pasture grassland and native forest fragments.

Methods

Study site and kiwi population

We carried out our research on South Ponui Farm, a sheep and beef farm on Ponui Island, New Zealand (1770 ha; latitude 36 55'S, longitude 175 11'E). Vegetation cover on the farm is a patchwork of pasture and unfenced native forest fragments. Within fragments, thick scrub and remnant kauri trees (*Agathis australis*) vegetate ridges. Forested gullies have a very open understorey due to browsing by stock. Raupo (*Typha orientalis*) swamps extend some distance along the gully floors. Shapiro (2005) provides further detail of vegetation cover.

The study was carried out in an area comprising two forested gullies (Red Stony Hill Gully, hereafter RSHG; Pipe Gully, hereafter PG) and surrounding pastureland in the southeastern corner of the largest forest fragment on the island (Fig. 1). Brown kiwi were translocated to Ponui Island in 1964 (Miles & Castro 2000) and kiwi density on the island is now estimated at ~1/ha (Cunningham et al. 2007). Between 30 and 36 kiwi within the study area carried radio-transmitters ('tagged kiwi') during the course of this study.

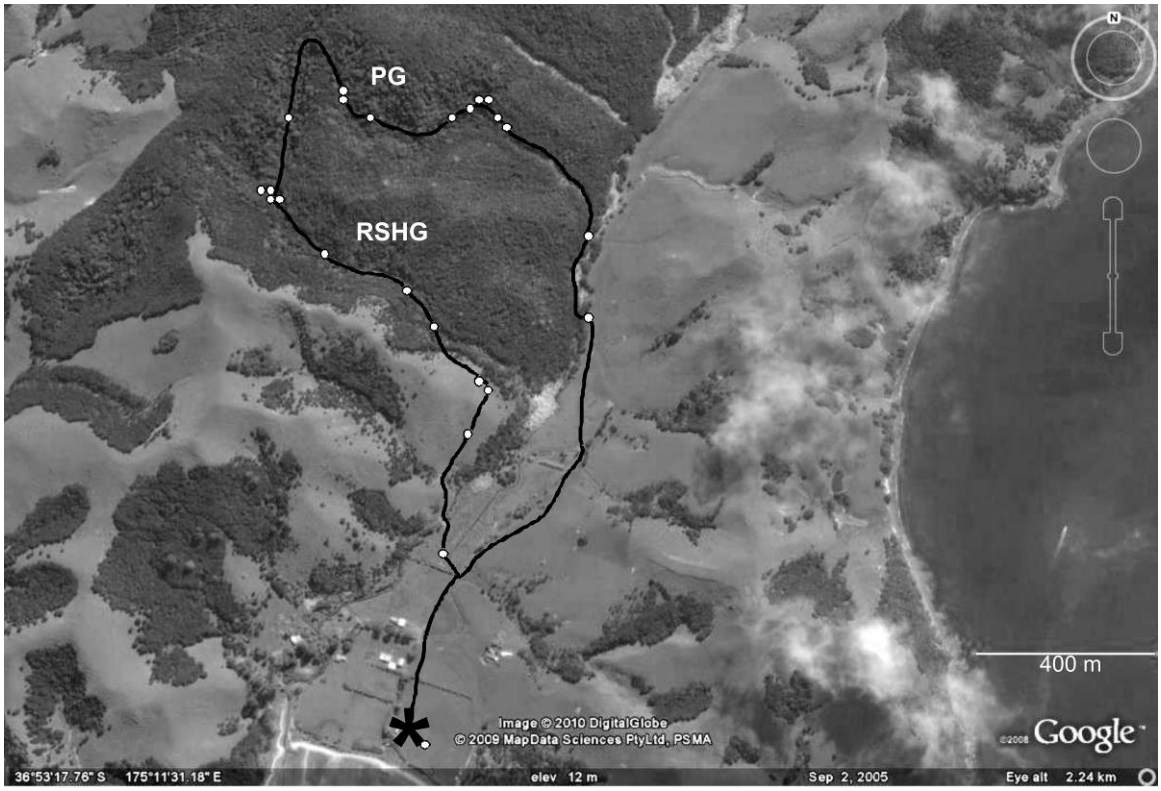


Figure 1. Aerial photograph of the study area, showing walking routes (black lines), locations of video-recorded kiwi encounters used in the analyses (white circles), and our campsite (marked with a black asterisk). PG = Pipe Gully, RSHG = Red Stony Hill Gully. (Photograph extracted from Google Earth).

Kiwi morphometrics

In April 2008 we recaptured 30 kiwi, including 13 females and 17 males that had been radio-tagged previously, for an annual transmitter change. Kiwi were located using radio-telemetry equipment (yagi aerial, TR4, Kiwitrack Ltd, Havelock North, NZ) and captured by hand whilst roosting during the day, following kiwi best practice guidelines (Robertson et al. 2003). Additionally to replacing old transmitters, we measured bill length (from the circle of the cere to the tip; following Robertson et al. 2003) and tarsus width to the nearest 0.1 mm using Kinchrome Vernier callipers, and weighed the birds using a Pesola spring balance to the nearest 5 g. Bill measurements were used to calculate average difference in length between males and females for use in comparing probing depths. Measurements were also used to assess degree of sexual dimorphism in this population.

Obtaining video-recordings of kiwi

We made eleven seven-day trips to the study site between January 2007 and September 2008 (approximately every second month) to video-record kiwi. Video-recording was

attempted for between four and eight hours from dusk every night (66 nights), except under severe adverse weather conditions. Recordings were made using Sony Handicams (DCR-HC40E and DCR-HC96E) with NightShot function and infrared spot lamps (IRLamp6, Bat Conservation and Management Inc.). Lamps and cameras were handheld; lamps were powered from 12V batteries carried in backpacks. Kiwi did not visibly react to the infrared light from the lamps.

Each evening, teams of two people walked set routes through the study site, including areas of open forest (2 km) and pasture (1.6 km) (Fig. 1). Walking routes avoided swamps and scrubby ridges due to the difficulty of obtaining clear footage in these areas, although transmitter signals indicated they were used by kiwi. This created an unavoidable habitat bias in our sample. Kiwi were located by the rustling sounds they made when walking or foraging. On hearing a kiwi, we stopped, switched off headlamps and scanned with the camera to locate the bird. Kiwi were recorded from the time they were first encountered until they moved out of view into thick vegetation or out of camera range. Kiwi were not followed when they left the area in which we were video-recording them, therefore footage length could be used as an indicator of the 'willingness' of the bird to stay in close proximity to researchers. Radio telemetry was used to identify kiwi observed to be wearing a transmitter.

Assigning sex to untagged kiwi

Male and female kiwi at our study site were highly dimorphic in terms of bill length (see results), and this feature can be used to sex kiwi (Robertson et al. 2003). However, we could not directly measure bill length from video-recordings due to the impossibility of determining an accurate scale. Instead we developed a measurement, the 'bill length ratio' (BLR) that allowed sexing kiwi from video footage. The BLR is the ratio of the distance between the cere and eye : bill length (Fig. 2). We chose this measure because the bill, cere and eye of kiwi reflect infrared light and are clearly visible in infrared footage. When applying the BLR, it is important to correctly locate the top of the cere and to ensure the bird is in full lateral view. We therefore chose the clearest lateral view still frames of each video-recorded kiwi and imported these into ImageJ (National Institute of Health 2008), which allows accurate measurement from photographs. We took five repeat eye-cere and bill length measurements from each bird and used the averages to calculate each individual's BLR.

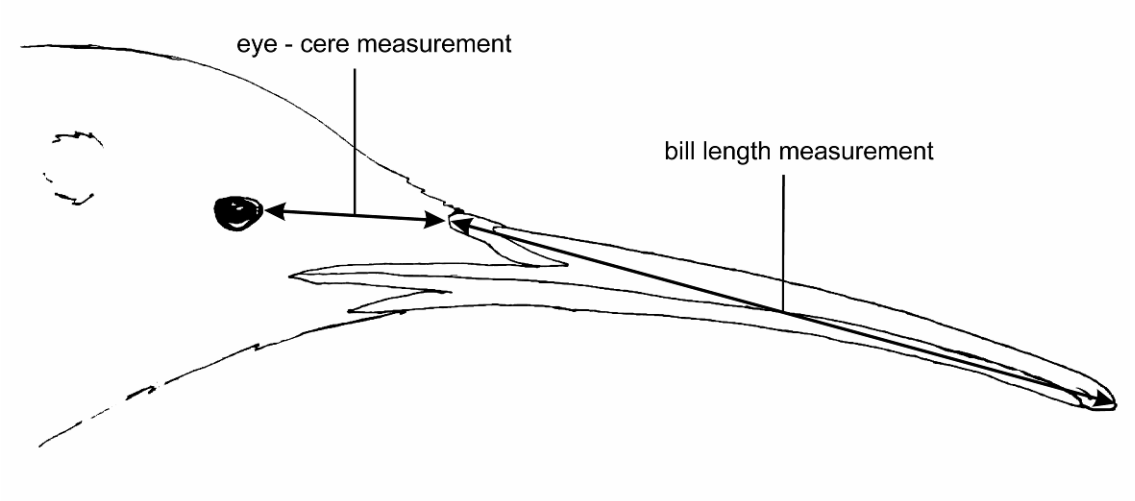


Figure 2. Diagram of a kiwi head showing eye – cere and bill length measurements used to calculate a bill length ratio for sexing untagged birds from video-recordings (females: bill length ratio ≥ 4.15; males/juveniles: bill length ratio < 4.15).

To calibrate the measurement, we compared the BLRs of adult tagged birds of known sex for which we had clear video still frames or photographs in lateral view (n = 6 males; 8 females). Male kiwi had an average BLR of 3.70 ± 0.22, range 3.40 - 3.99, 95% confidence interval 3.26 – 4.13; female kiwi average BLR 5.17 ± 0.52, range 4.28 – 5.88, 95% confidence interval 4.15 – 6.19. Because the average BLR of male and female kiwi was significantly different (Mann-Whitney U test, W = 21, p = 0.002) and there was no overlap in range or confidence interval, we felt it was safe to assign female sex to individuals with BLRs ≥ 4.15. Males and juvenile birds, which overlap in bill measurements, could not be distinguished using BLR, so these were treated as a single category.

Assigning identity to untagged kiwi

Untagged kiwi of the same sex, videoed on different occasions, were assumed to be the same individual unless plumage was distinctively marked (some had pale feathering on the head), size was markedly different, or they were filmed in widely separated areas (e.g. top versus bottom of a gully, different gullies).

Data extraction from video-recordings

All videos were screened for quality and bird identity before transcription. Poor quality videos (very dark, blurry, or kiwi obscured by vegetation) were discarded from analysis.

Where multiple videos of the same individual kiwi had been obtained, data was transcribed from the highest quality video only, in order to avoid pseudoreplication. Where videos of an individual were of similar quality, the longest sequence was used. Videos were transcribed using freeware video editing programme VirtualDub (Lee 2008) which allowed us to step through recordings frame by frame.

Habitat and season

Habitats were broadly described as 'pasture' or 'forest' based on vegetation type. The year was divided into two 'seasons' designated 'summer' (November – March) and 'winter' (May –September), as these categories better reflected the prevailing weather conditions on Ponui Island during our study, than did the standard four seasons (pers. obs.).

Behavioural variables

Behaviours were typified by close examination of video-recordings. Time spent engaged in each of six behaviour states (foraging – including prey handling, walking, comfort, vigilant, escape, investigation of obstacles) and detailed aspects of behaviours relating to these states were recorded (Table 1).

Foraging microhabitat variables

At the completion of each filming session leaf litter depths and soil penetrability were measured at 2 - 6 locations within the area in which the kiwi was foraging. Leaf litter depth was measured to the nearest mm using a metal ruler inserted vertically into the litter, and soil penetrability (psi) was measured for the top 5mm of soil using a hand penetrometer.

Kiwi foraged in a variety of different microhabitat types within forest and pasture habitats. We classified these as: litter (leaf litter away from tree trunks), tree roots (within one kiwi body length of a tree trunk, or where roots emerged from the soil), logs (fallen logs/branches), supplejack tangles (*Ripogonum scandens* –a liana, with stems that form dense tangles close to the ground, kiwi foraged beneath these), fallen epiphytes, banks, ditches, creek edges, swamp edges, grass roots, bare ground and cow dung. We extracted data on how long kiwi foraged in each of these microhabitat types from the video-recordings.

Statistical analyses

Footage length: Video footage length was normally distributed (Anderson Darling test $p > 0.05$) and was compared between sexes (female and male/juvenile kiwi), seasons ('summer' and 'winter') and habitat types (forest and pasture) using ANOVA tests.

Morphometric and behaviour data: Most behaviour data were not normally distributed (Anderson Darling tests $p < 0.05$). We therefore used non-parametric Kruskal-Wallis H-tests to compare behaviours between sexes, seasons and habitat types, and Wilcoxon paired-sample tests to compare related behaviours (e.g. the durations of successful versus unsuccessful probes). Morphometric variables were compared between male and female kiwi using Mann-Whitney U-tests.

Shannon-Wiener diversity indices were calculated for behavioural repertoires of female and male/juvenile kiwi; kiwi video-recorded during 'summer' and 'winter'; and kiwi video-recorded in pasture and forest habitats. This index takes into account both the number of different behaviours used and also the 'evenness' of use (i.e. how commonly each behaviour was performed). Indices were compared using Hutchison's t-test, following Zar (1999).

Foraging microhabitat use: Diversity of foraging microhabitat use within forest habitats was compared between sexes and seasons using Shannon-Wiener diversity indices followed by Hutchison's t-test. Proportion of time spent foraging in each microhabitat type was compared between sexes and seasons using Kruskal-Wallis H-tests.

Most analyses were carried out in Minitab 15. Data are presented as means ± SD. Results were considered significant when $p \leq 0.05$.

Permits

Kiwi handling was carried out under the Department of Conservation permit: AK-14971-RES.

Table 1. Ethogram of kiwi behaviours, including the aspects recorded and analysed and the % video-recordings in which the behaviour occurred (% occurrence). The % of time spent in each behaviour state was also recorded and used in analyses. One copulation sequence, one calling sequence and one fight sequence were recorded during the course of the study but were not included in the data analyses.

Behaviour state	Action	Description	% Occurrence	Fine scale aspects recorded and used in analyses
Foraging	Tap	Bill-tip sensory pad pressed to ground momentarily; can be directly ahead or slightly to one side; no prey capture.	100	− Tap frequency/min − Tap duration (s) − Ratio of taps (including tap walking, see below) to probes
	Rapid tap	Series of very short, rapid taps of the bill to the ground in a small area, bill not lifted far from the ground between taps; each series counted as one event; prey capture may follow.	60	− Rapid tap frequency/min
	Soil probe	Bill inserted into soil substrate, depth ranges from bill-tip only to entire bill and part of face. Head and bill may be repositioned via partial withdrawal of bill; vigorous rocking movement of whole body from legs sometimes occurs; bird may walk around bill during probe resulting in rotation of the bill within the ground; prey capture may follow. Conical probe holes left by deeper probes result from the rocking & rotation of the bill during probing.	88	− Probing frequency/min (total of soil & litter) − Duration of successful probes (total of soil & litter) (s) − Duration of unsuccessful probes (s) − Depth of soil probes relative to bill length (scale: 1 < half bill, 2 ≥ half bill, 3 entire bill including cere, 4 part of face in probe hole)
	Litter probe	Bill inserted into litter layer or matted grass roots in manner of a soil probe but angle often shallower. Bill may be shoved forward in the litter resulting in litter being shunted along, sometimes accompanied by the bird stepping forward. Head & bill movement and changes of angle may be apparent, but stepping around the probe & rocking of body not normally seen. Prey capture may follow.	96	− Depth of litter probes (scale as for soil probes) − Percent of probes into soil − Percent of probes into litter/matted grass roots − Prey captures/min − Percent of captures in litter/ matted grass roots − Percent of captures in soil
	Bill hover	Looks like tapping but bill does not contact ground, rather hovers above; usually occurs in a short back-to-back sequence. May be olfaction related i.e. smelling for prey or another scent on the ground.	24	− Bill hover frequency/min
	Squeaky beak	Non-vocal squeaking noise produced while probing, apparently as the bill is removed from the substrate	Unknown	− Not recorded as detection dependant on distance to bird
Prey handling	Withdraw	Measured from the beginning of bill withdrawal from substrate at the end of a successful probe/lifted from surface after successful rapid tap, until beginning of first swallowing movement	96	− Prey handling duration (sum of duration of withdraw, swallow and downtime) (s) − Number of bill flicks/capture
	Swallow	Rapid flicking/jerking of head and bill to toss the prey back within the bill. Sometimes also includes prey positioning movements of bill during which the lower bill moves sideways independently of the upper bill.	96	
	Downtime	Measured from the end of final bill flick in the swallowing sequence to beginning of next activity (e.g. start of next probe)	96	
Walking	Walk	Walking	100	− Percent of time walking
	Tap (walking)	As described above for tap but while the bird is walking. May be involved in orientation, assessment of terrain ahead.	100	− Frequency of taps/min while walking − Tap duration walking (s)

Table 1 cont…

Behaviour state	Action	Description	% Occurrence	Fine scale aspects recorded and used in analyses
Escape	Run	Running	20	− Percent of time running
	Jump	Escape behaviour when startled, also used to get down banks and off logs	8	− Jumps/min
Comfort	Preen	Running bill through feathers; sometimes results in consumption of ectoparasites through conspicuous swallowing movements of head and bill	32	− Percent of time preening
	Scratch	Scratching of body, head and neck with foot	32	− Scratching bouts/min
	Stretch	Kiwi stretches head & neck forward, one leg raised to horizontal and stretched behind the body	8	− Stretches/min
	Shake	Puffing of body feathers followed by vigorous shake. Separate head and bill shake sometimes follows on from body shake, or can occur independently.	40	− Shakes/min
	Leaf toss	Rapid shaking of the head from side to side to clear bill of leaf litter speared during probing. Can involve opening and shutting of beak.	52	− Leaf toss/min
	Defecation	Defecation	8	− Defecation/min
Vigilance	Freeze	Freezes in position following disturbance	12	− Freeze/min
	Head lift	Head lifted above the level of the back and bill held horizontally, often in response to a noise audible to the observer. Can involve several back-to-back head lifts.	100	− Headlifts/min
	Olfactory search (‘sniffing’)	Stereotyped upright body posture (like a penguin), head and bill repeatedly drawn upwards and backwards in a series of audible 'sniffs'. Often follows head lift behaviour. Described in detail by Castro et al. (in press: *Appendix 2*)	88	− “Sniffs” /min towards observer − “Sniffs” /min other directions (mostly other kiwi)
Investigation of obstacles	Bill reach	Reach forward with head and bill, tapping to investigate difficult terrain	24	− Bill reaches/min
Social / courtship	Call	Standing bird lifts head and neck and calls in a series of bars which involve wide opening of beak and lifting of upper bill to vertical while producing each bar	n/a	
	Grunting	"Courtship" vocalisation made by birds in close proximity while engaged in other behaviours e.g. foraging. On one occasion preceded copulation (see below)	n/a	
	Mewing	Vocalisation heard during pre-copulation grunting	n/a	
	Feather probing	Probing of the feathers of the other bird with the bill during courtship	n/a	
	Copulation	Female crouches on ground, male mounts briefly. In the sequence recorded, male’s parted feathers offered a clear view of his uropygial gland prior to mounting. After (during?) copulation he fell backwards from the female onto his back on the ground. Copulation was followed by the male chasing the female with his beak inserted among her back feathers (both birds running).	n/a	
	Chasing	Chase post copulation or during/after fighting	n/a	
	Fighting	Kicking and chasing accompanied by growling vocalisations.	n/a	

Results

Kiwi morphometrics

Female kiwi were significantly larger than males in all morphological variables measured (weight, tarsus width, bill length; Mann-Whitney U-tests all $p < 0.05$) (Table 2). Female bills were on average 30% longer than male bills at our study site and there was no overlap in bill length between the sexes.

Table 2. Morphological measurements of Ponui Island kiwi, April 2008. Data presented are means ± SD with range given in parenthesis.

	Males (n = 17)	**Females (n = 13)**
Weight (g)	1926 ± 221 (1400 – 2350)	2223 ± 326 (1550 – 2775)
Tarsus width (mm)	11.9 ± 0.7 (10.8 – 13.1)	12.7 ± 0.9 (10.9 – 14.2)
Bill length (mm)	92.3 ± 3.2 (88.1 – 98.5)	120.4 ± 8.3 (108.0 – 136.2)

Video-recordings: sample size and length of observations

A total of 96 separate kiwi observations (including multiple sightings of the same individuals) were made during the course of the study, and over 8 hours (500 minutes) of video footage were obtained. This included 73 separate observations made in summer, and 23 in winter. This bias towards observations made in summer was despite slightly more winter than summer trips being made to the study site (six and five, respectively). Kiwi were observed in both pasture (19% of observations in summer when long grass made viewing kiwi difficult, 26% of observations in winter) and forest (81% summer; 74% winter) habitats throughout the year.

After controlling for video quality and removing duplicate footage of individuals, almost 6 hours (355 minutes) of footage of twenty-five individual kiwi remained. Video-recording length ranged from 3.6 - 31.6 minutes (average 14.2 ± 7.8 minutes). The sample included 12 adult females and 13 males or juveniles, 17 summer and 8 winter observations, and 19 forest and 6 pasture observations. There was no difference in average video-recording length with sex, season, or habitat type (ANOVA all $p > 0.05$). Approximately equal numbers of male/juvenile and female kiwi were video-recorded in summer (n= 9 and 8, respectively) and winter (n = 4 and 4 respectively). Approximately equal numbers of male/juvenile and female kiwi were video-recorded in

forest (n= 9 and 10, respectively) and slightly more male/juvenile than females were recorded in pasture (n = 6 and 2 respectively). Fig. 1 shows the approximate locations where the sequences used in the analyses were obtained.

