Causes of low reproductive success of translocated takahe (*Porphyrio mantelli*) on predator-free islands

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CONTENTS

Abstract

We conducted a two-year investigation (1996–98) into the possible causes of low reproductive success of translocated takahe breeding on predator-free islands. Infertility rates are twice as high on islands as they are in the remnant population in Fiordland and substantially higher than most other endangered New Zealand birds. We found no evidence that high infertility was associated with age of breeders, whether pairs were closely related, or whether breeders were the original translocated birds or had been raised on the islands. We also found no similar pattern of high egg infertility among pukeko on Mana Island, suggesting that pesticides that were used in farm operations on islands are unlikely to be the problem. Analysis of dietary nutrients in blood samples showed that selenium levels were slightly higher on islands compared with Fiordland but manganese levels were lower although sample sizes were small. We also found no evidence that island takahe have difficulty regulating water loss inside their eggs or show unusual patterns of incubation, factors that could potentially contribute to poor hatching success. We found that fertility rates of individual pairs increased with second and third clutches, and hypothesise that reduced territorial aggression as the season progresses might improve sperm production in males. However, fertility rates and productivity were still low, even in re-nesting attempts. Molecular data indicate that island takahe have low genetic variation indicative of a historical bottleneck. We therefore suggest that takahe are suffering from inbreeding depression induced by translocating an inbred population adapted to the Fiordland environment to a substantially different habitat on lowland islands. The need to immediately implement a supplementary feeding programme on islands before further research is undertaken is questioned. A review of the policy of transferring birds between islands to promote outbreeding is suggested, and suggestions to improve nest monitoring, data collection and staff training are made.

1. Introduction

The takahe (*Porphyrio mantelli*) is an endangered flightless rail endemic to New Zealand. Early efforts to conserve the species in the one remaining population in the Murchison Mountains in Fiordland focused mainly on research and management in situ, including aspects of their habitat, competitors, population ecology and breeding biology (Crouchley 1994). More recently, techniques for captive-rearing and translocation have been developed. Since 1984, this has included establishing small populations of takahe on predator-free offshore islands, which provide insurance against loss of the Fiordland population due to unforeseen circumstances. These populations also have the potential to become a source of birds for release back into Fiordland or into alternative mainland sites (Crouchley 1994, Clout & Craig 1995), although there are some potential reproductive problems that must be overcome first.

Although the annual survival rate of adult and yearling takahe on islands is high (over 90%; Ryan & Jamieson 1998) reproductive success in terms of number of chicks produced per egg and number of juveniles produced per pair per year has been significantly lower than that of takahe breeding in Fiordland (Bunin et al. 1997). Furthermore, reproductive success on islands is lower than we might expect, given the absence of mammalian predators and competitors and a more favourable climate than that found in Fiordland. The main cause of low productivity on islands is the high proportion of non-viable eggs, particularly in first clutches of a season (Ryan 1996). The large proportion of addled eggs (20%; Ryan 1996) makes it difficult to attribute failures to either infertility or embryo death, a potentially important distinction. An additional factor that affects juvenile productivity is that a significant number of chicks die in the first few weeks following hatching.

This report aims to address some of the problems of managing island populations of takahe by: 1) by improving the quality and quantity of information gathered on islands, and 2) by providing direction for future research through preliminary studies of possible causes of egg and chick failure.

2. Environmentally-induced inbreeding depression

2.1 INTRODUCTION

The proportion of New Zealand's terrestrial bird species listed as rare or endangered is higher than in any other country and second only to the Hawaiian islands in terms of relative land area (Halliday 1978). New Zealand's long predator-free biogeographical isolation has rendered its endemic species of birds particularly vulnerable to introduced mammalian predators, including humans. Hence most conservation management effort has focused on either controlling predators on the mainland of New Zealand or establishing small viable populations on predator-free offshore islands (Bell 1991, Innes & Hay 1991).

Other factors which can limit population growth in endangered species such as high rates of infertility of eggs have received much less attention in New Zealand because direct predation on eggs, juveniles or adults can override any of them. Areas where introduced predators have been removed or are controlled allow us to ask whether high infertility is a serious problem in New Zealand species, especially in small populations where inbreeding might be common. Celebrated cases such as the recovery of the Chatham Island black robin (*Petroica traversi*) from one breeding female to a growing population of over 100 individuals (Reed & Merton 1991) would suggest not. Other examples of endangered passerine species being introduced in small numbers on to predator-free island refuges indicates that inbreeding may not be a significant factor in successful translocations (Craig 1991). However, the question remains of what would happen if inbred individuals were translocated to an environment or habitat that was substantially different to the one from which they came.

