Can microbes be contributing to the decline of the North Island Brown Kiwi (*Apteryx mantelli*)?

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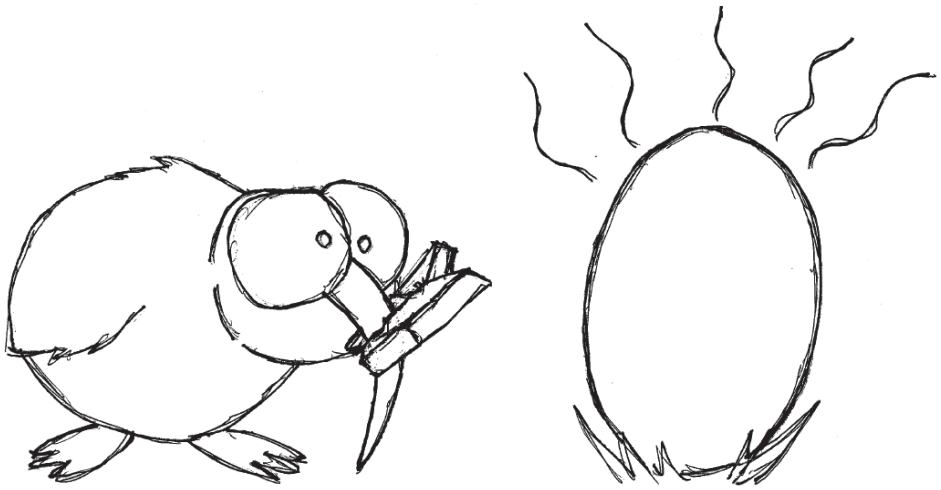
This thesis is dedicated to my daddy, who supports me through all my endeavours- even when they stink



Abstract

North Island Brown Kiwi (NIBK, *Apteryx mantelli*) are considered nationally vulnerable. Current conservation efforts concentrate on the predator vulnerable chicks, through both intensive predator control and Operation Nest Egg (ONE), a captive hatching and rearing scheme for wild eggs. While these methods are having a positive impact on some NIBK populations, they are expensive to maintain and many NIBK populations are dependent on this intensive management to maintain and increase numbers. Ideally, a point will be reached when less intensive management is needed to maintain NIBK populations. Therefore, ONE is not a permanent conservation strategy; the aim is to phase out intensive management when predator control is deemed sufficient to protect a majority of chicks.

However, even with intensive management, overall NIBK numbers are still declining. A potentially significant and previously overlooked factor in this decline could be that NIBK eggs experience high mortality. Indeed 60 per cent of NIBK eggs in the wild do not hatch. Both infertility and predators are unlikely to be major mortality factors in NIBK eggs. Consequently, predator control efforts do little to protect eggs. Research into why NIBK eggs experience such high hatching failure is needed and future conservation work needs to be adjusted in light of the results.

The overall objective of this project was to investigate if microbes could contribute to NIBK egg mortality. This project had two aims within this objective: 1. to determine if microbes that could impact hatching success are present on and in NIBK eggs; and 2. to use the results to direct future work and conservation efforts for NIBK. These aims were addressed using four studies, which together support each other in terms of conclusions and give an understanding of the microbes present at different stages in NIBK egg development, in locations throughout the population's range.

The first two studies used 16S rRNA sequencing and/or phenotypic identification methods to identify 1. the bacteria and 2. the fungi on the shells of wild NIBK eggs. Together these provided an understanding of the types of microbes that are present on living eggs during active incubation. In contrast, the third study used 16S rRNA sequencing to identify the bacteria present inside un-hatched infertile NIBK eggs,

collected from across the North Island. In the final study, a method was designed to determine if a target bacterium could penetrate through the NIBK egg's defensive shell. This method was not finalised because the NIBK eggshells could not be sterilised. However, this result showed that NIBK eggshells harbour bacteria that survive even through medical grade cleaning. The consequence of this may mean that bacteria can survive in the shell during adverse conditions, which may result in increased penetration when conditions become suitable.

Both the shell and the contents of NIBK eggs in this study had microbes present that could impact hatching success. Of these the most prevalent was *Staphylococcus,* and while no work has been done on the impact of *Staphylococcus* on NIBK, members of this genus have been shown to significantly impact the hatching of success of chickens and other birds. The prevalence of *Staphylococcus* in NIBK eggs indicates that it may be a significant factor in NIBK hatching success and warrants further, focused investigation.

That potentially pathogenic genera were isolated from NIBK eggs in this study has consequences for both fieldwork and NIBK conservation. NIBK are known to have dangerous and contagious pathogens in their blood and digestive tracts, such as *Cryptococcus* spp. Through this research, the potentially dangerous genera *Aspergillus, Staphylococcus*, *Streptococcus* and *Pseudomonas* are added to this list.

The Kiwi Best Practice Manual states that 'thin sterile latex gloves' should be worn when handing eggs, however, to use dry, bare hands 'rather than gloved' when collecting an ONE egg from the wild, to 'increase sensitivity to holding the egg ', as the eggs are cleaned upon arrival at the ONE facility. The eggshells in this project harboured bacteria that survive even through medical grade cleaning; therefore, the cleaning at ONE is unlikely to remove all bacteria. The conclusions of this project are that gloves should be worn at all stages of egg and bird handling, including collecting ONE eggs. This is because of the risk to the handler, as well as the egg. The results of this project also emphasise the need for all equipment used to be cleaned between individuals; this includes callipers, candling torches and weighing bags.

In regards to NIBK conservation, the results of this project suggest that predators are not the only factor in NIBK mortality. This project has shown that there are potentially

serious pathogens present on and in wild NIBK eggs that can kill avian embryos and could be contributing to NIBK egg mortality. We still do not know definitively what is causing the 60 per cent hatching failure in NIBK, but these results highlight the need for egg mortality and microbial factors to be factored in to NIBK conservation and recovery plans. Intensive management of NIBK should be phased out not only when predator control is deemed sufficient to protect the majority of chicks, but when researchers have a better understanding of what other factors contribute to NIBK mortality, at all stages of life. We need long-term, cost-effective ways to keep NIBK populations self-sustaining that protect the eggs as well as the chicks and adults. This means that phasing out of ONE needs to be considered in terms of egg mortality and not just chick survival.

More detailed studies are needed to both further identify the microbes present on wild NIBK eggs and to experimentally prove/disprove that NIBK embryos can be killed by these pathogens. This can be achieved by infecting eggs, or by cleaning them.

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