Behaviours observed and time budgets

Kiwi behavioural repertoire included behaviours related to foraging (nine behaviours), vigilance (three behaviours), social and courtship interaction with other kiwi (seven behaviours), walking and escape (four behaviours), comfort (six behaviours) and investigation of terrain (one behaviour) (Table 1). Social and courtship behaviours were observed on a very rare basis. One fight between two male kiwi, one female call, and one courtship – copulation sequence including female and male calls were video-recorded during the course of the study. Due to small sample size these behaviours are described in Table 1 but not used in analyses.

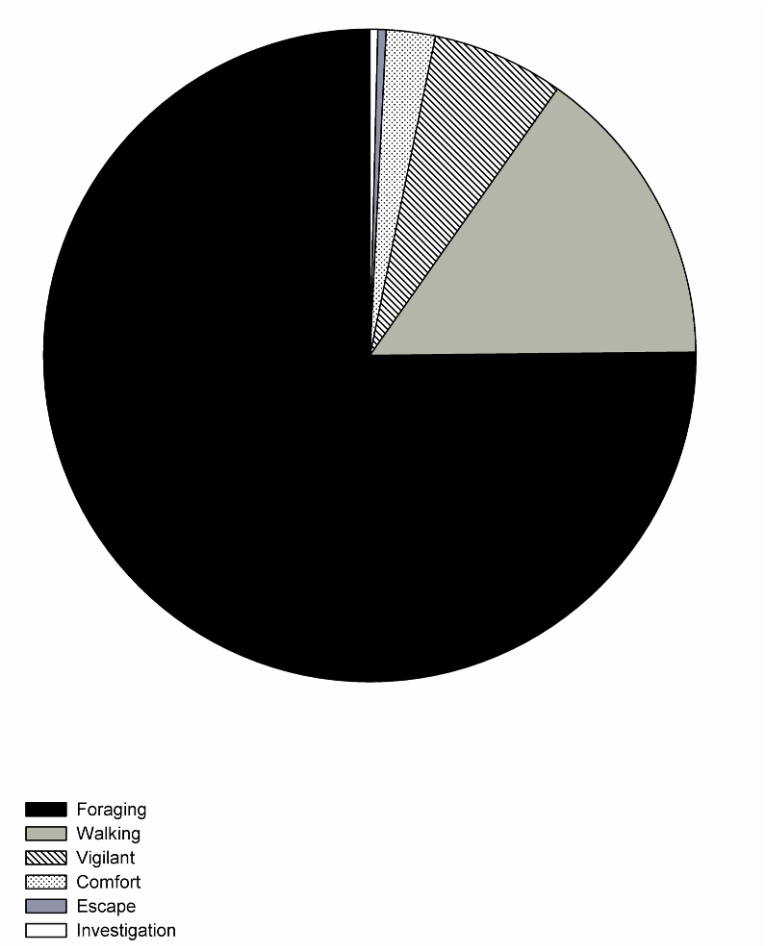


Figure 3. Average percentage of time kiwi spent in each of six behaviour states: foraging, walking, vigilant, comfort, escape, investigating obstacles. N = 25 kiwi.

The average % of time spent in each of the behaviour states foraging, walking, vigilant, comfort, escape, and investigation of obstacles, did not differ significantly between kiwi sexes, seasons, or habitat types (Kruskal-Wallis H-tests, all $p > 0.05$). Foraging was the activity the birds spent most time involved in during our observations (75 ± 17% of total observation time), followed by walking (15 ± 13%) (Fig. 3).

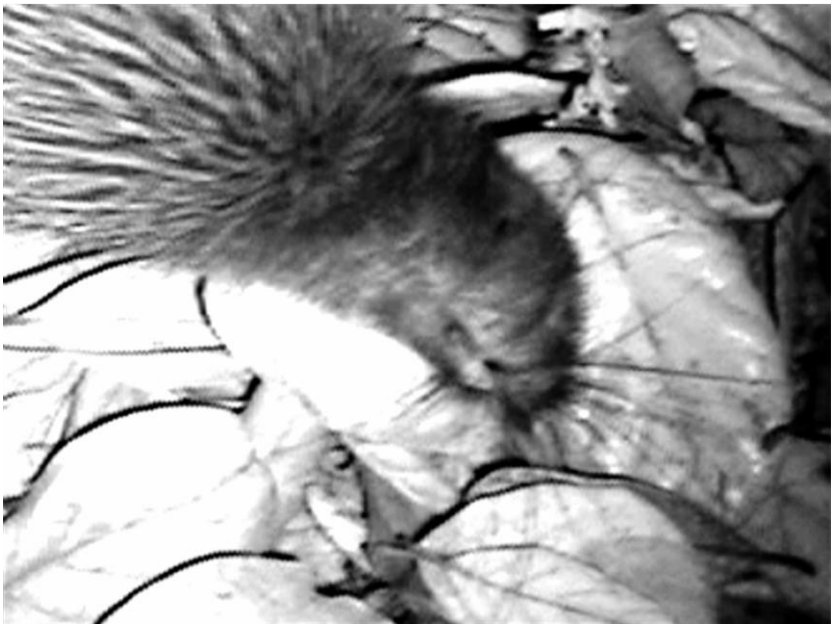


Figure 4. A still frame from a video-recording of a kiwi foraging in leaf litter. The head, neck and 'shoulders' of the kiwi are visible. The bird is facing to the right of the picture, with the majority of its bill inserted beneath the leaves (which look pale due to infrared reflection). The long bristle feathers are visible fanning out from around the base of the beak, and spreading out over the surface of the litter as the kiwi probes.

Foraging behaviour: All foraging behaviours observed are described in Table 1. The most commonly observed foraging behaviours were tapping the ground ahead ('tapping': 100% of kiwi) and probing (100% of kiwi were observed probing; 96% used leaf litter or matted grass roots and 88% used soil substrates). The average ratio of taps : probes was 1.69 ± 1.14 : 1. Rarer foraging behaviours included 'rapid tapping' which appeared to be an attempt to pick up prey items found on the surface (60% of kiwi) and 'bill hovering' where the bill tip was held close to the ground and moved back and forth with a motion similar to tapping (24 % of kiwi; see Table 1). In high quality footage, we were able to observe that foraging kiwi held their facial bristle feathers forward, forming a 'net' around the bill which contacted the surface of the leaf litter as the bird probed (Fig. 4). Kiwi never displayed pecking, or other obviously visually guided foraging behaviours.

Foraging kiwi probed at a rate of 9.87 ± 4.47 probes per minute. Probes were into leaf litter (forested habitats) or matted grass roots (pasture habitats) (57.7% of probes) and into soil substrates (both habitat types, 42.3% of probes). There were no differences between males/juveniles and females, or between seasons, in terms of the proportion with which these probing substrates were used (Kruskal-Wallis H-tests, all $p > 0.05$). Successful probes (resulting in prey capture) were longer in duration than unsuccessful probes (6.53 ± 2.89s and 2.66 ± 0.84s respectively; Wilcoxon paired-sample test $Z = 295$, $p < 0.001$). Some soil probes included a non-vocal squeaking noise as the bill was withdrawn from the substrate, perhaps caused by friction of the bill against the soil.

Probing depth relative to total bill length varied with sex. For example, male/juvenile kiwi used a greater proportion of their bill length on average when foraging in leaf litter than females (Kruskal-Wallis $H = 6.48$, $df = 1$, $p = 0.011$). The average proportion of bill length inserted during soil probes was similar for male/juvenile and female kiwi (Kruskal-Wallis $H = 0.50$, $df = 1$, $p = 0.481$), but a greater % of male/juvenile than female soil probes used the full length of the bill or more (e.g. a part of the face inserted into the probe hole), (male/juvenile kiwi, 37.5% of probes; female kiwi, 18.2 % of probes; Kruskal-Wallis $H = 4.28$, $df = 1$, $p = 0.039$). This was despite the fact that probing to this depth would allow female kiwi access soil strata that were inaccessible to males and juveniles.

Prey capture and handling: We observed 413 prey captures during the course of the study (average 16.5 ± 22.7 captures / kiwi). The majority of these followed probing into litter (19.6%), matted grass roots (14.3%), or soil (47.5%); although 11 prey items (2.7%) were captured directly from the surface using rapid tapping. The foraging strata could not be identified for 16.0% of prey captures, due to video-recording quality. Prey capture rate was higher in pasture (4.43 ± 2.52 captures/min) than in forest (1.25 ± 0.57 captures/min) (Kruskal-Wallis $H = 6.42$, $df = 1$, $p = 0.011$). Prey capture rate also tended to be higher in winter (2.98 ± 2.48 captures/min) than in summer (1.56 ± 1.59 captures/min), although this was not quite significant (Kruskal-Wallis $H = 3.26$, $df = 1$, $p = 0.071$).

We divided the kiwi prey handling sequence into three discrete parts: withdrawal of the bill from the substrate; swallowing the prey; and 'down time' which was measured from the end of swallowing to the beginning of the next activity (e.g. a new probe). Total handling times were 1.81 ± 0.53 s on average and did not vary significantly with sex, season or habitat (Kruskal-Wallis H-tests, all $p > 0.05$). Swallowing prey involved tossing the head and bill back repeatedly ('bill flicking') and kiwi took an average of 2.05 ± 0.64 bill flicks to swallow each prey item.

Walking and investigating terrain: Kiwi spent 15.08 ± 13.44 % of observation time walking, and 0.39 ± 1.36 % investigating obstacles such as steep banks and fallen logs in their path. When investigating obstacles, kiwi stretched their neck and reached forward with their bill repeatedly to touch the obstacle surfaces. The bird then either clambered over the obstacle or walked around it.

While walking, kiwi continually tapped the ground ahead with the bill-tip. This behaviour was not seen in birds that were running, or accelerating from a walk to a run. Kiwi tapped with greater frequency when walking in forested habitats (42.87 ± 29.78 taps/min) than when walking in open pasture (16.57 ± 10.43 taps/min, Kruskal-Wallis H-test $H = 5.76$, $df = 1$, $p = 0.016$).

Vigilance and escape behaviours: All video-recorded kiwi displayed some vigilance behaviour, ranging from 0.28 – 24.36 % of total observation time (average 6.53 ± 6.90 %). All kiwi displayed 'head lift' behaviour (pausing activities and raising the head above the level of the back). This behaviour was usually presented in response to a noise audible to the observer. Kiwi also commonly displayed olfactory search behaviour ('sniffing' see Table 1; 88% of kiwi); often but not exclusively following head lifts (42% of the time). Olfactory search behaviour (OSB) was directed towards the observer (55.60 % of OSBs) or towards odour sources other than the observer (where identifiable, other kiwi, on one occasion another kiwi's nest) (44.40 % of OSBs). There was a near-significant increase in the frequency of OSB towards sources other than the observer in winter (0.32 ± 0.72 OSBs/hr in summer; 1.22 ± 1.39 OSBs/hr in winter, Kruskal-Wallis $H = 3.43$, $df = 1$, $p = 0.064$). Kiwi sometimes also 'froze' in response to particularly loud or sudden disturbances nearby (observed on 3 occasions, 12% of video-recordings).

Escape behaviours following a disturbance included running away, either out of sight of the observer, or a short distance prior to resuming foraging (20% of kiwi); or jumping out of the way (2 kiwi, both in winter). On one occasion a kiwi leapt away from a twig which sprang from the leaf litter and hit the bird as it trod on it. No differences in vigilance or escape behaviours between sexes or in different habitats were observed (Kruskal-Wallis H-tests, all $p > 0.05$).

Comfort behaviours: Comfort behaviours exhibited by kiwi included tossing away dead leaves which had become speared on the beak during probing (only in forested habitats, 52 % of kiwi displayed this behaviour), shaking, stretching, preening and scratching, and defecation. We observed kiwi defecations more frequently during winter than summer (1.76 ± 2.66 / hour winter; 0.15 ± 0.63 / hour summer; Kruskal-Wallis $H = 4.40$, $df = 1$, $p = 0.036$). We found no other differences in comfort behaviour with sex, season or habitat.

Behavioural repertoire diversity

Kiwi behavioural repertoire was larger in winter (11.63 ± 2.07 behaviours/observation) than in summer (9.29 ± 1.65 behaviours/observation) (Kruskal-Wallis $H = 6.11$, $df = 1$, $p = 0.013$). Repertoire diversity also increased in winter (Shannon-Weiner H' winter = 1.239; H' summer = 1.127; Hutchison's $t_{0.05(2),\,198} = -3.219$, $p < 0.002$). The behaviours that increased most in frequency in winter were OSBs towards odour sources other than the observer, and the rarely observed behaviours defecation and jumping (see results above).

Behavioural repertoire size and diversity were similar between male/juvenile and female kiwi, and between pasture and forested habitats (sexes: Kruskal-Wallis $H = 0.47$, $df = 1$, $p = 0.492$; Hutchison's $t_{0.05(2),\,896} = -0.615$, $p > 0.50$; habitats: Kruskal-Wallis $H = 1.27$, $df = 1$, $p = 0.261$; Hutchison's $t_{0.05(2),\,94} = 1.548$, $p > 0.10$).

Foraging microhabitat use

Within forested habitats, kiwi used a more diverse range of microhabitats in winter than in summer (H' winter = 0.812; H' summer = 0.667; Hutchisen's $t_{0.05(2),\,827} = -6.992$, $p < 0.001$) (Fig. 5). Kiwi observed in summer spent the majority of their foraging time in open litter, tree roots or fallen log substrates (81.37 ± 20.67%). In winter, these three

microhabitat types became less important, with birds spending 50.17 ± 28.51% of their foraging time there, a near-significant decrease (Kruskal-Wallis H-test: H = 3.36, df = 1, p = 0.061). Supplejack tangles, ditches, banks, creek edges and fallen epiphytes together were used significantly more in winter than in summer, accounting for 49.82 ± 28.51% of time foraging in winter and 17.08 ± 20.44% of foraging time in summer (Kruskal-Wallis H test: H = 5.57, df = 1, p = 0.018). Soil was softer and more penetrable in winter (0.58 ± 0.13 psi) than summer (1.92 ± 1.62 psi; Kruskal-Wallis H = 5.21, df = 1, p = 0.022), perhaps allowing kiwi to use a wider range of microhabitats at this time.

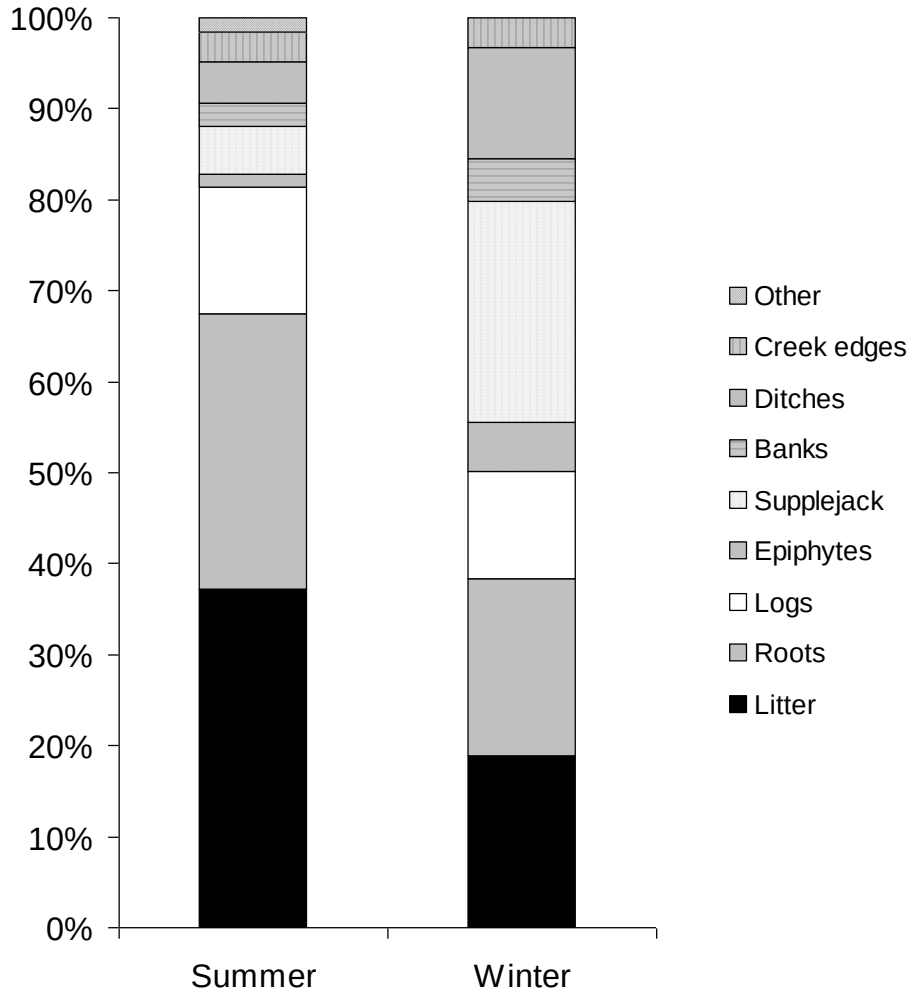


Figure 5. Foraging microhabitat use by kiwi in forested habitats in summer versus winter. Category "Other" includes swamp edges, bare ground and grass roots. Summer N = 13, winter N = 5.

There were no differences in microhabitat use or diversity of use related to sex: Kruskal-Wallis H tests, all $p > 0.05$; Shannon-Weiner H' male/juvenile = 0.796, H' female = 0.779; Hutchison's $t_{0.05(2),\,965} = 0.784$, $p > 0.20$.

Discussion

Here we describe nocturnal behaviour and foraging microhabitat use of wild brown kiwi from direct observation and in detail, for the first time. We found that kiwi spent the majority (~75%) of their time foraging, and only ~25% on other activities, including comfort, walking and vigilance behaviours. Direct social and breeding interactions between individuals were observed very rarely. Below, we discuss our results in terms of the sensory systems used by kiwi, the extent to which kiwi behaviourally partition their foraging niche in a dense population, and the importance of different habitat types in foraging.

Senses

Vision: Among birds, kiwi possess an unusual suite of sensory specialisations. Their visual fields and visual processing areas in the brain are greatly reduced and their bill-tip falls outside their visual field (Martin et al. 2007). Kiwi are therefore unlikely to guide their beak using visual cues (Martin et al. 2007). In keeping with this, we observed no behaviours in kiwi that were obviously visually guided. Kiwi did not peck at food items in the manner of visually guided foragers (e.g. short-billed shorebirds, Barbosa & Moreno 1999) but captured prey only by probing (into soil, matted grass roots and leaf litter) or by tapping repeatedly (‘rapid tapping’) at prey items on the surface they appeared to have located by touch or smell. Kiwi were observed on a number of occasions to ignore potential prey which walked past their bill tips as they probed, and on at least one occasion a rapid tap sequence was unsuccessful due to the prey item (a large spider) escaping from under the bill tip and walking away. The kiwi commenced to search for this lost prey item by tapping with the bill and did not seem able to see it (although it continued to be visible to the observer). Kiwi occasionally probed into tree trunks and banks, but did not glean prey items from vegetation surfaces, a possibility raised by Reid et al. (1982).

Touch: Kiwi possess a vibration-sensitive bill-tip organ similar in structure to that of sandpipers (Cunningham et al. 2007). Enlarged centres in the brain for the relay and processing of tactile information from the bill, suggests that touch-related senses are highly important to kiwi (Martin et al. 2007). Trials in captivity show that kiwi use

remote touch (a remote tactile sense mediated by their bill-tip organ), together with olfaction to find buried prey (Cunningham et al. 2009: *Chapter 2*). We observed behaviours which we believe provide evidence that kiwi also use tactile cues in exploration and navigation. Kiwi in difficult terrain (steep banks, root bolls of fallen trees) used 'bill reach' behaviours, in which they stretched the neck forward and tapped with the bill, presumably to investigate the topography ahead before clambering over it. Kiwi employed similar tapping behaviours (minus the neck stretch) both when foraging and when walking. The frequency of taps per minute when walking in forest habitats where they were likely to encounter tree trunks and undergrowth was significantly higher than when walking in open pasture, providing further support for the idea that kiwi use their sensitive bill-tip to assess obstacles in their path. This observation lends support earlier observers' notes that kiwi use their bill to tap ahead in a way similar to 'a blind man's walking stick' (e.g. Haeusler 1923). We assume that tapping and bill reach behaviours are primarily tactile. However, kiwi nostrils are placed at the tip of the beak and it is likely the birds may gain some olfactory information when performing these behaviours as well. The information from both senses could be integrated to create a more complete picture of the surrounding environment.

Brown kiwi facial bristle feathers ('whiskers') are long (Baker et al. 1995, see also *Chapter 6*) and often held back away from the beak when the birds are handled (author's pers. obs.). We observed kiwi holding their facial bristles forward during foraging, forming a 'net' around the bill which contacted the leaf litter during probing. It is possible therefore that the bristles have a tactile function in prey-detection. Kiwi facial bristle follicles are surrounded by Herbst corpuscles supporting this idea (*Chapter 6*). However, on at least one occasion we observed a potential prey item walk through the bristle feather net apparently without being perceived by the kiwi. Facial bristles may also be used in tactile investigation of the immediate environment, for example undergrowth or burrow walls.

Olfaction: Kiwi nostrils are uniquely placed at the tip of the long bill, and their olfactory chamber and olfactory bulb are extensively developed and large (Bang 1971; Martin et al. 2007). Kiwi are known to use olfaction for foraging (Wenzel 1969, 1971; Cunningham et al. 2009: *Chapter 2*) and could potentially use olfaction for a variety of other purposes as well (Jenkins 2001, Castro et al. in press: *Appendix 2*). We observed

kiwi using stereotyped olfactory search behaviour (OSB) as described by Castro et al. (in press: *Appendix 2*) on numerous occasions (88% of video recordings). We also observed a new behaviour which seems likely to be related to olfaction: bill hovering. When performing this behaviour, kiwi move the beak back and forth close to the ground without contacting it. We classified this behaviour as a foraging behaviour, but kiwi may use it to detect scents other than prey odour close to the ground.

Kiwi directed stereotyped OSBs both towards observers and in directions other than the observer. Where odour source other than the observer could be identified, it was almost invariably another kiwi (identified by rustling sounds distinctive to kiwi activity), although on one occasion it was another kiwi's nest. Our observations corroborate Castro et al.'s (in press: *Appendix 2*) contention that odour is used by kiwi in environmental exploration and social interactions. The near significant increase in OSBs towards other kiwi during winter provides some evidence that odour may be used in the context of breeding, as kiwi in our study population commenced breeding early in winter (late May, early June) and continued breeding throughout the winter months (May – September) (Ziesemann in prep., authors' pers. obs.). Rarely observed behaviours also increased in frequency in winter as did overall behavioural repertoire size and diversity, and there was a near significant increase in prey capture rates. It is therefore possible that the increased frequency of olfactory search behaviours towards other kiwi, along with increase in other behaviour types, was a result of reduced foraging pressure on kiwi during winter together with an increase of social activities necessary for successful breeding.

Hearing: Trials in captivity show that kiwi use olfaction and remote touch to locate buried prey, but do not seem to use auditory cues for this purpose (Cunningham et al. 2009: *Chapter 2*). We observed no overt behaviour related to auditory prey detection in our study. Other auditory foragers, for example American robins (*Turdus migratorius*) and Australian magpies (*Gymnorhina tibicen*) distinctively cock their heads towards source of sound (Floyd and Woodland 1981, Montgomerie & Weatherhead 1997). Kiwi did not perform such behaviours, although this does not preclude them using hearing in foraging. We did see behaviours related to the use of hearing in other parts of kiwi life – 'head lifts' seemed to be auditory-related behaviour as they often occurred in response to the observer, or a kiwi other than the focal individual, making a sound. Kiwi also

have loud calls, cochlea structure similar in some aspects to barn owls (*Tyto alba* - auditory specialists) and hearing range shifted to higher frequencies (Corfield 2009). Corfield (2009) suggests the shift to higher frequencies would be ideal for hearing invertebrate rustlings in leaf litter. We suggest kiwi's high frequency hearing may also be used in detection of conspecific footsteps on leaf litter, or similar disturbances in the environment.

Foraging niche partitioning

Female kiwi at our study site had on average 30% longer bills than males, with no overlap in bill length between the sexes. Despite this, male/juvenile and female kiwi in our study showed no separation in foraging substrate, microhabitat or habitat use, or any behaviour relating to foraging except for probing depth. When probing into 'surface' substrates (leaf litter or matted grass roots), male/juvenile kiwi used a greater average proportion of their bill length than females, presumably allowing them to reach the same depth. This implies all kiwi were likely to be foraging in direct competition within surface substrates.

When probing into soil, however, all kiwi inserted the same average proportion of the bill, presumably resulting in females probing on average 30% deeper than males. 18.18% of female soil probes used the full length of the bill, reaching soil strata completely unavailable to males. Interestingly, male/juvenile kiwi probed to the full length of their bills significantly more often than females (37.38% of probes), even though they would still be in competition with females at this depth. Deep probes are presumably more energetically costly than shallower ones, and the percentage of probes reaching maximum depth may relate to the foraging pressure kiwi are under. Our results may therefore indicate that male and juvenile kiwi were under greater competitive pressure than females who can exploit deeper strata. Alternatively, deep probes may simply be less costly for shorter billed kiwi than for adult females.

Other bird species which are sexually dimorphic in terms of bill length seem to show greater partitioning of their foraging niches than kiwi. For example, western sandpiper (*Calidris mauri*) females have 15% longer bills, forage in drier microhabitats and have different foraging behaviour patterns than males (Fernandez & Lank 2008). Female bar-tailed godwits have 24% longer bills than males. When wintering in Queensland,

Australia, female godwits (*Limosa lapponica*) used both sand flats and sea grass habitats for foraging, while males used only sea grass (Zharikov & Skilleter 2002). Male green woodhoopoes (*Phoeniculus purpureus*) have bills which are 36% longer than females, use different foraging microhabitats (wider tree branches), substrates and techniques than females, resulting in partitioning of diet between the sexes (Radford & du Plessis 2003).

Kiwi may partition their foraging niche more in times of great food stress (e.g. during droughts) and we may not have observed such a period during our study. They might also use further mechanisms to reduce intraspecific competition, which we could not detect with our sampling method. For example, in most cases were could not identify prey items captured by kiwi. Diet studies suggest kiwi are generalists that take prey in proportion with availability (e.g. Kleinpaste 1990; Miles 1995), however it is currently unknown whether sexes specialise on different types of prey. Further, we could not distinguish untagged juvenile females from untagged males in our study. If foraging differences exist between kiwi sexes that are independent of size and bill length, we would therefore have been less able to detect these. Kiwi have a slow metabolism compared to similar sized birds (Calder & Dawson 1978) which may ease competition for food at high densities.

Sexual selection has been proposed as a mechanism for sexual bill-length dimorphism in species where evidence for foraging niche partitioning is minimal (e.g. Cory's shearwater, *Calonectris diomedea*, Navarro et al 2009). It is possible that sexual selection may be partially responsible for bill-length dimorphism in kiwi, but further work needs to be carried out to provide evidence for this.

Habitat use

Kiwi foraged in forest and pasture habitats throughout the year. The majority of observations (76%) were made in forest habitats. This likely reflects the length of our walking routes (longer in forest), and the fact we found it easier to detect kiwi in open forest where we could hear their footsteps on leaf litter clearly, than in pasture where their movements were quieter.

Fewer kiwi were video-recorded in winter than summer, despite more winter than summer visits being made to the study site (6 and 5, respectively). This suggests that birds may have been using habitats we did not visit (densely vegetated ridges; swamps) more often during the winter. Alternatively, kiwi may have been more cryptic in winter, perhaps due to dampened leaf litter muffling their movements and/or breeding males spending time incubating eggs and therefore being unavailable to view.

Prey capture rate was significantly higher in pasture than in forest. Previous studies of kiwi living in areas including both exotic and native vegetation patches have concluded that native vegetation is preferred by kiwi (Taborsky & Taborsky 1995) and has higher soil invertebrate availability than exotic vegetation (Colbourne and Kleinpaste 1983). Taborsky & Taborsky (1995) and Colbourne & Kleinpaste (1983) also found that kiwi territories encompassing fragments of native vegetation were smaller than those which did not, suggesting that native vegetation increases territory quality. Our observation of higher foraging success in exotic pasture is therefore in direct contrast with previous work. Most pasture observations were near a forest edge and higher invertebrate diversity due to edge effects could help explain high prey-capture rates in this habitat type (see review Didham 1997). Black field crickets (*Teleogryllis commodus*) were seasonally abundant in the pasture in summer (author's pers. obs.), and earthworms could be found in higher abundance in pasture than within the forest year round (Shapiro 2005). The forest fragment itself is unfenced, and is severely affected by livestock (sheep and cattle) browsing and trampling (authors' pers. obs.). Recent research shows that, in New Zealand, forest fragments accessible to livestock have 10 to 100-fold lower densities of leaf litter invertebrate species compared to forest reserves from which livestock are excluded (Didham et al. 2009). Forest leaf litter was an important foraging stratum for kiwi in this study (almost 20% of prey captured were extracted from litter). Therefore, the impact of livestock may depress kiwi prey capture rates in our forest fragment, helping explain the differences between our study and those of others.

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Chapter 6

Facial bristle morphology and function in insectivorous birds in New Zealand

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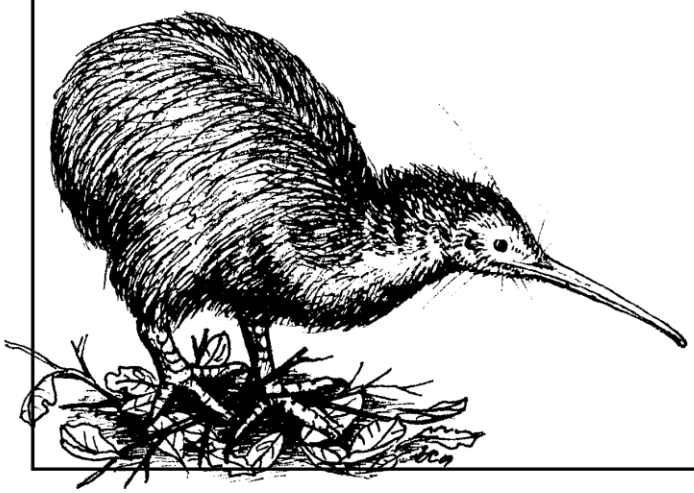

Abstract

Little is known about the histological structure or the function of avian facial bristle feathers. Here we provide information on their morphology and histology, with inferences for function, in five insectivorous birds from New Zealand. We chose species with varying ecologies, including: brown kiwi (*Apteryx mantelli*), morepork (*Ninox novazealandae*), hihi (*Notiomystis cincta*), New Zealand robin (*Petroica australis*) and New Zealand fantail (*Rhipidura fuliginosa*). Herbst corpuscles (vibration and pressure sensitive mechanoreceptors) were found in association with bristle follicles in all species, suggesting that facial bristles in general have at least some tactile function. We found that facial bristles were generally shorter in diurnal than nocturnal species, and that nocturnal and hole-nesting birds had more heavily encapsulated Herbst corpuscles than diurnal open-nesting species. Our results from the New Zealand fantail lend some support for the hypothesis that aerial insectivores use their facial bristles as insect scoops or to protect the eyes from limbs of captured prey and particles during flight, whereas nocturnal and ground dwelling birds such as brown kiwi may use their facial bristles for tactile exploration of the environment, or prey detection.

Introduction

Many bird species possess bristle feathers on the head and around the face, particularly at the base of the bill and nares, the lores, malar and rictal regions and forehead, and sometimes taking the form of 'eyelashes' (Stettenheim, 1973). These bristles can be named by location (e.g. 'rictal,' 'lorial,' 'narial,' Lucas & Stettenheim, 1972) and take a variety of forms in different species. For example, short stiff rictal bristles are found in many insectivorous and hawking species while longer bristles resembling mammalian vibrissae are found in some nocturnal and hole-nesting species such as kiwi (*Apteryx* spp.). Lucas & Stettenheim (1972) suggest that the term 'facial bristles' should apply to bristles found on the sides of the head, but here we use this term generally to mean all bristles on the face of the bird including those found in defined regions such as the rictus and lores.

The structure of avian bristles tends to be simple, consisting of a single (occasionally double) shaft, sometimes with feather barbs present at the base and with tips at times branched (Jany, 1955; Stettenheim, 1973). The bristle rachis is generally tapered, stiff and dark-coloured particularly at the tip (Stettenheim, 1973). It is thought that increased melanin content in bristles may contribute to their stiffness (Lucas & Stettenheim, 1972; Stettenheim, 1973). A gradation of bristle types exists - from the contour feathers from which they are derived through variously branched "semibristles" to stiff, unbranched bristles such as those seen around the gape of many aerial insectivores (Chandler, 1914; Lucas & Stettenheim, 1972; Stettenheim, 1973). Very little work has been done on the histological structure and connections of bristles with nerves, muscles and connective tissue within the dermis. It is known, however, that bristle follicles tend to be operated by feather muscles, and that bristle follicles in some owl species at least are accompanied by avian lamellar corpuscles (mechanoreceptors, probably Herbst corpuscles) (Küster, 1905; Stettenheim, 1973).

The function of avian bristles has been the cause of much speculation, with many hypotheses put forward, but few empirical tests of these hypotheses carried out. Major ideas as to the function of bristles in birds are: (a) bristles around the mouth of insectivorous species act as a 'scoop' or 'net' to help in the capture or retention of flying

insects (reported often in popular literature, also Welty, 1962; Van Tyne & Berger, 1976); (b) bristles around the gape and face act to protect the eyes from particles in flight, or from broken off or struggling parts of insect prey during handling (Dyer, 1976; Conover & Miller, 1980), or from vegetation (Brush, 1967; Hill, 1967) and bristles around the nares and ear openings may protect these orifices (Stettenheim, 1973); (c) long bristles on the face and head of nocturnal and hole-nesting species have a tactile function similar to mammalian vibrissae (Küster, 1905; Lucas & Stettenheim, 1972); and (d) stiff facial bristles prevent soiling of the facial plumage during feeding (Chandler, 1914). Jany (1955) even suggested that bristles along the base of the upper bill may act as chemoreceptors.

Of these hypotheses, there is some observational and experimental evidence that rictal bristles do protect the eyes of aerial insectivores from flying particles / kicking legs of prey (Dyer, 1976; Conover & Miller, 1980). The presence of lamellar mechanoreceptors found associated with the follicles of the facial bristles of some owl species (Küster, 1905; Stettenheim, 1973), strongly suggests a tactile function. As yet, there is no empirical evidence for the intuitive supposition that bristles around the gape of insectivorous birds act as an insect scoop, and species in which this function might be expected, have not been observed to use their bristles in this way (Lederer, 1972; Conover & Miller, 1980). Leisler & Winkler (1985) found a correlation between greater gape width, longer rictal bristles and aerial feeding in birds, however, and Keast & Saunders (1991) noted that in kinglets, rictal bristles of hawking species tend to be longer than those of gleaning relatives, perhaps suggesting a function as a scoop for flying insects. Longer bristles might also provide greater protection for the eyes during flight. There is no structural or histological evidence that bristles can act as chemoreceptors (Stettenheim, 1973).

We conducted a morphological and histological investigation of rictal, and some other facial, bristles of five insectivorous bird species including representatives from each of the groups: hole nesters; aerial feeding (‘hawking’) & non-hawking insectivores; volant and flightless nocturnal species. We compared innervation and bristle structure between these bird species with respect to the ecological differences between them. We predicted that bristle follicular structure would be related to apparent ecological function and therefore formulated the following hypotheses:

a) that in hole-nesters and nocturnal birds, bristles would have a tactile function like that of mammalian vibrissae. We would therefore expect a high degree of innervation including the presence of mechanoreceptors associated with the follicle. We would expect these bristles to be longer than those possessed by diurnal, open-nesting hawking and non-hawking insectivores.
b) that stiff rictal bristles in hawking and non-hawking insectivores will be less well innervated than tactile bristles due to their apparent function in the protection of the eyes of these birds – therefore we expected to find fewer mechanoreceptors in diurnal insectivores than in the facial bristles of nocturnal and hole-nesting birds. We also expect these bristles to be thicker and perhaps shorter than those of nocturnal, hole-nesting birds.

Methods

Study species

Five avian species were used. Three were either nocturnal and hole-nesting (brown kiwi *Apteryx mantelli*, morepork *Ninox novazealandae* (a small owl)) or diurnal and hole-nesting (hihi *Notiomystis cincta*); one was a diurnal aerial insectivore which forages by hawking (New Zealand fantail *Rhipidura fuliginosa*; hereafter fantail) and one was a diurnal ground feeding and gleaning insectivore, less reliant on hawking for prey (New Zealand robin *Petroica australis*; hereafter robin). Details of species ecology and sample sizes are given in Table 1.

Specimens for morphology and histology were sourced from the National Wildlife Mortality Database collection based at Massey University, New Zealand, where they had been archived in 10% formalin for research purposes following initial necropsy.

Table 1: Species investigated, family, and aspects of ecology. N = number of specimens used.

Species	Family	Aspects of ecology	N (morphology)	N (histology)
Brown kiwi *Apteryx mantelli*	Apterygidae	Nocturnal Flightless Hole/burrow nesting Insectivore (probe foraging)	2	2
Morepork *Ninox novazealandae*	Strigidae	Nocturnal Flighted Hole nesting Insectivore (hawking/ground)	1	1
Hihi *Notiomystis cincta*	Notiomystidae	Diurnal Flighted Hole nesting Nectivore/ insectivore (gleaning)	2	1
New Zealand fantail *Rhipidura fuliginosa*	Monarchidae	Diurnal Flighted Open nesting Insectivore (hawking)	2	1
New Zealand robin *Petroica australis*	Eopsaltriidae	Diurnal Flighted Open nesting Insectivore (ground/gleaning)	3	1

Morphology

Detailed drawings of facial bristle location were made for each species. Samples of two to eleven bristles from each distinct area of the specimen's head were measured to the nearest half millimetre, from emergence from the skin to the tip, using a stainless steel ruler. Bristles from different parts of the face were detached for closer examination of structure under a dissecting microscope. Classification of facial bristles as 'bristles' or 'semibristles' was made in accordance with Lucas & Stettenheim (1972). Bill length and gape width were measured using Vernier callipers. Average bristle length: gape width ratios were calculated.

Histology

Clusters of bristles and semibristles, including the whole of the dermal layer in which they were embedded, were trimmed from formalin-fixed specimens, processed routinely, and embedded in paraffin. Paraffin blocks were soaked for 30 minutes in

Molliflex (BDH) prior to sectioning to soften keratinaceous bristle shafts. 3μm sections were cut from each block and stained with haematoxylin and eosin (Luna, 1968).

Stained slides were examined and the presence of Herbst corpuscles (vibration and pressure sensitive mechanoreceptors) associated with bristle follicles was recorded. The average number of Herbst corpuscles visible per bristle was calculated, and length, width, diameter of inner core, outer zone and capsule were measured for each Herbst corpuscle. Widths of the bristles at the skin surface was also measured, and bristle width: bristle length ratios calculated.

Permits

All histology work on protected species was carried out under the following permits issued by the New Zealand Department of Conservation: NO-19321-DOA, WE-322-RES.

Results

Morphology

In the morepork and especially in the passerine species (hihi, fantail, robin), bristles occurred in well differentiated groups in different parts of the head (Fig 1). Bristles within a group were generally structurally similar, but differences in structure between groups were common. All passerine species possessed bristles with varying numbers of umbilical or distal barbs, rictal bristles having fewer barbs than those in other areas. Most barbules were denoted “reduced” and probably represented the stylet type, although some had small nodules or hooks and may be reduced plumulaceous or pennaceous types (see Lucas & Stettenheim, 1972). In the robin and hihi, upper rictal bristles were longer on average than bristles in other areas of the head. In the fantail, both the upper and the lower rictal bristles were long in comparison to other bristle groups.

All facial bristles we located on the morepork were structured like semibristles, although those on the eyelids and interramal region had extended rachis tips and may be

intermediate in form between semibristles and bristles. In kiwi, bristles, semibristles and intermediates of varying lengths were interspersed and distributed around the entire facial region including the interramal region, rictus, lores, orbital region, and forehead. Length of bristles was much more varied than in the other species. The longest bristles were found around the rictus, lores and forehead (Table 2, Fig. 1). Kiwi bristles were simple in structure and did not possess umbilical barbs.

Facial bristle morphology, colour, and placement in each species are described in detail in Table 2.

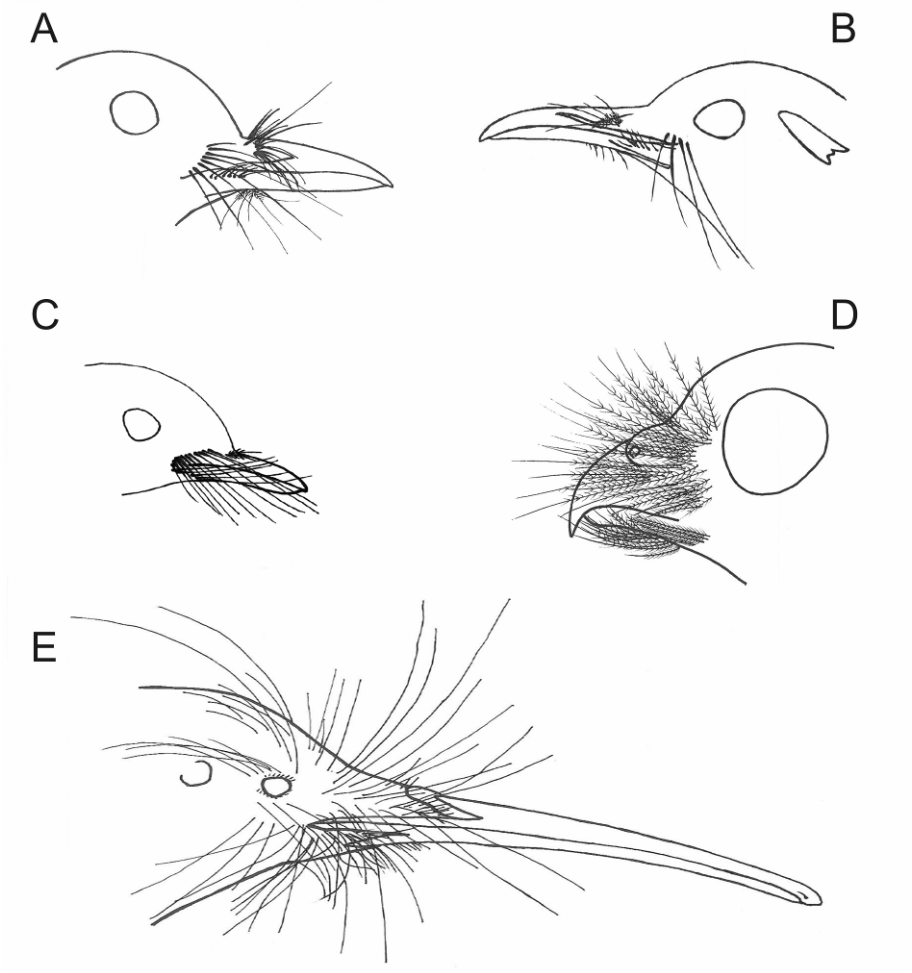

Figure 1: Facial bristle locations in five New Zealand bird species. (A) New Zealand robin; (B) Hihi; (C) New Zealand fantail; (D) Morepork; (E) Brown kiwi.

Table 2: Bill length, gape width and details of facial bristle morphology in five New Zealand birds

Species (number examined)	Bill length in mm (n)	Gape width in mm (n)	Bristle length : gape width ratio	Bristles					
				Region of head	Structure	Orientation	Colour	Average length in mm (total no. bristles measured)	Range (mm)
Brown kiwi (2)	80 (1)	23.26 (1)	1.1	Forehead	Long, stiff bristles, tapering to a fine point. Very fine barbs lie close to the shaft proximally.	More rostral point forward, more caudal curve upward and backward over the head	Dark brown/black	51.9 ± 22.7 (14)	24 - 95
				Lores	Long bristles, some with very tiny barbs emerging from rachis	Forward and upwards	Dark brown/black	32.6 ± 18.3 (11)	12 – 70.5
				Superorbital	Long with small barbs on rachis	Upwards, outward	Dark brown/black	26.4 ± 13.5 (13)	15.5 – 58.5
				Eyelashes	Short, fine.	Upwards	Dark brown/black	3.3 ± 2.1 (5)	1 - 7
				Suborbital	Short semibristles	Upwards	Dark brown/black	7.8 ± 3.5 (8)	5 – 12.5
				Rictus (upper)	Both true bristles and semibristles with very tiny barbs, no barbules. Long, thick.	Forward and outward	Dark brown/black	39.5 ± 22.1 (12)	8 - 77
				Rictus (lower)	Semibristles and intermediates, long with short barbs, increase in length caudally. Some long bristles without obvious barbs present. Long caudal bristles have short proximal barbs at the base.	Forward and outwards. Longest most caudal bristles point sideways and down.	Dark brown/black with light brown barbs	20.0 ± 22.7 (16)	4 - 71
				Rictal corner	Long bristles and shorter semibristles	Forward and upward	Dark brown/black	29.3 ± 14.1 (9)	15.5 - 50
				Interramal	Bristles, semibristles and intermediate forms. Long single tips, barbs lacking barbules	Point forward and variously outward	Brown, paler barbs	14.9 ± 9.8 (19)	4.5 - 41.5
Morepork (1)	20.08 (1)	11.42 (1)	1.0	Eyelashes	Semibristles with fine extended, bare rachis and distal barb tips, stylet barbules on distal parts of barbs, large tuft of umbilical barbs with long barbules	Outwards	Tips of rachis and distal barbs black, dark barbules on distal barbs, proximal and umbilical barbs and barbules and proximal region of rachis, white.	8.2 ±1.3 (11)	6.5-10.5
				Lores	Long semibristles, barbs along length. Proximal portions of rami have fine, long stylet barbules. Proximal part of rachis has short, fine barbules between rami of barbs.	Upward and forward	Black rachis, white barbs and barbules	17.4 ± 2.8 (21)	11 – 20.5
				Interramal	Semibristles with extended rachis, widely spaced barbs lacking barbules.	Upward and forward, covering mandible	Translucent barbs and rachis. Dark rachis tip.	9.3 ± 1.0 (11)	7.5 - 11

Table 2 cont…

Species (number examined)	Bill length in mm (n)	Gape width in mm (n)	Bristle length : gape width ratio	Bristles					
				Region of head	Structure	Orientation	Colour	Average length in mm (total no. bristles measured)	Range (mm)
Hihi (2)	17.68 ± 0.2 (2)	7.99 ± 0.77 (2)	0.7	Nares	Fine bristles with long single tips, occasional bristle longer than the others. Small groups of umbilical barbs and barbs on proximal part of rachis. Very reduced barbules lie close to rami towards the tips.	Upward and forward	Black, except for paler bases of rami	4.8 ± 1.8 (12)	2.5 – 8.5
				Rictus (upper)	Long, fine bristles, few fine barbs lie close against the rachis base. Set in a row of four either side of the head	Forward and downwards	Black	11.3 ± 2.2 (16)	7.5 -14.5
				Rictus (lower)	Tiny, fine bristles. Cluster of short umbilical barbs with very short, fine reduced barbules.	Upward along mandible	Brown/black	2.6 ± 0.5 (18)	1.5 – 3.5
				Interramal	Fine bristles, long single tip. Clump of umbilical barbs with short reduced barbules lying against rami.	Loose cluster point down and forward	Brown/black	3 ± 1.2 (6)	1.5 - 5
NZ Fantail (2)	7.05 ± 0.66 (2)	6.06 (1)	0.9	Nares	Long tips, basal barbs with fine reduced barbules	Point upwards	Brown/black	3.8 ± 1.0 (7)	2.5 – 5.5
				Rictus (upper)	Bristles, bulge noticeably at base of rachis. Pale plume(s) close to base, but no barbs	Row of bristles fan outward and forward, overlap but do not interweave with lower rictal bristle fan	Brown/black	7.5 ± 1.3 (17)	6 – 10.5
				Rictus (lower)	Bristles, bulge noticeably at base of rachis. Pale plume(s) close to base, but no barbs	Row of bristles fan upward and strongly forward, following line of mandible	Brown/black	8.9 ± 1.9 (16)	5 - 11
				Interramal	Short rachis, long basal barbs with reduced barbules.	Down and outward	Brown/black	2.6 ± 0.9 (6)	1.5 - 4
NZ Robin (3)	14.57 ± 2.84 (3)	7.87 ± 0.89 (3)	0.8	Nares	Long rachis emerging from a nest of umbilical barbs with reduced barbules. Barbules also present on rachis.	Forward and down across nares	Black rachis, tips of barbs black, pale rami, black barbules	5.6 ± 0.7 (24)	3.5 – 6.5
				Loral point	Long rachis, many barbs and umbilical barbs with reduced barbules, barbules present on rachis	Dorsal placement, forward and upward	Black rachis, tips of barbs black, pale rami, black barbules	5.9 ± 1.2 (14)	4.5 - 8
				Rictus (upper)	4 -5 fine, long bristles, few (~3) umbilical barbs with reduced barbules	Row of bristles, extend forward and down across gape	Black rachis, tips of longer barbs black, white rami and barbules	11.6 ± 2.9 (19)	5 - 16
				Rictus (lower)	Short bristles, few (~2-3) umbilical barbs with reduced pennaceous barbules. Slightly curled	Upward and forward along gape	Black rachis, tips of longer barbs black, white rami and barbules	4.1 ± 0.9 (25)	2.5 - 5
				Interramal	Very similar to narial bristles	Forwards and outwards	Black rachis, tips of barbs black, white rami and barbules	5.7 ± 0.8 (19)	4 - 7

Histology

Herbst corpuscles: Herbst corpuscles were found alongside and between bristle follicles in all the birds examined. Numbers, features and dimensions of the Herbst corpuscles varied between species as outlined in Table 3. Each corpuscle consisted of an outer capsule of perineural tissue which enclosed concentric rings of collagen fibrils ('outer zone'). This surrounded an elongated axon which was oval to circular in cross section. The axon was enclosed within an inner core of modified Schwann cells. Schwann cell nuclei were aligned neatly in two rows along opposite sides of the axon, as typical of Herbst corpuscles generally. In the robin, fantail, and hihi, all corpuscles were sectioned longitudinally, with the axons running approximately parallel to the rictal bristle shafts (Fig. 2a, b). In the morepork and kiwi, Herbst corpuscles were sectioned on many angles, with axons in various alignments from parallel with bristle shafts to perpendicular to them (Fig 2c, d). In these latter species, thick (45 µm morepork, 70 µm kiwi) bundles of nerves could be seen supplying the Herbst corpuscles (Fig 2c, d). These nerve bundles ran roughly parallel to the bristle shafts.

Nocturnal and hole-nesting species (brown kiwi, morepork and hihi) had significantly more heavily encapsulated Herbst corpuscles than diurnal open nesting species (fantail, robin) (in nocturnal and hole nesting birds, capsules were 23.3 ± 4.9% of total Herbst corpuscle width; in diurnal open nesters, capsules were 16.2 ± 1.5% of total Herbst corpuscle width; Kruskal-Wallis $H = 3.94$, $df = 1$, $p = 0.047$) (Table 3; Fig. 2). No other consistent anatomical differences in Herbst corpuscle structure were found between species.

Facial bristle follicles: Nocturnal birds had the longest bristles relative to gape width, with bristle length: gape ratios > 1. Of the diurnal birds, the fantail had the longest bristles, with a bristle length: gape ratio of 0.9 (Table 2). Width: length ratios of bristles were similar across species, but fantail and robin rictal bristles were the thickest relative to their length, while kiwi forehead bristles were the thinnest (Table 3).

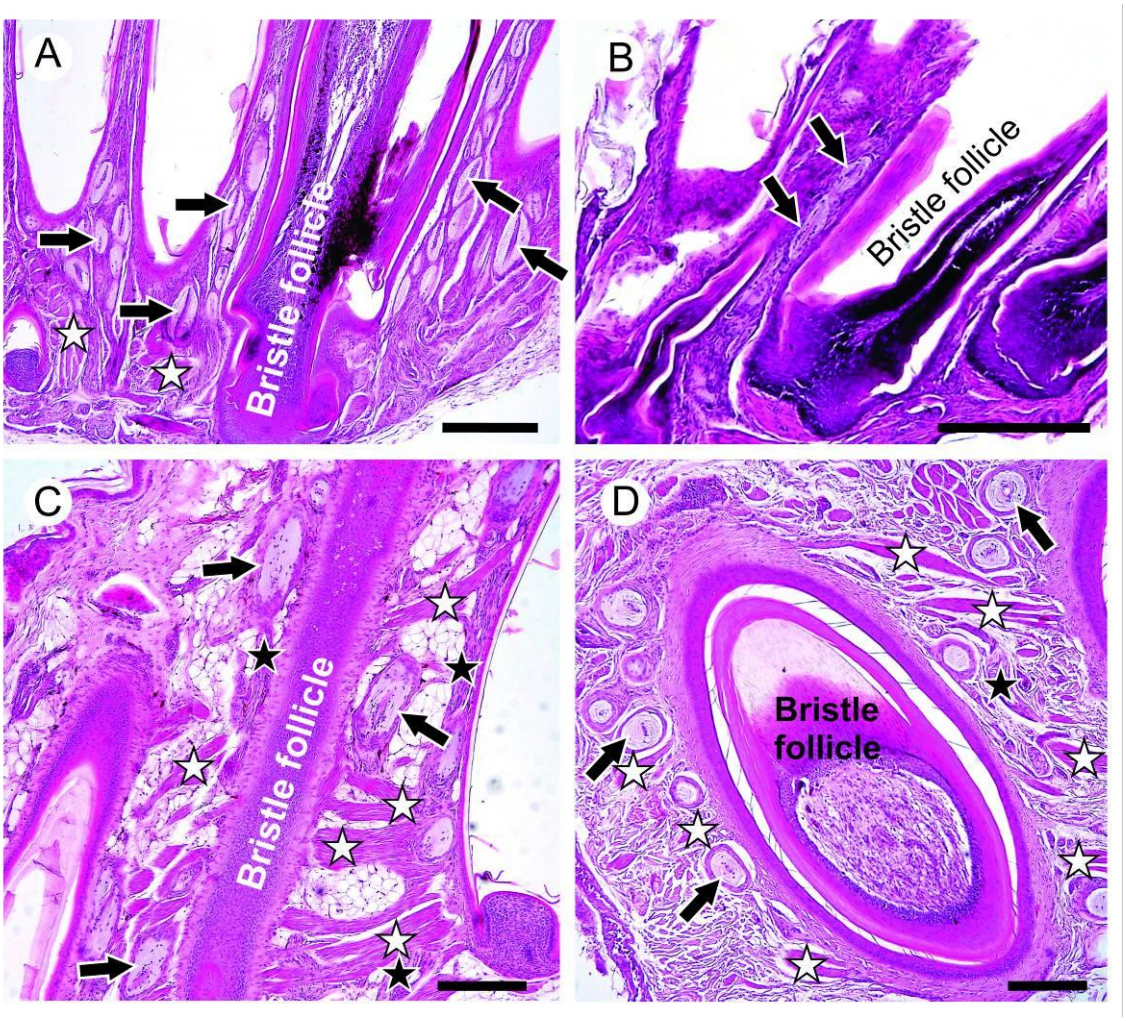

Figure 2: Facial bristle follicles and associated Herbst corpuscles from four insectivorous bird species: (A) New Zealand robin; (B) New Zealand fantail; (C) morepork; (D) brown kiwi. Black arrows indicate examples of Herbst corpuscles; black stars indicate nerve bundles, white stars indicate muscles. Note thick capsules of Herbst corpuscles in brown kiwi and morepork. Stain: haematoxylin and eosin. Scale bars = 200 µm.

In the robin and hihi, muscle blocks were situated at and beneath the bases of the bristles and were sectioned transversely making it difficult to ascertain where or if they attached to the bristle follicles (Fig. 2a). In the fantail, muscles attached at the base of the bristles and ran parallel to the skin surface. In the morepork, multiple small muscles (~ 70 µm in diameter) were attached at the base of, and along the bristle follicles. These muscles ran perpendicular to the shaft, and parallel to the skin surface (Fig 2c). In brown kiwi, thin (~ 50 µm) strips of muscle were attached to the bristle follicles near the base and to about half way between the base and the skin surface. Like the morepork, these ran perpendicular to the bristle shaft and parallel to the skin surface.

Table 3: Details of facial bristle histology, and structure of associated Herbst corpuscles.

Species (n)	Bristles sectioned (number of bristles)	Bristle width (mm)	Bristle width : length ratio	Herbst corpuscles				
				No. per bristle	Length x width (µm)	Inner core as % total width	Outer zone as % total width	Capsule as % total width
Brown kiwi (2)	Forehead (2)	0.51	0.01	5.00	143 x 113	30.9	46.9	22.2
	Lower rictal (1)	0.60	0.03	13.00	153 x 131	26.0	46.8	27.3
Morepork (1)	Lorial (5)	0.33	0.02	2.20	189 x 85	59.5	25.0	15.5
	Interramal (4)	0.32	0.03	4.00	250 x 102	32.3	40.3	27.4
Hihi (1)	Upper rictal (4)	0.19	0.02	1.75	87 x 34	40.0	36.0	24.0
NZ Fantail (1)	Upper rictal (6)	0.28	0.04	1.17	103 x 38	46.4	35.7	17.9
NZ Robin (1)	Narial (2)	0.19	0.03	4.00	78 x 35	52.0	32.0	16.0
	Upper rictal (6)	0.35	0.03	5.20	100 x 41	33.3	51.9	14.8
	Lower rictal (1)	0.21	0.05	5.00	260 x 138	43.3	39.2	17.5
	Interramal (2)	0.09	0.02	0.50	111 x 46	55.9	29.4	14.7

Discussion

Here we describe aspects of facial bristle feather morphology and histology in five species of insectivorous birds with a range of ecologies. We found Herbst corpuscles associated with bristle follicles in all birds examined, regardless of species or ecology. This suggests that bristles in insectivorous birds generally have a tactile function. Except for kiwi, bristles were found in discrete groups around the face. These groups varied in location between species. The differences in histology and morphology of bristles between nocturnal and hole-nesting birds and diurnal open nesters are discussed below.

Nocturnal and hole-nesting birds

We expected nocturnal and hole nesting birds to have well innervated bristle follicles and long bristles to aid in navigation through cluttered habitat at night and underground (e.g. kiwi), and to negotiate nest crevices and feed chicks (e.g. morepork, hihi). Both these predictions were supported by our results: nocturnal birds tended to have longer bristle: gape ratios than diurnal birds, and both nocturnal birds and hole-nesting species had Herbst corpuscles associated with their bristle follicles. We were unable to ascertain from our sample whether nocturnal and hole-nesting birds had significantly more Herbst corpuscles per bristle follicle than diurnal open-nesting species. With the possible exception of kiwi, however, the number of mechanoreceptors per bristle appeared to vary independently of these factors.

In kiwi, high numbers of Herbst corpuscles were combined with very long bristles. These bristles were found all around the head and face, rather than in discrete areas as in the other species examined. Kiwi are able to move their facial bristles somewhat forward and backward as a group, holding them forwards while foraging, and backwards when being handled (*Chapter 5*). Our histological preparations have identified the muscles involved in this movement. Length, placement and innervation of kiwi bristles all suggest a strongly tactile function, either in navigation through undergrowth or underground, as recently shown for long facial feathers in the whiskered auklet (Seneviratne & Jones, 2008) or in prey detection. In morepork, bristles cluster around the lores and interramal region, extending forward to surround and obscure the beak. They are well innervated with heavily encapsulated Herbst corpuscles, suggesting they may also have a tactile function, perhaps in prey manipulation or in helping the bird locate offspring in cavity nests.

There was a difference in Herbst corpuscle structure between diurnal (robin, fantail) and nocturnal & hole-nesting birds (morepork, kiwi, hihi): the latter had more heavily encapsulated Herbst corpuscles than the former. The outer capsule of the Herbst corpuscle is composed of layers of flat, perineural cells (Gottschaldt, 1985). The functional significance of the thicker capsules of Herbst corpuscles in kiwi, morepork and hihi is unknown, but we speculate that it may affect the mechanoreceptive properties of the corpuscles. The thick capsules of these species appear to communicate

closely with the surrounds of the facial bristle follicles, perhaps increasing the sensitivity of the corpuscles to movements of the bristle.

Diurnal and open-nesting birds

We expected diurnal and open-nesting species to have shorter and less extensively innervated bristles than nocturnal and hole-nesting species. There is some evidence that rictal bristles of diurnal insectivorous birds have a function in protecting the eyes (e.g. Dyer, 1975, Conover & Miller, 1980), and that hawking species have longer bristles than gleaning species (e.g. Keast & Saunders, 1991). Therefore, we also expected bristles in diurnal species to be thicker (i.e. more resistant to insect limbs) than in nocturnal species, and longer with respect to gape width in the fantail (a hawking bird) than in the hihi or robin (gleaning species). New Zealand fantail rictal bristles fitted these predictions exactly. They were shorter and thicker than those of nocturnal species, but longer with respect to the gape width than those of other diurnal insectivores (hihi and robin). The fantail appeared to have only few small Herbst corpuscles per bristle, and the upper and lower rictal bristles fanned out and overlapped, forming a stiff net around the gape. These features are both in keeping with hypotheses that the rictal bristles of hawking species are used as insect scoops or to protect the eyes, rather than as specifically tactile structures.

New Zealand robin bristles, also as predicted, were shorter and thicker than the bristles of nocturnal species. However, they were well innervated, suggesting they have a significant tactile function and probably do not act simply as ‘eye guards’. Further, robin bristles were found in distinct upper and lower rictal, narial and interramal groups. The upper rictal bristles were noticeably longer than the lower, and these did not overlap these to form a net in the manner of the fantail.

In the hihi, bristles were found in similar groupings to the robin. Hihi had groups of only four long, fine upper rictal bristles on each side of the head. These rictal bristles had few, small, heavily encapsulated Herbst corpuscles. The small number of Herbst corpuscles was surprising given that hihi are hole-nesters, and the long rictal bristles might be expected to have an important tactile function in navigation inside the nest cavity. We were able to section only one male hihi specimen and quality of that specimen was low. It is possible that more Herbst corpuscles might be found with

examination of further individuals. Hihi bristles in general were shorter than those of nocturnal birds and finer than those of fantails and robins.

Concluding remarks

Each of the five species we examined possessed facial bristle feathers innervated with tactile mechanoreceptors (Herbst corpuscles). The structure and degree of innervation of the bristles, and structure of the Herbst corpuscles varied from species to species. We found some evidence in support of the hypothesis that rictal bristles may act as an insect scoop or eye protection in the fantail, and evidence that facial bristles have an important tactile function in nocturnal birds, particularly kiwi, as suggested by Küster (1905).

Acknowledgements

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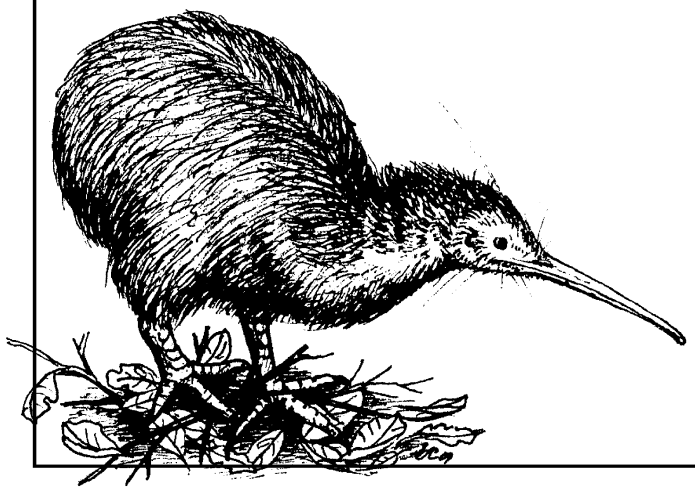
Discussion



Discussion

Humans rely predominantly on vision in our day-to-day lives. For us, it is hard to imagine living in a world which is not experienced primarily by how things look, but instead by how things feel, sound or smell. Even our colloquialisms about perception tend to be visually biased (for example, we all hold different out*looks* on life, *view* things from different *perspectives*, and yet attempt to *see eye-to-eye*). Studying animal species in which vision is not the dominant sense in all situations is like opening a window into another world – it gives us access to a completely different point-of-view.

In this thesis I have examined the occurrence and use of a sense completely unfamiliar on a personal level to humans – the avian sense of remote touch, mediated by the scolopacid-type bill-tip organ. I have also explored the sensory systems of kiwi, a bird in which vision is so unimportant as to be essentially "foregone" altogether (Martin et al. 2007). Here I discuss my findings in the context of convergent/parallel evolution and the links between sensory systems and ecology, structure and function. I make a note of where each thesis objective, as outlined in the introduction, is covered.

Evolution, structure and function of the scolopacid-type bill-tip organ

Evolution of the scolopacid-type bill-tip organ

All birds possess Herbst corpuscles within their bill-tips (Gottschaldt 1985). Most birds also have at least some pits ('neurovascular foramina') within the beak bones for the passage of nerves and blood vessels (Bolze 1968). These pits are often arranged as a row near the cutting edges of the mandible and premaxilla, sometimes also accompanied by a sparse scatter of further pits across the bill-tips (Bolze 1968, *Chapter 1*). Evidence that this type of bill-tip structure might be ancestral to the scolopacid-type bill-tip organ comes from the Rallidae. There is a large radiation of rail species across the Pacific, all thought to be descended from a common ancestor, the banded rail *Gallirallus philippensis* (Olson 1973). The banded rail possesses some minimal pitting of the bill-tip similar to that just described (*Chapter 1*). Within New Zealand, members of the banded rail radiation include weka *Gallirallus australis* and the extinct Hutton's rail *Cabalus modestus* from the Chatham Islands (Olson 1973) – each with bill-tip pitting similar to the banded rail (*Chapter 1*). Cranial features highly specialised for probe-foraging make the relationship of the extinct New Zealand snipe-rail *Capellirallus karamu* to other rails unclear, but it probably also belongs to the banded rail group (Tennyson & Martinson 2006). The snipe-rail possesses a dense arrangement of pits in the bill-tip, reminiscent of a simple scolopacid-type bill-tip organ (Olson 1975, *Chapter 1*). If this species is indeed derived from the banded rail, then it has evolved this arrangement of dense pits from a simple bill-tip structure such as that possessed by the banded rail and many other avian species (*Chapter 1*). My data suggest that the process of developing a honey-combed Scolopacid-type bill-tip organ from an ancestral 'banded rail type' bill occurs fairly readily in probe-foraging birds, resulting in independently evolved bill-tip organs (like those of the brown kiwi, *Apteryx mantelli*, and bar-tailed godwit, *Limosa lapponica*, *Chapter 1*) that are remarkably similar in morphology, although they may differ in fine details of structure.

Distribution of the scolopacid-type bill-tip organ and the use of remote touch

Prior to its discovery in kiwi (Apterygidae; Cunningham et al. 2007) the scolopacid-type bill-tip organ was known only from the shorebird family Scolopacidae. We are

now aware that it also occurs in ibises (Threskiornithidae; Cunningham et al. in press: *Chapter 3*) and possibly in a simplified form in some probe-foraging rails (Rallidae; *Chapter 1*) (objective (b)). In all families in which it has been described so far, the scolopacid-type bill-tip organ functions for remote touch prey-detection uring probe-foraging (shorebirds: Gerritsen & Meiboom 1986, Piersma et al. 1998; kiwi: Cunningham et al. 2009: *Chapter 2*; ibises: *Chapter 4*) (objective (c)). Pitting suggestive of a scolopacid-type bill-tip organ occurs in the aquatic sweep-foraging spoonbills (also Threskiornithidae), for which there is also anecdotal behavioural evidence of remote touch foraging (Swennen & Yu 2005). We can therefore confidently predict that the scolopacid-type bill-tip organ should be functional for remote touch in any further species in which it may be discovered.

The Apterygidae, Scolopacidae, Threskiornithidae and Rallidae are widely separated within the phylogeny of birds (Hackett et al. 2008, see also the *Introduction*). The independent evolution of the scolopacid-type bill-tip organ in the first three, possibly all four, unrelated families of birds suggests that remote touch is strongly favoured by a probe-foraging life style.

The role of the Herbst corpuscles in bill-tip organ function

In order to detect the presence of a swimming or burrowing prey item at some distance from the bill-tips using remote touch, a bird must be able to sense vibratory stimuli of frequencies that match those produced by the prey. In the case of the red knot *Calidris canutus*, they must be able to detect the pressure disturbances in wet sand caused by sessile bivalve prey (Piersma et al. 1998). The clusters of Herbst corpuscles found within the scolopacid-type bill-tip organ are generally accepted to mediate the reception of vibration and pressure signals during remote touch (Gerritsen & Meiboom 1986, Zweers & Gerristen 1997, Piersma et al. 1998, Barbosa & Moreno 1999, Nebel et al. 2005). Direct evidence of the role of the Herbst corpuscles, in the form of experiments recording the electrophysiological responses of Herbst corpuscle afferents in a bird actively foraging by remote touch, is lacking – probably due to the logistical and ethical difficulties of carrying out such research. Nevertheless, the circumstantial evidence for the role of the Herbst corpuscles in remote touch is compelling.

As stated in the *Introduction*, Herbst corpuscles are rapidly adapting mechanoreceptors sensitive to the acceleration components of vibratory stimuli (i.e. pressure wave stimuli) and responsive to a range of signal frequencies which correspond well to measured seismic (pressure) waves produced by burrowing invertebrates (Heppner 1965, Floyd &Woodland 1981, Gottschaldt 1985, see *Introduction*). Herbst corpuscles form large and obvious clusters in the scolopacid-type bill-tip organ and the presence of these clusters is one of the defining features of this organ. Herbst corpuscles are the only mechanoreceptors yet shown to be present in the bill-tip organ which have the correct electrophysiological response properties to collect information about substrate-borne vibrations or pressure gradients (Zweers & Gerritsen 1997). Light pressure applied directly to the bill-tip of anesthetised dunlins (Scolopacidae: *Calidris alpina*) causes a measurable electrophysiological response in the brain attributable to the 'firing' of the Herbst corpuscles (Pettigrew & Frost 1985). It is likely the bill-tip organ should respond similarly when the bill intercepts pressure waves travelling in the probing substrate. This is supported by the behavioural evidence for the use of the remote touch sense by birds possessing a scolopacid-type bill-tip organ.

Degree of reliance on remote touch by shorebirds, kiwi and ibises

Remote touch is one of a suite of senses available to probe-foraging birds for prey detection. For example, in aviary trials shorebirds in the genus *Calidris* have been documented using vision and chemo-detection to find prey, as well as remote touch, depending on the quality of the cues available (Van Heezik et al. 1983, Gerritsen et al. 1983, Gerritisen & Van Heezik 1985; Gerritsen & Meiboom 1986).

Like *Calidris* sandpipers, captive kiwi integrate more than one remote sense when probe-foraging, using both olfaction and remote touch to discover buried live prey in foraging trials in captivity (Cunningham et al. 2009: *Chapter 2*, objective (c)). They show a preference for using olfaction and switch to remote touch only in the absence of olfactory cues. The normal diet of captive kiwi is an odoriferous meat-based mix presented on a plate. It is therefore possible that captive kiwi may be conditioned to rely most heavily on olfaction as a foraging cue (as occurs in some other birds; Roper 2003, Mennerat et al. 2005). Free-living kiwi are exposed to a greater range of foraging

conditions than captive birds and may more readily integrate other senses (e.g. remote touch and possibly hearing) with olfaction to find prey.

Remote touch appears to be the only sense (other than chance detection by direct contact with the bill-tips) used by captive ibises to detect buried prey (*Chapter 4*, objective (c)), although they also seem to use vision to capture prey items on the surface (pers. obs.). This observation is supported by recent data on Northern bald ibis *Geronticus eremita* visual fields, showing that they have the characteristics of species that use vision to guide bill position (G. R. Martin pers. comm.). The bill-tip organs of ibises from aquatic habitats provide evidence that they can use remote touch when foraging in water as well as in granular substrates (Cunningham et al. in press: *Chapter 3*, objective (b)).

Structure and function of the bill-tip organs of different species

The scolopacid-type bill-tip organ is similar, but not identical, in structure in all species in which it has been described. For example, kiwi (brown kiwi) and shorebird (bar-tailed godwit) bill-tip organs have the same basic components, but differ in structural detail related to the shape of bill, the pattern of innervation, and the structure of the Herbst corpuscles (*Chapter 1*, objective (a)). While some of these differences in structure will be due to the independent evolution of the organ in different families of birds, many are likely to have functional implications.

Differences in bill-tip organ structure that have functional implications may represent adaptations to the different habitats and ecologies of the species involved. For example, the bill shape of kiwi may be adapted to probing in relatively hard substrates, as opposed to the soft mudflats used by shorebirds. Kiwi bill-tip organ Herbst corpuscles are different from those in other birds, in that they have broad disc-shaped, instead of cylindrical, central axons and the collagen fibrils of the outer zones are densely coiled (*Chapter 1* objective (a)). However, the Herbst corpuscles surrounding kiwi bristle feather follicles look similar to those in the bill-tip organs of the bar-tailed godwit and Australian white ibis *Threskiornis molucca* (*Chapters 1, 3, 6*). This suggests that, rather than resulting purely from phylogenetic differences, the unusual features of kiwi bill-tip Herbst corpuscles may be an adaptation for detecting prey signals in damp soil (instead of wet sand or water). If this is indeed the case, we might expect similar modifications

in the bill-tip organ Herbst corpuscles of ibises such as the Madagascar Crested ibis *Lophotibis cristata*, which are ecologically similar to kiwi in terms of habitat use (Keith et al. 1974, Hino 2002).

Within the ibis family Threskiornithidae, aquatic-foraging ibises have more extensive and densely pitted bill-tip organs than terrestrial-foraging ibises (objective (b)). This pattern extends to the terrestrial-foraging kiwi family (Apterygidae) in which all five species have relatively small bill-tip organs (~12.5 % bill length pitted; Cunningham et al. 2007, *Chapter 1*). The tendency for increased bill-tip organ size in aquatic habitats is taken to its extreme in the obligate aquatic-foraging spoonbills (Threskiornithidae), in which the bill-tip is completely flattened, providing a wide surface area for large numbers of sensory pits. In the black-faced spoonbill (*Platalea minor*) these pits extend to >50% of the bill length (Swennen & Yu, 2004). I offer two tentative hypotheses which may explain why more aquatic species may possess more extensive and densely pitted bill-tip organs than their terrestrial counterparts.

1. Phylogeny: The internal phylogeny of the Threskiornithidae, other than its division into the subfamilies Threskiornithinae (ibises) and Plataleanae (spoonbills), is not well resolved (Matheu & del Hoyo 1992). Even this traditional division is now in question; with recent work suggesting spoonbills are in fact nested within the ibises phylogenetically (Chesser et al. 2010). This taxonomic uncertainty is important when examining the link between aquatic habitat use and bill morphology in ibises, because the most aquatic group of four ibis species I examined in *Chapter 3* share several features related to the bill-tip organ, but are also all members of the same two genera, *Plegadis* and *Eudocimus.* Until the phylogeny of ibises is properly resolved, the possibility that these two genera are sister groups, and the shared features of their bill-tip organs are due to shared inheritance rather than the shared selective pressures of an aquatic habitat, cannot be ruled out. Phylogeny does not provide an entirely satisfactory explanation for the observed pattern of increasing extent and density of pitting in the bill-tip organ with increasingly aquatic habitat however, given the extension of this pattern to the unrelated Apterygidae.

2. Adaptation to different substrates and prey types: Individual Herbst corpuscles respond to pressure/vibration signals only when the amplitude (intensity) of the signal

exceeds a critical level, which varies from receptor to receptor (Gottschaldt 1985). By grouping together corpuscles of different intensity thresholds within the sensory pits of the bill-tip organ, a bird can begin to measure the amplitude of a given pressure wave within the substrate in which it is probing. Given a sufficient number of Herbst corpuscle clusters within sensory pits positioned around the beak tips, the bird may gain information about the loss in amplitude of the signal from one part of the bill to the other and hence the direction from which it originates (Zweers & Gerritsen 1997). Zweers & Gerritsen (1997) reason that a bill-tip organ with high numbers of sensory pits housing large clusters of Herbst corpuscles should be better able to resolve both distance and directionality of a prey signal than a bill-tip organ with a smaller number of corpuscle clusters. They support this argument by pointing out that long-billed birds highly specialised for deep probing (e.g. snipe, *Gallinago gallinago*) would benefit from, and possess, more extensive and densely pitted bill-tip organs (and larger numbers of Herbst corpuscles per sensory pit) than shorter billed probers (red knots and dunlin) (Zweers & Gerritsen 1997). Zweers & Gerritsen's (1997) hypothesis that an increase in the number of mechanoreceptors in the bill-tip is an adaptation for probe-foraging is supported by Barbosa & Moreno's (1999) ecomorphological study of the evolution of foraging strategies in shorebirds.

I hypothesise that differences between substrate types (e.g. soil and water) might also result in a need for different levels of sensitivity in the bill-tip organ. Both signal transmission and prey behaviour differ widely between soil and water substrates. Pressure wave transmission is unpredictable in granular media such as soil (Liu & Nagel 1992), but soil-dwelling prey items must burrow relatively slowly to move through this substrate and directionality of signals they produce will be fairly constant. Pressure wave transmission in water is more predictable, but aquatic prey of ibises (such as fish and crayfish, Kushlan 1979) can move much more rapidly in this medium, producing constantly changing signals. The remote touch mechanisms required to localise prey under these very dissimilar foraging conditions must also differ widely. If Zweers & Gerritsen (1997) reason correctly that greater numbers of Herbst corpuscle clusters (housed within sensory pits) are linked with a greater ability to detect signal distance and directionality, perhaps the extensive, densely pitted bill-tip organ of aquatic ibis species is adapted to resolve signals from fast moving prey, distance and direction of which rapidly change? Birds probing in soil substrates may require fewer

corpuscle clusters (and therefore fewer sensory pits) in order to localise signals from slower moving burrowing prey. There is presumably an energetic cost to maintaining large numbers of mechanoreceptor clusters in the bill-tip organ, and the associated signal processing centres in the brain. Therefore, specialisation for locating fast moving prey in the water column should only be maintained in aquatic habitats, perhaps explaining the pattern in the degree of pitting of the bill-tip organ that we see within the Threskiornithidae and Apterygidae.

In order to further tease apart the roles of phylogeny and adaptation in shaping the differences seen in the bill-tip organs of probe-foraging birds, it would be helpful to (a) research further the physical requirements for remote touch in a variety of substrate types, (b) examine the histology of the bill-tip organ in a broader range of species within families and (c) clarify the intra-family phylogeny of the Threskiornithidae.

Differences in foraging between kiwi and ibises in aviary trials

Madagascar crested ibises foraged noticeably faster than brown kiwi in the aviary trials presented in *Chapter 2* and *Chapter 4.* Ibises probed into the experimental trays more times per minute and therefore had higher prey capture rates per minute than kiwi, although captures per probe were similar. This may have been to do with the housing of the birds: ibises were housed with conspecifics and other African bird species whereas kiwi were housed alone. Ibises were therefore under pressure to complete the trials quickly to avoid loss of mealworms to other individuals (on completing a trial, ibises tended to run to probe in the trays of other ibises that had not yet completed their trial). There is also a possibility that kiwi probed more slowly than ibises because kiwi have lower metabolic rates than predicted for non-passerine birds their size (Calder & Dawson 1978). Kiwi used both olfaction and remote touch to find prey whereas ibises used remote touch only. Kiwi tapped at the surface, as well as probing, possibly using this behaviour to gather olfactory and tactile information without using the energy required to probe. Perhaps this behaviour caused them to probe fewer times per minute than the ibises.

Other sensory specialisations for probe-foraging

Although remote touch and the scolopacid-type bill-tip organ appear to be favoured by a probe-foraging lifestyle and to be developed fairly readily, not all probing birds possess

them. For example, I could not find any sign of this organ in the bill-tips of the oystercatcher (*Haematopus ostralegus*), hoopoe (*Upupa epops*), stilt (*Himantopus himantopus*) or many long-billed rails (with the exception of *Capellirallus karamu* and *Rallus madagascariensis*) (objective (b)). Oystercatchers are known to use visual cues on the surface to find some buried prey, but also to be efficient direct-touch hunters for bivalves (Hulscher 1982). Their manner of opening bivalves by hammering or stabbing (Durell et al. 1993), and the flexibility of their bill-shape associated with this (Swennen et al. 1983), may preclude the development of a sensitive bill-tip organ. Senses used by other probe-foraging species lacking a bill-tip organ remain unexplained. Some non-probing species use hearing to detect buried prey (e.g. Australian magpies, *Gymnorhina tibicen*: Floyd & Woodland 1981; American robins, *Turdus migratorius*: Montgomerie & Weatherhead 1997), and this may, therefore, be a specialised sense in some probing species, too.

Kiwi senses

Kiwi are olfactory, tactile (direct and remote) and auditory specialists, with limited vision. Kiwi have a nocturnally-adapted retina (Corfield 2009), but their eyes are reduced in size compared to other birds, and the areas of their brain for dealing with visual information are also small relative to other birds (Martin et al. 2007). They display no obviously visually guided behaviours in the wild (*Chapter* 5, objective (d)). In contrast, the ears and auditory centres in the brain are specialised, and the processing centres for touch and smell are enlarged (Corfield 2009).

Kiwi use hearing at long range, responding with ‘head lift’ behaviours to sounds audible also to observers (*Chapter 5*). They use stereotypic odour sensing behaviours, which look like mammalian ‘sniffing’, to further investigate the sources of sounds (*Chapter 5,* Castro et al. in press: *Appendix 2,* objective (d)). Kiwi also use both olfaction and remote touch to locate prey (see above, Cunningham et al. 2009: *Chapter* 2, objective (c)). Kiwi facial bristle feathers are well innervated with Herbst corpuscles (like those of other New Zealand birds) and are exceptionally long and numerous (Baker at al. 1995, *Chapter 6*). They are somewhat mobile and can be angled forward during foraging (*Chapter 5*), expanding the area of touch sensitivity. Kiwi constantly tap ahead with the

sensory pad of their bill-tip as they move through their habitat. Tapping occurs with greater frequency in cluttered than in open habitats, and likely aids kiwi in building a picture of their immediate surroundings and in negotiating obstacles (*Chapter 5*, objective (d)). It would appear that kiwi integrate the information from their ears, olfactory system, facial bristle feathers and bill-tip to create a 'picture' of their world that would be unrecognisable to us.

Niche partitioning in brown kiwi

Male and female brown kiwi are significantly dimorphic in terms of size and bill-length, with females being larger than males (Robertson et al. 2003). Many other avian species which display sexual size dimorphism, particularly in bill length, also display behaviours suggestive of foraging niche partitioning (e.g. Western sandpipers *Calidris mauri*: Fernandez & Lank 2008; bar-tailed godwits: Zharikov & Skilleter 2002; and green woodhoopoes *Phoeniculus purpureus*: Radford & du Plessis 2003). On Ponui Island, female brown kiwi probed on average 30% deeper into soil substrates than males or juveniles, and reached soil strata beyond the maximum probing depth of males 18.18% of the time (*Chapter 5*, objective (d)). Despite a high population density (Cunningham et al. 2007) and significant sexual bill-length dimorphism at this site (*Chapter 5*), I could find no further evidence for behavioural niche partitioning between the sexes (*Chapter 5*, objective (d)). This level of niche partitioning may be sufficient to ease intraspecific competition under normal conditions, and kiwi may partition their foraging niche more in times of great food stress (e.g. during droughts). Further, kiwi have a slow metabolism compared to similar sized birds (Calder & Dawson 1978) which also might ease competition for food at high population densities. Sexual selection has been proposed as a mechanism for sexual bill-length dimorphism in species where there is only limited evidence for foraging niche partitioning (e.g. Cory's shearwater, Navarro et al 2009). It is possible that sexual selection may be partially responsible for bill-length dimorphism in kiwi, but further work needs to be carried out to provide evidence for this.

Future research directions

Remote touch and the associated sensory organ in birds was a topic unexplored outside of the shorebird literature (Bolze 1968, Gerritsen & Meiboom 1986, Zweers & Gerritsen 1997, Pettigrew & Frost 1995, Piersma et al. 1998, Barbosa & Moreno 1999 and Nebel et al. 2005), prior to our paper first describing the existence of a scolopacid-type bill-tip organ in kiwi (Cunningham et al. 2007). This thesis considerably expands knowledge of the structure of the scolopacid-type bill-tip organ in kiwi, ibises and the bar-tailed godwit, the function of the organ in kiwi and ibises, and the distribution of the organ and associated remote touch sense among birds.

The main focus of this study was on describing the scolopacid-type bill-tip organ and experimentally verifying its function in prey-detection outside of the Scolopacidae, for the first time. The data I present here therefore provide the foundation for what could become a new field of research into seismic prey-detection in birds. I list some suggestions for future research in this field, below:

(a) what is the functional significance of the differences in Herbst corpuscle structure in godwits and kiwi – are they simply due to phylogeny, or are the broad axon and dense outer zone of kiwi bill-tip Herbst corpuscles specific adaptations for remote touch in soil substrates? Would we find similar differences in Herbst corpuscle structure in the bill-tips of ibises from aquatic versus terrestrial habitat types?

(b) Is the correlation between extent and density of pitting in the bill-tip organ and habitat use in the ibises adaptively significant, or an accident of phylogeny?

(c) Related to (b), how does the scolopacid-type bill-tip organ function in water as opposed to in granular substrates, and what structural modifications are required for remote touch sensing in these different substrate types? Can we learn anything about this by studying the bill-tips of spoonbills, obligate aquatic foragers related to ibises, which also possess sensory pits (Swennen & Yu 2004)?

(d) What is the histological structure of the bill-tip organ in rails, and how does the functioning of this less-densely pitted organ compare to that of the scolopacid-type bill-tip organ in other families of birds?

(e) Independent evolution of the scolopacid-type bill-tip organ seems to be favoured by a probe-foraging lifestyle. Why, then, do we find specialist probe-foraging birds (e.g. oystercatchers, stilts, hoopoe, probe-foraging rails other than *Rallus madagascariensis* and *Capellirallus karamu*) that have not developed it? What are the alternative sensory specialisations of these species?

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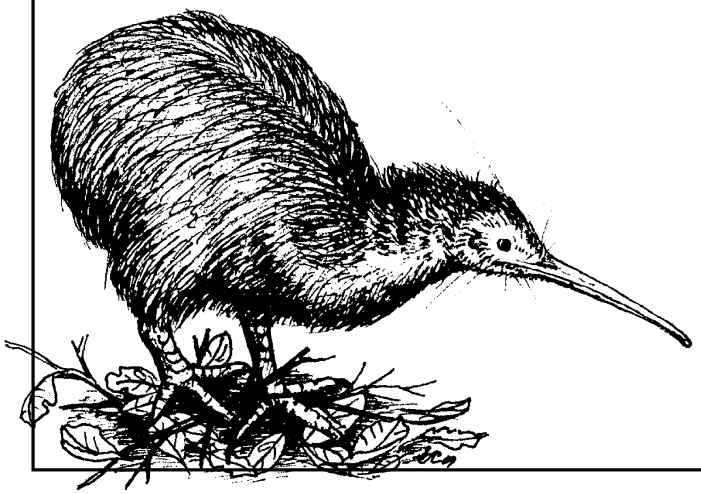
Appendix 1

Effects of leg-mounted radio-transmitters in adult brown kiwi

Chapter reference: Cunningham, S. J. and Castro, I. Effects of leg-mounted radio-transmitters in adult brown kiwi. *Formatted for the New Zealand Journal of Ecology.*



Abstract

Kiwi are endangered, nocturnal, flightless birds endemic to New Zealand. Research and conservation on kiwi is usually carried out via radio-tracking, employing 20g (< 2% body-weight) leg-mounted radio-transmitters. We aimed to assess the impact of these transmitters on everyday behaviour and risk of injury to adult kiwi. We monitored 43 wild adult or sub-adult brown kiwi (*Apteryx mantelli*) carrying transmitters (tagged kiwi) on Ponui Island, NZ, for 1 – 3 years each and documented all transmitter-related injuries/mortality. We also video-recorded both tagged and untagged kiwi exiting burrows and whilst active at night. We used the night videos to compare fine scale behaviours and time budgets between tagged and untagged birds. There was no significant difference in the behaviour of these birds, apart from a non-significant tendency for tagged birds to spend less time engaged in comfort behaviours. Tagged kiwi, males significantly more than females, displayed discomfort behaviours (exaggerated stretching the transmitter-bearing leg) on exiting burrows, but not when active away from roosts. We think this may be due to pressure exerted on the leg by the transmitter band while the bird is roosting. Thirty percent of tagged kiwi, again predominantly males, experienced at least one transmitter-related injury/problem, mostly chafing or blisters under the transmitter band. One female kiwi died due to entanglement of the transmitter-bearing leg in vegetation but the contribution of the transmitter to the entanglement was unclear. Overall, the main effects of wearing leg-mounted radio-transmitters on kiwi at our study site were small injuries due to abrasion under the band and discomfort on leaving roosts. This study did not address longer term impacts of transmitters on life history traits or energetics of kiwi.

Introduction

Radio-telemetry is an important technique in the study and management of many wildlife populations. It allows researchers and managers to monitor animals remotely and aids the collection of data on difficult to study, cryptic or nocturnal species (White & Garrott 1990). The basic assumption of all radio-tracking studies is that carrying a radio-transmitter (hereafter transmitter) has very little effect on the behaviour, movements and life history of the individuals studied – that is, that data collected from tagged individuals is representative of the population as a whole (White & Garrott 1990).

A number of studies have aimed to address this assumption, with mixed results. For example, Göth and Jones (2001) found no impact of carrying a transmitter on the behaviour patterns of brush turkey chicks (*Alectura lathami*), Petrie and Rogers (1996) found only minimal effects (an increase in % time spent preening) of carrying a transmitter in whitefaced ducks (*Dendrocygna viduata*), and Nussberger and Ingold (2006) found no impact of radio-collars on the behaviour or social interactions of alpine chamois (*Rupicapra rupicapra*). Negative effects of transmitters have been found in other studies, however. Radio-collar transmitters have a negative impact on pair bonding and breeding success in snow geese (*Anser caerulecens atlanticus*) (Demers et al. 2003). Carrying data loggers reduced parental care effort in thick billed murres (*Uria lomvia*) (Paredes et al. 2005), and back mounted transmitters altered the sound and appearance in flight, and perhaps the escape behaviours of sharp-tailed grouse (*Tympanuchus phasianellus columbianus*), resulting in increased predation (Marks & Marks 1987). External transmitters may also increase the risk of injury or entanglement in vegetation for an animal carrying them (e.g. Zschille et al. 2008). Impacts of transmitters on day to day behaviour and survival are therefore possible and may jeopardise the welfare of tagged individuals, and the validity of data collected from them.

Brown kiwi (*Apteryx mantelli)* are secretive, flightless, nocturnal birds that often live at sparse densities in thickly vegetated habitats. For this reason, the majority of studies of wild brown kiwi biology have been carried out from a distance using radio-tracking

(e.g. McLennan et al. 1987; Potter 1990; Miles 1995; Taborsky & Taborsky 1995; Grant 2003, and others). Conservation efforts for this endangered and iconic bird also rely heavily on radio-tracking techniques to monitor breeding individuals (e.g. Operation Nest Egg, Colbourne at al. 2005). Guidelines set out in the Kiwi Best Practice Manual (Robertson et al. 2003) and followed by kiwi practitioners nationwide state that adult kiwi should be fitted with leg-mounted transmitters weighing approximately ~20g (< 2% kiwi body weight) containing an internal aerial. Chicks carry transmitters with external whip aerials weighing ~6g (Robertson et al. 2003). Effects of transmitters on kiwi behaviour are poorly known although it is assumed transmitters may affect everyday life by protruding from the bird's body (Robertson et al. 2003). Kiwi, particularly young kiwi, are at risk of mortality through the transmitter package becoming entangled in vegetation, although this seems to be a rare occurrence (Robertson et al. 2003; Colbourne et al. 2005). There is a single record in the literature of a joint dislocation in a kiwi caught up by its transmitter package as it fell through thick vegetation (Robertson et al. 2003).

We aimed to assess the effects on adult or sub-adult wild brown kiwi of wearing standard, ~20g, leg-mounted transmitters. We observed fine scale behaviour patterns of wild brown kiwi using infrared sensitive cameras to video record the birds while active at night, and while exiting burrows, on Ponui Island, New Zealand. Our study was part of a wider programme of research into kiwi biology on the island and some (but not all) of the kiwi in the study population wore transmitters as part of this research. We took advantage of this fact to compare data from tagged and untagged kiwi in order to assess transmitter effects on everyday behaviours. We also examined three years of data collected when tagged kiwi in the study population were captured to change transmitters, in order to assess any physical impacts (e.g. injuries) to the birds caused by the transmitter package.

Methods

Study site and kiwi population

Our research was carried out on South Ponui Farm, a sheep and beef farm on Ponui Island, New Zealand (1770 ha; latitude 36 55'S, longitude 175 11'E). Vegetation cover

on this farm is a patchwork of rough pasture and remnant native forest fragments (Shapiro 2006). This study was carried out in an area comprising two forested gullies (Red Stony Hill Gully (RSHG); Pipe Gully (PG)) and surrounding pastureland in the southeastern corner of the largest forest fragment on the island (Fig. 1). Brown kiwi were translocated to Ponui in 1964 (Miles and Castro 2000) and kiwi density on the island is now estimated at approximately one bird per hectare (Cunningham et al. 2007).

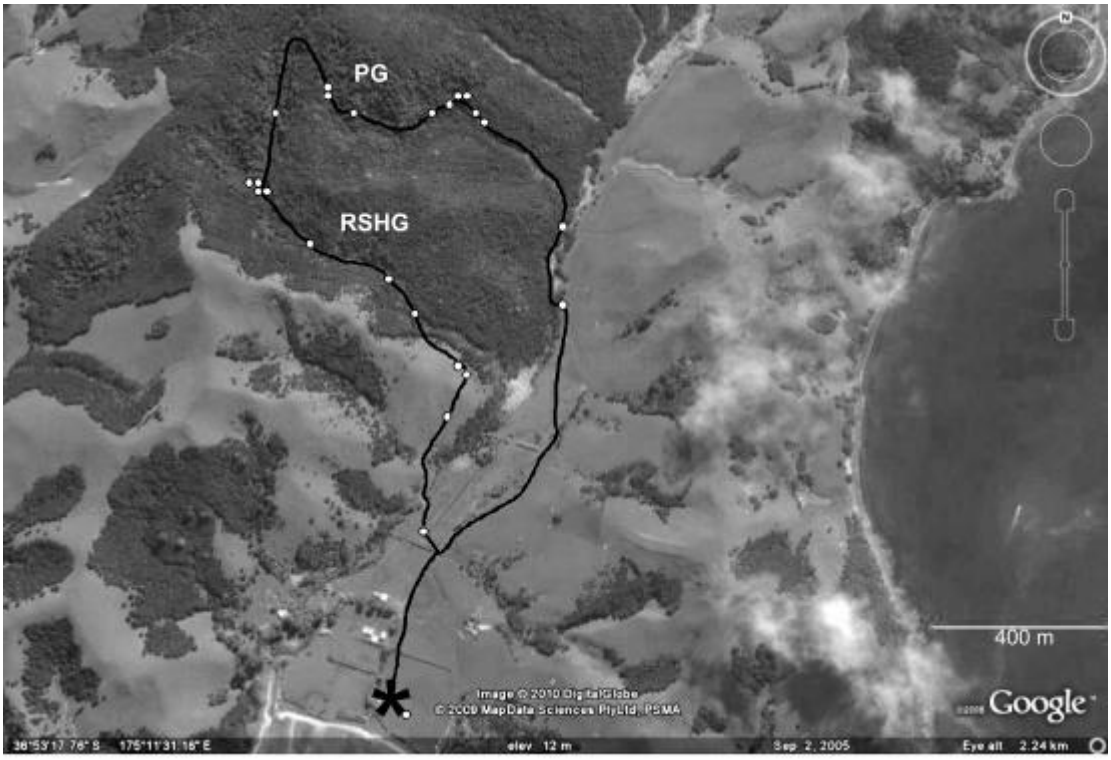


Figure 1: Aerial photograph of the study area, showing walking routes (black lines), locations of video-recorded kiwi encounters used in the analyses (white circles), and our campsite (black asterisk). PG = Pipe Gully, RSHG = Red Stony Hill Gully. (Photograph extracted from Google Earth).

Transmitter attachment and assessment of transmitter-related injury

Radio-transmitters used at the study site were obtained from KiwiTrack™, Havelock North, New Zealand. They were standard ~20g two-stage leg-mounted transmitters with internal aerial as used for adult kiwi throughout New Zealand (Robertson et al. 2003). Transmitters were fitted to the tibia of each kiwi, above the joint with the metatarsus (tarsus), using a hospital identification band and 8 rounds of black electrical tape, as set out in the Kiwi Best Practice Manual (Fig 2; Robertson et al. 2003). Researchers attaching the transmitters were all certified with the New Zealand Department of Conservation, having been trained by other certified professionals.

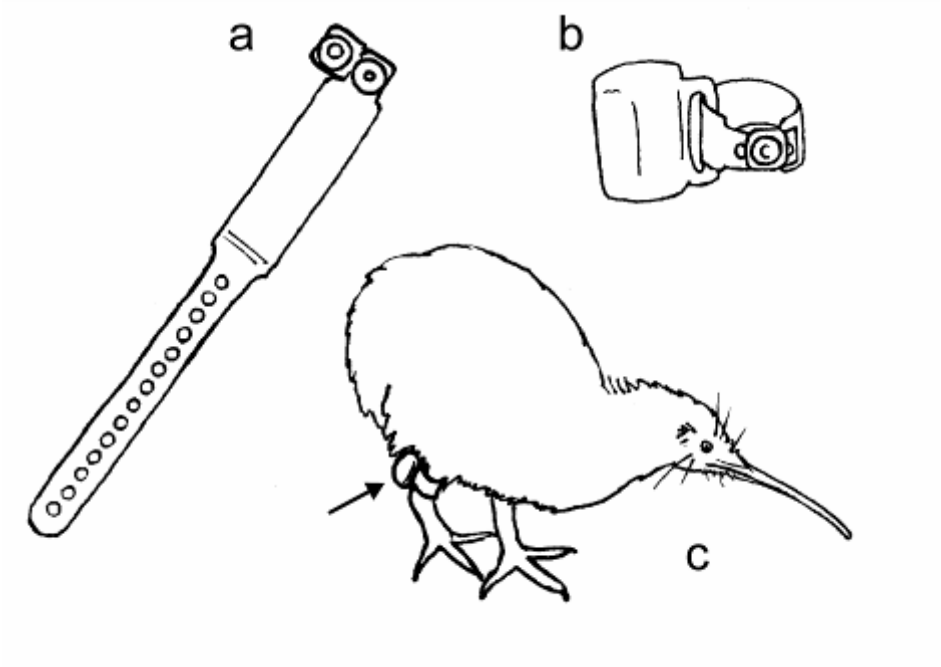


Figure 2: (a) Hospital identification band used to attach transmitters to kiwi; (b) transmitter shown with the hospital band fastened through the attachment loop and trimmed; (c) kiwi wearing a transmitter, indicated by an arrow. Drawings by S. Cunningham.

The authors and other kiwi research team members monitored 43 kiwi (22 male, 21 female) wearing transmitters for varying lengths of time over 3 years (2007-2009) (Table 1). At any given time 30 – 36 kiwi in the study area were carrying transmitters (13 – 16 females, 17-20 males – hereafter 'tagged kiwi'). Female kiwi weighed 2319 ± 297 g on average (range 1510 – 2700 g); male kiwi weighed 1997 ± 169 g on average (range 1675 – 2325 g). Transmitters were 0.88 ± 0.14 % of female body weight (range 0.14 – 0.74%), and 1.00 ± 0.09 % of male body weight (range 0.86 -1.19), below the 3% maximum set out in the Kiwi Best Practice Manual (Robertson et al. 2003). Differences between body weight, and the weight of the transmitter as a percent of body weight, were significant between males and females (Kruskal-Wallis H tests, $p < 0.001$).

Tagged kiwi were located (but not captured) at least once per month to check their location and status (e.g. whether they were still alive). Transmitters were checked and replaced once every year, and any injuries to the site of transmitter attachment were noted at this time.

Table 1: Number of kiwi carrying transmitters for 1 year, 2 years or 3 years of this study.

	Number of years carrying a transmitter			Total number of tagged kiwi
	1 Year	2 Years	3 Years	
Male	5	1	16	22
Female	9	0	12	21
Total	14	1	28	43

Obtaining video recordings of kiwi

Recordings were made using Sony Handicams (DCR-HC96E, DCR-HC40E, DCR-SR42 and DCR-SR45) on NightShot mode and infrared spotlamps (IRLamp6, Bat Conservation and Management Inc). Lamps were powered from 12V sealed lead acid batteries. Kiwi did not visibly react to the infrared light from the lamps.

Observations of kiwi away from burrows: Between January 2007 and September 2008 we made eleven seven-day trips to the study site to video record kiwi. Video recording was attempted every night (66 nights) except under severe adverse weather conditions. Lamps and cameras were handheld and batteries for the lamps were carried in backpacks. Teams of two people, one holding the video gear and one holding telemetry gear, walked set routes through the study site for up to 8 hours from dusk each evening (Fig. 1). Walking routes included areas of open forest and pasture. We avoided densely vegetated ridges and swamps due to the difficulty of obtaining clear footage in these areas (although transmitter signals indicated kiwi used these areas).

In order to avoid bias in our sample, radio telemetry was never used to find tagged kiwi for the purposes of video recording them, but was only used to identify kiwi observed to be wearing a transmitter. Kiwi were located by the rustling sounds they made when walking or foraging. On hearing a kiwi, we stopped, switched off headlamps and scanned with the camera and infrared light to locate the bird. Kiwi were recorded from the time they were first encountered until they moved out of view into thick vegetation or out of camera range. Untagged kiwi were categorised as adult female or male/juvenile using the bill length ratio described in *Chapter 5*.

Burrow exits: We also video-recorded kiwi exiting burrows at night using the above mentioned cameras and infrared spot lamps mounted on tripods every night from June 2 - 15, 2009. This camera set up was located 8-10 m from burrow entrances where tagged birds were roosting, sometimes in company with other, untagged kiwi. Kiwi were located during the day using their transmitter signals. Cameras were set up and turned on shortly before dusk to capture kiwi behaviours when exiting their roost site.

Data extraction from video recordings

Videos of kiwi away from burrows were screened for quality and bird identity before transcription. Poor quality (very dark, blurry, or bird obscured by vegetation) videos were discarded from analysis and data were transcribed from the highest quality video only in cases where multiple videos of the same individual kiwi had been obtained, in order to avoid pseudoreplication. Where multiple videos of an individual were of similar quality, the longest sequence was used. Burrow exit videos were all of excellent quality, and repeat recordings of the same individuals from subsequent nights were used to calculate the percentage of exits in which each kiwi displayed behaviours related to its transmitter. All videos were transcribed using freeware video editing programme VirtualDub™ (Lee 2008) which allowed us to step through recordings frame by frame.

Behavioural data

Away from burrows: Time spent engaged in each of six behaviour states (foraging, walking, comfort, vigilant, escape, investigation of obstacles), behavioural repertoire (number of different behaviours observed) and detailed aspects of each behaviour were extracted from video recordings, following the definitions used in *Chapter 5* (Table 2). Kiwi were not followed when they left the area in which we were video recording them, therefore footage length was used as an indicator of the 'willingness' of the bird to stay in close proximity to researchers.

Burrow exits: Birds were observed from exiting the burrow entrance until they had walked 2-3m away when they moved out of sight of the camera. We recorded any instances of stretching and any movements of the legs different from walking or running, specifying the side of the body, and calculated the percentage of burrow exits in which each individual displayed each of these behaviours.

Table 2. Behaviour states and actions recorded and used in analyses. Duration (minutes) of each behaviour state was also recorded. Actions were defined as in *Chapter 5*.

Behaviour state	Action	Aspects recorded and used in analyses
Foraging	Tap	− Frequency/min − Duration (s) − Ratio taps to probes
	Rapid tap	− Frequency/min − Whether successful
	Soil probe	− Frequency/min − Duration (s) − Depth relative to bill length (scale: 1 < half bill, 2 ≥ half bill, 3 entire bill including cere, 4 part of face in probe hole) − Whether successful
	Litter probe	− Frequency/min − Duration (s) − Depth relative to bill length (scale as for soil probe) − Whether successful
	Bill hover	− Frequency/min
	Handling time	− Duration (s)
Walking	Walk	− Duration (s)
	Tap (walking)	− Frequency/min − Duration (s)
Escape	Run	− Duration (s)
	Jump	− Frequency/min
Comfort	Preen	− Frequency/min
	Stretch	− Frequency/min
	Scratch	− Frequency/min
	Shake	− Frequency/min
	Leaf toss (clearing beak of leaf litter)	− Frequency/min
	Defecation	− Frequency/min
Vigilance	Freeze	− Frequency/min − Duration (s)
	Head lift	− Frequency/min
	Odour sensing behaviour (‘sniffing’)	− Frequency/min − Direction (towards observer, other directions)
Investigation of obstacles	Bill reach	− Frequency/min

Statistical analysis

Away from burrows: The majority of behavioural variables were not normally distributed (Anderson-Darling tests, $P < 0.05$), therefore these data were analysed using non-parametric Kruskall-Wallis H-tests. Footage length data were normally distributed and analysed using ANOVA tests.

Shannon-Wiener diversity indices were calculated for behavioural repertoires of tagged and untagged kiwi. This index takes into account both the number of different behaviours used and also the 'evenness' of use (e.g. how commonly each behaviour was performed). Indices were compared using Hutchisen's t-test, following Zar (1999).

Burrow exits: Data on transmitter-directed behaviours was normally distributed (Kolmogorov-Smirnov test, D = 0.17; P = 0.75). Independent t-tests were carried out to compare the effects of transmitters on discomfort behaviour as well as to compare behaviours between males and females.

Data are presented as means ± 1 standard deviation.

Permits

Kiwi handling and transmitter attachment was carried out under the Department of Conservation permit AK/14971/RES and Banding Office permit 2007/11.

Results

Injuries and problems related to carrying transmitters

We observed 18 problems or injuries related to transmitter use, involving 13 (5 female, 7 male) of our 43 tagged birds (30% overall: 24% of females, 32% of males). The majority of these related to abrasion of the leg by the transmitter band (Table 3). One female kiwi became entangled in a climbing fern (mangemange, *Lygodium articulatum*) and subsequently died. The role of the transmitter was uncertain as the bulk of the entanglement, although around the tibia, was below the transmitter band (Fig 3). We felt the presence of the transmitter probably exacerbated this entanglement if it did not cause it, and therefore we have included it here.

The most common type of transmitter related injury was blistering under the transmitter band and this affected males (6 cases) more commonly than females (one case: Table 3; Fig 4). We also recorded two cases of chafing (both males) and two cases of scabbed sores (1 male, 1 female). Sores may have been tick bites irritated by the band, given the

presence of further scabs elsewhere on the birds' bodies which were associated with ticks. In three cases (7%) transmitters slipped from the tibia to the tarsus of the kiwi. This caused no injury, but may have increased the risk of entanglement. In two cases (two separate individuals) a mark or wound was caused by a too snug band. Both these kiwi were growing juvenile females misidentified as adult males, one as a result of a mistaken genetic sexing. At least some kiwi presented feather loss under the transmitter band, although we did not record this information systematically (e.g. Fig. 4).

Table 3: Frequency of transmitter-related injuries and other problems in tagged kiwi between 2007 and 2009.

Injury/problem	**Occurrences 2007-2009**		
	Males	**Females**	**Total**
Transmitter slipped over hock	1	2	3
Chafing under transmitter band	2	0	2
Blisters under transmitter band	6	1	7
Scabbed sore under transmitter band	1	1	2
Mark on leg from too snug transmitter band	0	1	1
Lumps/swelling associated with transmitter band	1	0	1
Infected wound from too snug transmitter band	0	1	1
Fatal entanglement, role of transmitter uncertain	0	1	1
Total injuries/problems	11	7	18
Number of birds involved	7	5	13
Total number of birds carrying transmitters	22	21	43

Kiwi video-recorded away from burrows

We filmed 25 kiwi away from their burrows at night, including 8 tagged (3 male, 5 female) and 17 untagged (10 male, 7 female) birds. Untagged birds were filmed in both pasture (6 kiwi) and forest (11 kiwi), but tagged birds were filmed only in forest, perhaps because all tagged birds were initially captured there.

Time budgets: The percentage of time spent in each behaviour state (foraging, walking, vigilant, comfort, investigating obstacles, escape: see Table 2) did not differ significantly between tagged and untagged kiwi overall (Table 4); nor between tagged and untagged males, or tagged and untagged females (Kruskal-Wallis H tests, all $p > 0.05$). Nevertheless there was a near significant tendency for untagged birds to spend a greater percentage of time on comfort behaviours than tagged birds. However, there

were no significant differences between tagged and untagged birds in frequency of occurrence of any of the finer scale aspects of comfort behaviour recorded.

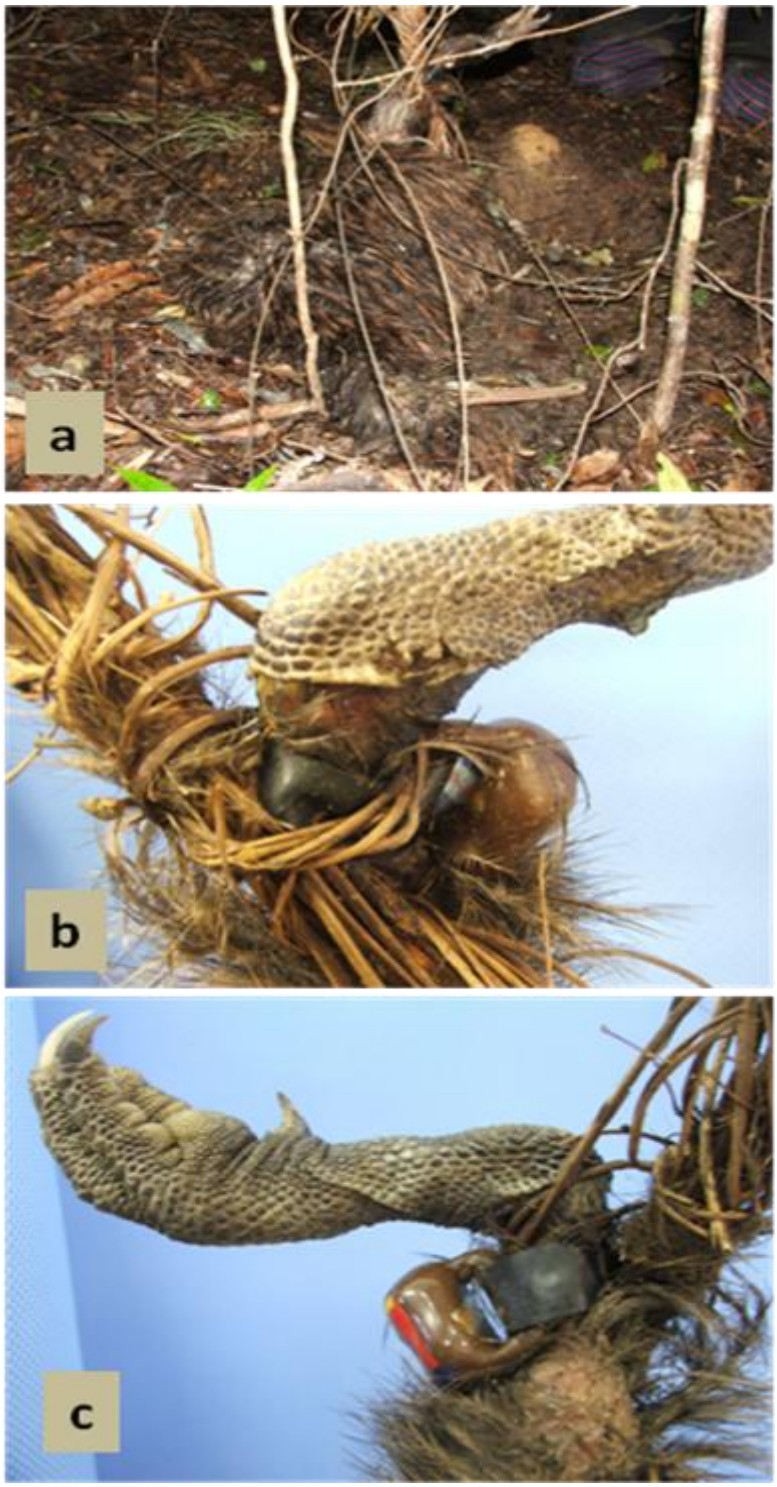


Figure 3: Kiwi found entangled in *Lygodium articulatum.* (a) the bird was found dead hanging from the legs; (b) view of the entangled leg from the true left; (c) view of the entangled leg from the true right. Photographs I.Castro.

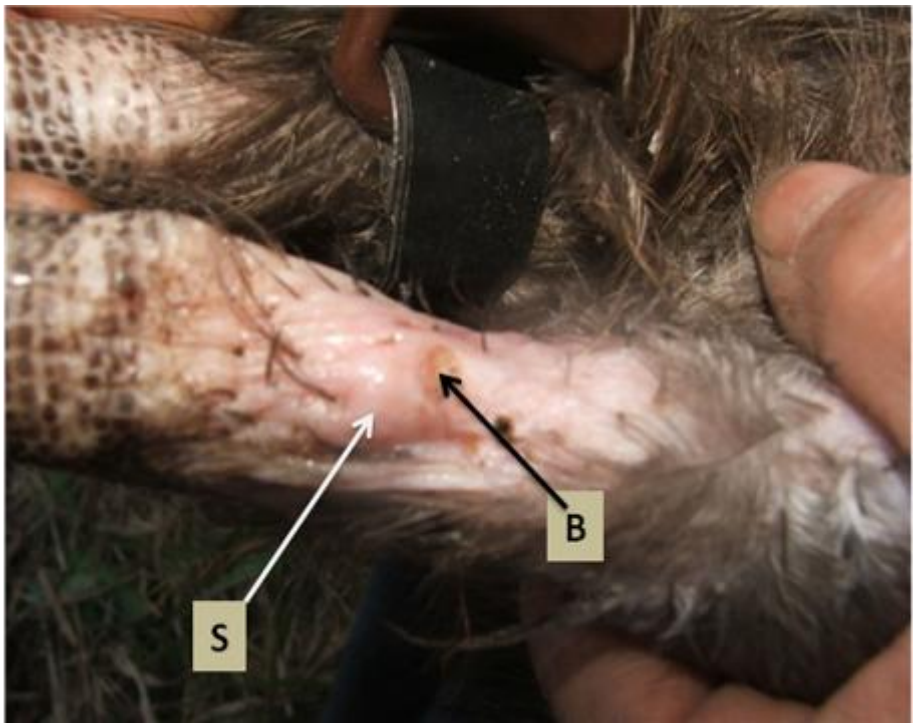


Figure 4: Scabby blister (B) and associated skin swollenness (S) caused by the transmitter band on a kiwi tibia. Transmitter battery lasts approximately 14 months and transmitters are changed annually. Each year the transmitter is placed on the alternative leg to prevent permanent injuries to the birds. In this photo the second tibia already has the new transmitter on. Notice the feather loss on the blistered leg in comparison to the newly banded leg. Photo by I. Castro.

Behavioural repertoire size and diversity: There was no significant difference in the size of behavioural repertoire between tagged and untagged kiwi (10.50 ± 1.85 and 9.82 ± 2.19 behaviours per observation, respectively). Shannon-Weiner diversity indices (H') of behavioural repertoire also did not differ significantly between tagged and untagged birds (H'= 1.153 tagged birds; H' = 1.160 untagged birds; Hutchisen's $t_{0.05(2),\,175}$ = 0.224, $p > 0.05$).

Fine scale aspects of foraging behaviour: Tagged kiwi caught a higher percentage of their prey in soil substrates (rather than in leaf litter, on the surface, or in grass roots) than untagged birds. (Tagged kiwi: 53.8 ± 31.3%; untagged kiwi 17.46 ± 26.24%; Kruskal-Wallis H = 5.17, df = 1, p = 0.023). Recordings of tagged kiwi were biased to forested gully floor areas with soft soils (75% of recordings, versus 41% of untagged kiwi recorded in these areas). When data from kiwi recorded in pasture and on forested slopes (59% of untagged kiwi, 25% of tagged kiwi) were removed from the analysis,

the difference in percentage prey caught in soil substrates disappeared (Kruskal-Wallis H = 0.81, df = 1, p = 0.368). No other aspect of foraging behaviour differed significantly between tagged and untagged kiwi.

Table 4: % of time spent in each of six behaviour states by tagged and untagged kiwi. Data were analysed using Kruskal – Wallis H-tests, df =1.

Behaviour	**% Time (± SD)**		***H***	***P***
	Transmitter (n=8)	**No transmitter (n=17)**		
Foraging	77.28 (± 7.81)	74.17 (± 3.69)	0.49	0.485
Walking	18.00 (± 6.90)	13.71 (± 2.30)	0.00	1.000
Vigilant	3.91 (± 1.08)	7.76 (± 1.91)	0.34	0.560
Comfort	0.27 (± 0.14)	3.44 (± 1.50)	3.12	0.077
Investigating obstacles	0.14 (± 0.14)	0.51 (± 0.40)	0.39	0.532
Escape	0.41 (± 0.41)	0.41 (± 0.25)	0.60	0.437

Reaction of tagged and untagged kiwi to observers: We video-recorded twice as many untagged as tagged individuals, despite searching for both tagged and untagged kiwi in the same way. We believe this reflects the proportion of the kiwi population wearing transmitters at this site, although we cannot rule out that the tagged birds were avoiding us. There was no difference in the length of video footage obtained from tagged and untagged kiwi (ANOVA: $F_{1,23}$ = 0.05, p = 0.821). No differences were detected in any aspects of vigilance behaviour, whether directed towards observers or towards other disturbances (Kruskal-Wallis H-tests, all p > 0.05) and there was no difference in the percent of time spent vigilant between tagged and untagged kiwi (Kruskal-Wallis H = 0.34, df = 1, p = 0.560).

Other fine scale aspects of behaviour: No other fine scale aspects of behaviour (as described in Table 2) drawn from footage of kiwi away from burrows differed significantly between tagged and untagged birds (Kruskal-Wallis H-tests, all p > 0.05). Neither did any of these aspects of behaviour differ significantly between tagged and untagged males, or tagged and untagged females (Kruskal-Wallis H-tests, all p > 0.05).

Burrow exits and discomfort behaviour related to the transmitter band

We filmed 16 kiwi, including 3 untagged and 13 tagged birds, while exiting burrows from 1 to 15 times (median= 9; 25th percentile = 2.75). Of the tagged birds six were females and seven were males. All untagged birds were male/juvenile. One untagged

male had a metal band and another of the untagged males was captured and radio tagged during the study.

The most common leg behaviour observed was the lifting up of the leg followed by throwing it backwards, resulting in an exaggerated elevation of the hip with a small final kick. One bird lifted her leg and compressed it against her body for a couple of seconds before putting back on the ground. These behaviours were performed only with the leg carrying the transmitter and the male who was tagged during the period of observations only performed the described exaggerated stretching behaviour after the transmitter was attached. For these reasons we considered them discomfort behaviours caused by the presence of the transmitter. Both behaviours made the birds limp as they walked and in some instances the birds skipped while walking and even lost their balance.

Legs were also fully stretched towards the back in three occasions; two of those involved tagged birds, one which did not use the transmitter leg. The third bird was not tagged. A similar-looking behaviour was also observed in both tagged and untagged birds away from burrows, where we included it in the same category as preening, scratching, shaking or other types of stretches. This behaviour seems unlikely to be related to the transmitter when away from burrows. However, we included it as ‘discomfort’ behaviour in this section, because it suggests that the birds required stretching on exiting the burrow.

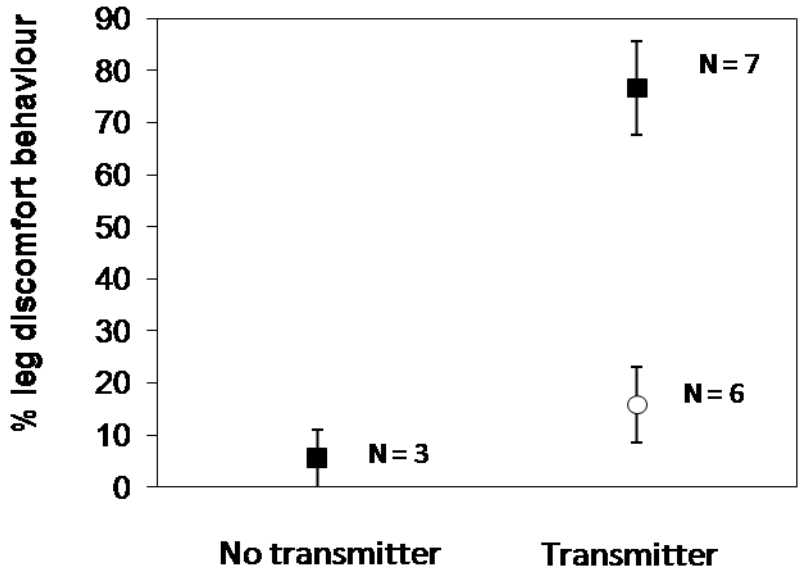


Figure 5: Percent of burrow exits where kiwi showed leg discomfort behaviours (see text for description of behaviours). Black squares = males; white circles = females. Whiskers are standard errors.

All the tagged males and three of the females showed discomfort behaviours on exiting burrows in the evening (Fig 5). On average, tagged males showed discomfort in 76 ± 23.56 % of burrow exits (n = 7) and females in 15.78 ± 17.69 % of burrow exits (n = 6). These differences were significant (t-test T = -2.286; df = 14; P = 0.038). Untagged birds by comparison showed discomfort on only 5.5 ± 9.60 % of the exits (n = 3). The difference in the percentage of exits involving discomfort behaviours by tagged and untagged birds was nearly significant (t-test T = -1.923; df = 14; P = 0.075; average tagged: 48.56 ± 37.50 %, n = 13; untagged = 5.55 ± 9.62 %, n = 3). This is most likely the case because half of the tagged females did not show any discomfort behaviours. Discomfort caused by the transmitter band on exiting burrows appeared to be transient, as, with one exception, kiwi video recorded away from burrows later in the evening showed no such transmitter-directed behaviours.

In one instance a transmitter with an unusually wide loop for band attachment was fitted to a female kiwi. A video recording of this kiwi foraging showed that the fault in the transmitter package was causing the transmitter to shift and bump against the tarsus of the bird with each step. This individual repeatedly stretched her transmitter-bearing leg

up and backwards as she walked, a behaviour not seen in other kiwi filmed away from burrows. The bird was recaptured and the faulty transmitter replaced.

Discussion

Radio-tracking is central to kiwi research and conservation efforts throughout New Zealand and the use of transmitters has revolutionised the study and monitoring of kiwi (Robertson et al. 2003). We consider that effects of the transmitters shown in this paper are short term because the transmitter packages were changed annually. Thus they generally did not cause permanent injuries to the birds, or even permanent behavioural changes. The short term impacts of transmitters on kiwi behaviour and welfare, at our study site, are mostly related to irritation under the transmitter band and transient discomfort on leaving roosts. Kiwi are long-lived and the long-term effects of prolonged continuous radio-tagging of individuals over several years or decades were not investigated in this study.

Transmitter-related injuries and mortality

Thirty percent of tagged kiwi in our study experienced at least one transmitter related issue or injury. Chafing, blisters and scabs under the transmitter band accounted for 85% of all problems/injuries and mostly occurred in males (9 instances, as opposed to 2 in females). These injuries do not appear to be incapacitating, but kiwi are likely to suffer discomfort from them, especially if they get infected or become open sores. Changing the transmitter band from one leg to the other more often (e.g. every 6-8 months rather than annually) may decrease the chance of kiwi developing these injuries. However, the benefits of avoiding band injuries need to be considered in conjunction with the timing of the breeding season and the stress caused by extra handling.

Too loose, slipped transmitters (3 cases) did not cause injury to the birds but may increase the risk of entanglement. The hospital bands used to attach the transmitters to kiwi are fastened using a dome clipped together through fixed perforations in the band. The tibia circumference of some kiwi is narrower than the minimum size attainable using these hospital bands. Some larger kiwi have tibia circumferences that fall between hospital band perforations, so that it is hard to determine the ideal perforation to use for

fastening the band. The three birds which had slipped transmitters in this study included one such small male (1800 g) and two females with 'in between' leg sizes. For birds in these categories the hospital band must be tightened by tensioning the electrical tape to achieve the desired size. However, this can be difficult and in the cases in question was probably not fully achieved. More care when fitting transmitters to such kiwi is necessary, or else the development of another method of attachment.

The worst transmitter-related injury we observed was an infected wound on a young female kiwi which had been misidentified as an adult male. This bird was still growing and the transmitter band had become too tight during the year since it was placed. This kind of injury is avoidable if unknown caught birds that are considered 'adult males' at tagging are caught again within six months of transmitter placement (even when genetic sexing returns as male) to ensure they are not growing. Genetic sexing for kiwi does not always provide consistent results for the same individual (authors' pers. obs.), perhaps due to sample quality. Two of 79 birds from our study site were assigned to the wrong sex more than once by genetic sexing using naturally shed feathers.

During the course of the study we lost a single adult female kiwi to fatal entanglement of the transmitter-bearing leg in vegetation. The bulk of the entanglement was around the tibia, but below the transmitter package. The role of the transmitter in this entanglement is therefore uncertain, but it is likely to have exacerbated the problem. This incident highlights the risk of entanglement for kiwi carrying transmitters, as emphasised before in New Zealand Department of Conservation publications related to kiwi conservation (Robertson et al. 2003, Colbourne et al. 2005). Although entanglements appear to be rare, they are generally fatal (Robertson et al. 2003, Colbourne et al. 2005). The benefits to the research or conservation project of tagging kiwi must therefore be assessed against the risk of entanglement and of other types of injuries as described above.

Transmitter impacts on kiwi behaviour

Kiwi carrying transmitters, particularly males, often showed discomfort behaviours on exiting burrows in the evening. This may indicate comfort issues with carrying a transmitter, perhaps related to compression of the leg by the transmitter band while the

bird is roosting. Kiwi sit with the tarsi laid flat on the ground, the tibia and femur crouched above these. The transmitter is strapped to the tibia, and we think that the band might put pressure on the tibio-tarsal joint when the bird is sitting, restricting blood flow to the lower leg. If this is the case, the differences observed between males and females may be explained by the fact that female kiwi are larger and probably have longer tibiae, perhaps resulting in less compression of the transmitter band between the tibia and tarsus when roosting.

For the most part, tagged kiwi video recorded away from burrows later in the evening did not display any discomfort behaviours related to the transmitter, suggesting that the discomfort is transient. The only exception to this was a single individual carrying a faulty transmitter on which the loop though which the attachment band passed was too wide.

Kiwi are not the only avian species to experience difficulties with tagging attachments placed on the legs. For example, foot and leg injuries caused directly by bands have been documented in North Island robins (*Petroica longipes*) (Berggren & Low 2004), and severe leg injuries have been caused by colour bands abrading and cutting into the legs of willow flycatchers (*Empidonax traillii*) (Sedgewick & Klus 1997) and hihi (*Notiomystis cincta*) (Armstrong et al. 1999). The current system for attaching kiwi transmitters (a hospital band wrapped in tape) results in a flexible band which should bend under pressure when the bird is sitting, rather than cutting into the legs. Transmitter bands removed from kiwi at our study site after being worn for 12 months are often bent, roughened and broken at the edges, suggesting such bending is occurring (authors' pers. obs.). We recommend that any future changes to transmitter fittings for kiwi should take into account the discomfort issues we document here, and use a flexible, rather than a hard, transmitter band.

The effects of carrying a transmitter on everyday nocturnal behaviour of kiwi once away from their burrows appear to be minimal. We found no effect of transmitters on time budgets apart from a near-significant decrease in the time spent on comfort behaviours by tagged kiwi (although not in the frequency of any individual comfort behaviour, e.g. preening, scratching). There was no increase in time spent foraging or in foraging rate (probes/minute or captures/minute) between tagged and untagged kiwi

which we assume might be expected if kiwi were experiencing any energetic cost of carrying the transmitter.

Conclusions

The impact of carrying a standard transmitter, as used in kiwi projects throughout New Zealand, seems to be higher in male than in female kiwi. This is likely because male kiwi are significantly smaller than females, meaning that the transmitter itself, as well as the band for attachment, are proportionately larger on males. The most commonly observed physical impacts of the transmitter package involved small injuries caused by abrasion (predominantly affecting males). More rarely observed problems included: slipped transmitters (males and females); over-tight bands (only growing females); and one fatal entanglement (a female). Carrying a transmitter did not seem to affect the fine scale behaviour patterns of either sex, except for the temporary discomfort displayed immediately upon leaving burrows - again particularly by males.

To reduce injuries caused by abrasion we could investigate the effect of changing the transmitter from one leg to the other approximately halfway through the battery life. To prevent injuries caused by over-snug transmitter bands, it is imperative that tagged 'adult male' kiwi whose sex has not been confirmed, be checked regularly to ensure they are not growing females. Extra care needs to be taken when fitting transmitters to kiwi with very narrow legs, or legs which are intermediate in size between perforations in the hospital band, in order to prevent the transmitter slipping over the tibio-tarsal joint ('hock'). Alternatively, a new transmitter attachment could be designed with a continuously adjustable band, to remove this issue altogether. To ensure future, modified, transmitter fittings do not cut into the legs of kiwi, we think it is important to retain a flexible band for attachment.

Assessing long term and broad scale effects of transmitters on kiwi behaviour and life history traits were outside the scope of this study, as was the assessment of transmitter effects on kiwi chicks. Data from other species show that transmitters can negatively affect adult survival (e.g. rock ptarmigans *Lagopus mutus*: Cotter & Gratto 1995; and sharp tailed grouse: Marks & Marks 1987) and breeding success (e.g. snow geese: Demers et al. 2003; and least terns *Sterna antillarum browni*: Massey et al. 1988). Lapwing (*Vanellus vanellus*) chicks have been shown to be negatively affected by the

frequent handling and disturbance associated with radio-tracking and handling (Sharpe et al. 2009) and tufted puffin (*Fratercula cirrhata*) chicks with tagged parents and those handled more often also suffered from higher mortality (Whidden et al. 2007). Carrying transmitters may also increase the energy costs of individuals, even if no measurable behavioural differences are observed, as shown in a study of takahe (*Porphyrio mantelli*) carrying backpacker transmitters (Godfrey et al. 2003). We therefore recommend that further research be carried out to investigate the possibilities that transmitters may impact long term survival and breeding success or energetics of kiwi.

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Appendix 2

Olfaction in birds: a closer look at the kiwi (Apterygidae)

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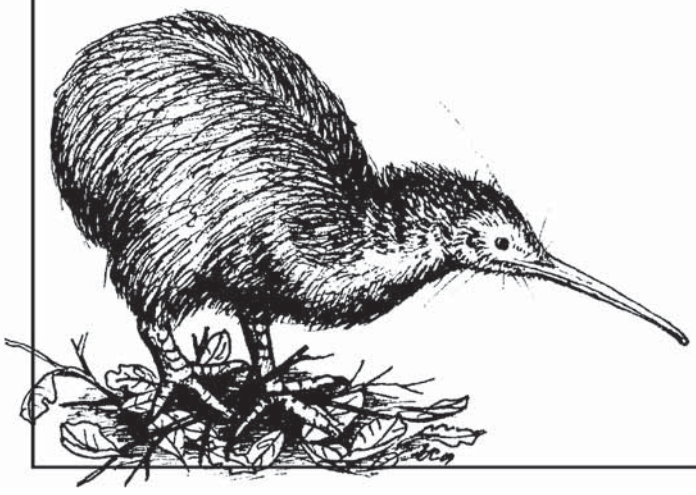

Abstract

Here we present a summary of our present knowledge of avian olfaction followed by a more detailed overview of olfaction in North Island brown kiwi (*Apteryx mantelli*). The article argues in favour of the importance of bird olfaction using evidence from the literature as well as our own observations.

Olfaction in birds

The evidence concerning the olfactory capability of birds and thus its potential use in their everyday life is now overwhelming. Over time however, it has been the source of much disagreement. In the early days of research into avian olfaction, the "small size" of most avian olfactory bulbs was taken as evidence that this sense was unimportant (Hill 1905; reviewed in Strong 1911). However, Strong (1911) dissected the olfactory organs of 65 species of birds (27 orders) and concluded that based on their size, the sense of smell could not be considered non-functional. One hundred years (mid 19th to mid 20th century) of behavioural observations and poorly designed experiments on a handful of bird species produced contradictory and confusing results which failed to elucidate a possible use of olfaction by birds (e.g. earlier papers reviewed by Strong 1911, including Audubon 1826; Darwin 1901; and Benham 1906; later work including: Meek 1922; Williams 1922; Soudek 1927; Marples 1931; Walter 1943; Hamrun 1953).

The first controlled experiments to test the function of the olfactory system in birds were carried out by Michelsen (1959) and showed that pigeons were able to learn food discrimination using odours as cues. A great deal of work on the anatomy and physiology of the olfactory apparatus and olfactory capabilities of birds followed this finding. Bang (1960) presented a description and photographs of the well developed olfactory apparatus of turkey vultures (*Cathartes aurea),* Trinidad oilbirds (*Steatornis caripensis),* Laysan (*Diomedea immutabilis),* and black-footed albatrosses (*D. nigripes),* offering behavioural observations to support the use of olfaction by each species. She followed this with a more detailed and extensive document in 1971 examining the anatomy of the olfactory system of avian species in 23 orders and providing evidence that olfaction should be a functioning sense in birds (Bang 1971). In 1968, Bang and Cobb reviewed the studies on olfactory bulb size and the comparative studies of the nasal fossa of birds, showing enormous variation between different groups of birds in these measurements.

Tucker (1965) and Wenzel (1965) measured the electrophysiological response of the olfactory tissue of several species of birds when stimulated by odorants, concluding that

all birds tested had a physiologically functional olfactory apparatus. Further extensive electrophysiological and behavioural work with more bird species continued to support this result (most of this work was done by Wenzel et al. but also Roper et al.; reviewed in Roper 1999).

Electrophysiological and anatomical work firmly established the functionality of the olfactory system in birds. However, the degree of olfactory sensitivity remained unknown. To date, olfactory thresholds have only been measured in a few species of birds. Results show that the birds' range of sensitivity to odours is similar to values obtained for mammals such as rats and rabbits (Stattleman et al. 1975; Snyder and Peterson 1979; Smith and Paselk 1986; Walker et al. 1986; reviewed in Waldvogel 1989 and Clark et al. 1993). In more recent times, researchers have searched the avian genome for the presence and extent of olfactory receptor (OR) genes (Gray and Hurst 1998; Leibovici et al. 1996; Nef et al. 1996; Niimura and Nei 2005; Steiger et al. 2008; Steiger et al. 2009a) which are associated with odorant detection in vertebrates. The most recent research shows that the number of OR genes in 9 species of birds correlates with the size of the olfactory bulb (Steiger et al. 2008). Furthermore, the sizes of the OR genes in kiwi (*Apteryx. australis*), kakapo (*Strigops habroptilus*) and jungle fowl (*Gallus gallus*), resemble those of mammalian genomes (Steiger et al. 2008). More interestingly, although the size of the olfactory bulb and the number of OR genes in the starling (*Sternus vulgaris*) are small (Steiger et al. 2008), this species is able to discriminate odours and use olfaction to select specific herbs (Gwinner and Berger 2008).

Experimental behavioural studies regarding the use of olfaction in birds have found that several species of procellariformes (e.g. work by Bang et al., Wenzel et al., Benvenuti et al., Bonadonna et al., and others; and reviewed in: Waldvogel 1989; Roper 1999 and Nevitt 2008); pigeons (research mainly by Papi et al; Wallraff et al.; Wiltschko et al; Benvenuti et al.; and reviewed in Papi 1990; Wiltschko 1996; Wallraff 2003; more recently: Mora et al. 2004; Gagliardo et al. 2007; Jorge et al. 2009); and starlings (Wallraff et al. 1995) use olfaction in navigation and/or long-distance prey finding (reviews in: Papi 1990; Wallraff 2003; Rajchard 2008). Holland et al. (2009) have

recently provided evidence that catbirds (*Dumetella carolinensis*) may use the olfactory sense in migratory orientation. Kiwi have been shown to be able to find food using the sense of olfaction alone or in combination with remote touch (Wenzel 1968; Cunningham et al. 2009: *Chapter 2*). Olfaction has also been suggested, and in some cases found, to be implicated in defence, either by deterring predators (Weldon and Rappole 1997; Dumbacher and Pruett Jones 1996; Weldon 2000) or parasites (Weldon 2000; Petit et al. 2002; Douglas 2004, 2008), or by making birds aware of possible poisons (Marples and Roper 1996; Skelhorn and Rowe 2006), and in predator recognition (Amo et al. 2008).

The use of odours in avian social communication has also received recent consideration (reviewed by Hagelin 2007; Hagelin and Jones 2007; Balthazart and Taziaux 2009). For example, several species of birds, mostly procellarids but also chickens (*Gallus domesticus*), have been found to respond to the smell of their partner or other conspecifics (reviews above plus: Bonadonna et al. 2007; Jouventin et al. 2007; Mardon and Bonadonna 2009), and others have been found to be able to recognise their burrow as well as their own odour (reviews above plus: Smith and Conway 2007; Mennerat 2008; O'Dwyer et al. 2008; Mardon and Bonadonna 2009). In a recent experiment Whittaker et al. (2009) found that incubating female juncos (*Junco hyemalis*) reduce incubation bout length in response to heterospecific's preen gland secretion suggesting that odour may affect parental care.

The kiwi

Kiwi (*Apteryx* spp.) are ideal candidates for studying the use of olfaction in birds because we already know that they have a highly developed and functional olfactory system (Bang and Cobb 1968; Wenzel 1968, 1971; Bang 1971). It is also known that kiwi use olfaction to find food (Wenzel 1968, Cunningham et al. 2009: *Chapter 2*). In recent years there has been an increase in our knowledge of kiwi brain anatomy and senses. For example, Martin et al. (2007) found that kiwi have reduced eyesight and visual field topography, which are reflected in the small area of their brain associated

with this function. In contrast, the areas of the brain concerned with touch, olfaction (Martin et al. 2007) and cognition (Corfield et al. 2008; Corfield 2009) are highly developed. Cunningham et al. (2009: *Chapter 2*) found that kiwi use a tactile sense called remote touch to find food, in addition to, or in combination with smell. Steiger et al. (2009b) have recently found that kiwi have a large number of OR genes and that those genes are correlated with nocturnality. Corfield (2009) studied the auditory capabilities and associated brain areas in kiwi, finding that kiwi have high frequency specialisations similar to that of barn owls (*Tyto alba*). Kiwi are nocturnal, ground dwelling and forest species and therefore it makes sense for them to primarily use senses other than vision in their everyday lives.

It is our belief that the olfactory and tactile senses of kiwi are likely to be used for more than one purpose (e.g. not just for foraging), due to their high degree of development. Some evidence in support of this idea comes from the work of Jenkins (2001). She examined, in an uncontrolled situation, the reactions of captive North Island brown kiwi (*Apteryx mantelli,* here referred to as kiwi) to self and unfamiliar kiwi faecal odour. She found behavioural responses ranging from attraction, to avoidance and escape, which suggested a territorial use of faecal scent marks. Further support for this idea comes from our field observations of wild kiwi. We used motion-picture recording to video at least 30 wild kiwi in their natural environment. Kiwi performed olfactory search behaviours aimed towards research equipment, other kiwi and people that were outside their burrows or nearby while foraging.

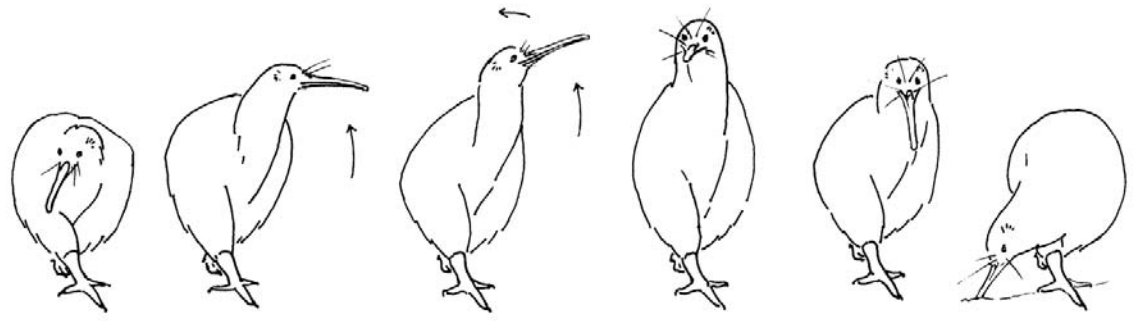

Figure 1. Brown kiwi displaying olfactory search behaviour. Head is thrown up and back during each audible *sniff*. During olfactory search the bird assumes a stereotyped erect posture (central drawings). Drawings at either end show kiwi in alert (far left) and normal foraging postures (far right) with the head held below the level of the back. Disturbed kiwi often freeze in position (in this case mid-step) before displaying olfactory behaviour.

Kiwi olfactory behaviours are reminiscent of mammalian sniffing. Individuals inhale air noisily through the nostrils, with an exaggerated lifting of the beak, pointing the bill several times in the direction of interest and moving the bill in a small arc in the air (Figure 1)[1]. Similar behaviours have been reported for procellarid species (e.g. DeBose and Nevitt 2008; O'Dwyer et al. 2008). For example, Leach's storm petrel chicks (*Oceanodroma leuchorhoa*) presented with odour choices sweep their heads in broad arches around their body while making coughing noises and rapid biting movements close to the source of odour (O'Dwyer et al. 2008). Our observations of wild kiwi suggest that olfaction is used in conjunction with hearing (at longer distances) and touch (at close proximity). For example, if an observer made a noise, the observed bird would lift its head and then perform the olfactory search behaviour. In some instances kiwi approached objects and people and touched them with their beak. Kiwi have their nostrils at the end of their beak, a unique characteristic among birds, and getting close to the objects may allow a better understanding of the source of odour (or taste or chemosense), which would otherwise be hampered by the very long bill. The bill-tip organ of kiwi (Cunningham et al. 2007) together with the sense of olfaction/taste may interact to allow kiwi to build a mental picture of the object and its location.

The loud inhaling associated with olfaction in kiwi may be a mechanism used to transport fluids containing the odour back to the olfactory concha in the upper reaches of the bill (Bang 1971). The loudness of the inhaling may reflect the effort necessary to transport the scent of interest, particularly if there is urgency to determine the source/composition of the smell. For example, female beaks may reach 12 cm in length in great spotted kiwi (*A. haastii)* and some populations of brown kiwi (Sales 2005). Kiwi also make a snorting sound with unknown function. In our videos, snorting was associated with both foraging and olfactory search. Wenzel (1971) suggested that snorting functions to clear the nostrils after probing or tapping. We suggest that snorting in the context of olfactory search has an additional function. Kiwi produce abundant nasal secretions which are often visible when the birds are caught as they 'blow

[1] The 'sniffling' sound has been reported in the past by many (Buller 1888, Wenzel 1971; and many kiwi fieldworkers) in relation to foraging, but never in terms of other functions, and the behaviour associated with it has to our knowledge never been described.

bubbles' through their nostrils. We associate this 'bubble blowing' with handling stress (Wenzel 1971; authors' pers. obs.) because 'bubble blowing' has never been observed in the wild. However, we have video recorded instances of sniffing or snorting wild kiwi sounding as if they had fluid in the nasal chamber. We think that the production of nasal drip together with the loud sniffing and snorting could be a way to aid the spread of nasal drip, in which the odours could be dissolved, from the nasal glands at the top of the bill to the nostrils and back. We suggest that nasal secretion should be produced more abundantly when the bird actively 'sniffs' particularly if the scent source is far from the bird or during stressful situations.

Use of olfaction in kiwi social interactions

The observations described above suggest that kiwi may be using olfaction to gather information about other organisms in their environment, for example predators or competitors, and conspecifics. Like petrels, kiwi emanate a notably unusual odour which Buller (1888) referred to as "a peculiar earthy smell". Kiwi faeces also have a strongly pungent smell that incorporates this 'earthy' body scent with the smell of ammonia (authors' pers. obs.). Using gas chromatography Antarctic prion (*Pachyptila desolata*) feathers have been recently found to have individual chemical profiles (Bonadonna et al. 2007). We hypothesise that kiwi odours, like those of petrels, may be specific to individual kiwi, providing an opportunity for social communication.

To examine this possibility, we undertook a preliminary examination of the chemical composition of kiwi faeces to determine the presence of substances which may provide faeces with their characteristic scent and which may allow kiwi to leave chemical messages. Kiwi faeces were collected in our study site in 2004 and 2006, stored in glass vials and frozen at -20ºC. We extracted volatiles from five faeces using hexane, dichloromethane and methanol. Extracts were examined by gas chromatography and mass spectrophotometry and compounds were identified by interpretation of mass spectra, comparison to database library and/or using retention data from authentic standards.

Table 1: Compounds found in excreta of brown kiwi from Ponui Island after extraction with hexane, dichloromethane and methanol.

Compound
Phenol
Phenol-4-methyl
Bencenecarboxylic acid
Caprolactam
Cinnamaldehyde
Bicyclo(2,2,1)heptane-2-carboxylic acid 3,3-dimethyl
Decanoic acid
Tetradecane
alfa-Cubebene
Pentadecane
Butylated hydroxytoluene
Dodecanoic acid
Hexadecane
Pentadecane-2,6,10-dimethyl
Heptadecane
Tetradecanoic acid
Octadecane
16-octadecenal
Isopropyl myristate
Pentadecanoic acid
Nonadecane
9-hexadecenoic acid, methyl ester
Hexadecanoic acid, methyl ester
17-norkaur-15-ene, 13-methyl-(8beta, 13beta.)-
1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester
Eicosane
Octadecanal
Heptadecanoic acid
Kaur-16-ene
Heneicosane
9,12-octadecadienoic acid (Z,Z) methyl ester
9-octadecenoic acid, methyl ester
Octadecanoic acid, methyl ester
9,12 octadecadienoic acid (Z,Z)
Oleic acid
Octadecanoic acid
2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester
Docosane
3-tricosene
Ferruginol
Tetracosane
Pentacosane
Octacosane
Nonacosane
Triacontane
Hentriacontene
Cholestan-3-ol, (3beta,5beta)-
Cholest-5-en-3-ol (3beta)
Cholestan-3-ol
Ergosta-5,22-dien-3-ol
Ergosterol
Campesterol
Dotriacontane
Ergosta-5,8-dien-3-ol, (3.beta)
Sitosterol

The chemical compounds found in kiwi excreta (Table 1) were common hexane-soluble hydrocarbons of the kind previously described by Jacob (1982) from secretions of the kiwi uropygial gland and which are found in sebaceous glands and most pheromone secreting glands in other animals. Wild kiwi faeces are often found in conspicuous places such as on logs and roots (J. Miles pers. comm.; authors' pers. obs.), on tracks (authors' pers. obs.), and also are accumulated inside some burrows (J. Miles, pers. comm.; authors' pers. obs.). Kiwi may be depositing information-loaded faeces in key locations where conspecifics may encounter them to provide information about breeding condition, burrow occupancy, or activity, as many mammals (e.g. reviewed in Johnson 1973; Goszczynski 1990; Feldman 1994; Ralls and Smith 2004; Barja et al. 2005) and reptiles do (e.g. Horne and Jaeger 1988; Bull *et al.* 1999; Carpenter and Duvall, 1995).

The similarity between the compounds in faeces and those found in the uropygial gland secretion examined by Jacob (1982) led us to begin investigating the uropygial gland of kiwi as a source of scents. Pycraft (1910) and Paris (1913; in Jacob and Ziswiler 1982) suggested that the uropygial gland of birds was a scent gland because many of the constituents of the waxes produced in the glands are very odorous. Uropygial gland secretions are typically preened through the feathers in most bird species, distributing their compounds all over their body and potentially spreading a chemical message on the bird's feathers which could be interpreted by conspecifics. The composition of the uropygial gland in female mallard ducks (*Anas platyrhynchus*) varies dramatically during the breeding season. The main change is caused by the production of pheromones (Kolattukudy and Rogers 1987) which were found to be influenced by sex hormones (Bohnet et al. 1991; Hiremath et al. 1992). Using gas chromatography Jacob (1982) found the same type of compounds which are precursors of the pheromones present in female mallards during the breeding season, in the uropygial gland secretion from kiwi. We are currently examining the composition of individual kiwi preen gland secretions to determine whether there are differences and if these are also associated with sex and season.

Figure 2. Brown kiwi uropygial gland. The head of the bird is located to the left of the photograph and the pygostyle to the right. C = Cloaca; U = Uropygial gland.

The kiwi uropygial gland is very large and located adjacent to the cloaca (Beddard 1899; Figure 2). The location of the uropygial gland leads us to hypothesise that chemicals may be produced in the gland and then deposited on the faeces to be used as social markers. This hypothesis remains to be tested. Although the uropygial gland is an obvious candidate for the production of scented markers, we cannot exclude the possibility that anal glands, also located in close proximity to the birds' cloaca (Coil and Wetherbee, 1959), may be involved.

Kiwi characteristic body odour could also be produced in at least two other types of glands that could manufacture scented products which could be used in chemical communication: the entire skin of many birds contains numerous cells capable of sebaceous secretions (Lucas and Stettenheim, 1972; Menon and Menon, 2000; Stettenheim 2000); and the external ear sebaceous glands (Lucas and Stettenheim, 1972). In addition, Douglas (2008) has recently described the production of fragrant aldehydes, used for alloanointing by crested auklets (*Aethia cristatella*), by secretory wick feathers.

Concluding remarks

We consider there is enough evidence that birds can use olfaction and that some species do so regularly; it is now important to determine to what extent this sense is used in the group Aves. In most of the studies above, the interest has been in how individual species use the sense of olfaction for a particular purpose such as navigation or foraging. It seems to us that the approach in these studies has generally been to show that the sense of smell *alone* is used for a particular purpose. However, the evidence suggests that birds use the sense of smell in conjunction with other senses (Strong 1911; DeBose and Nevitt 2008). Experiments therefore should aim to decipher the role of smell when used in combination to other senses (DeBose and Nevitt 2008) and sometimes this will mean that new senses need to be discovered first (e.g. Cunningham et al. 2007; Cunningham et al. 2009: *Chapter 2*).

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Author contributions: Conceived and designed ideas: IC, SC with contributions from AG and KJ. Field Work: SC, IC, AG. Laboratory work: KJ, AC, CL. Wrote the paper: IC with comments from all other authors and much advice from editors.

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