In a single relict population of about 120 takahe in the Murchison Mountains of Fiordland, numbers appear to be limited by predators and harsh alpine weather conditions (Bunin & Jamieson 1995; Maxwell & Jamieson 1997). Nine juvenile takahe (five males and four females) raised in captivity were translocated to predator-free Maud Island by the New Zealand Wildlife Service in 1984 and 1985. Five additional birds were released on Mana Island as yearlings or adults in 1987. The first breeding attempt occurred in 1986 (takahe normally start breeding at two years of age) and by 1991 there were a total of eight pairs breeding on four islands (Maud, Mana, Kapiti, and Tiritiri Matangi Islands; see Fig. 1). With no predators and a more benign climate, survival of independent juveniles and adults on islands has been high compared with that of the source population in Fiordland. However, island birds have produced significantly fewer juveniles per egg due to extremely poor hatching success—hatching failure is twice as high on islands as it is in Fiordland (Bunin et al. 1997).

The exact cause of the low hatching success on islands is not known. During the period covered by the analysis (1991–1995) by Bunin et al. (1997), the first generation of island-raised takahe were just beginning to breed. Thus the number of breeding pairs that were closely related was small (n = 5) and had no worse mean reproductive success than pairs that were not closely related

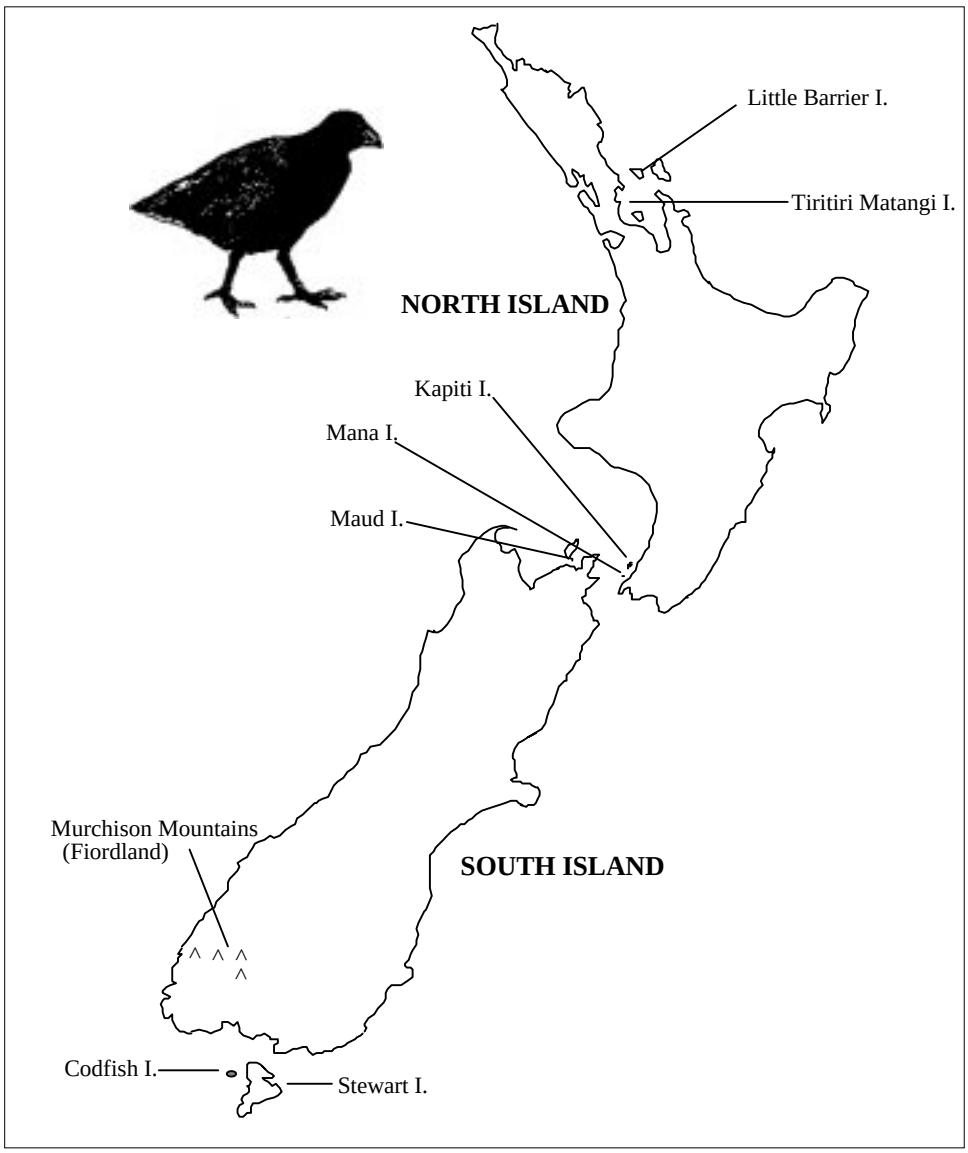


Figure 1. Map of New Zealand showing natural and translocated populations of takahe and kakapo.

(n = 20). Overall, island populations of takahe did not appear to be any more inbred than the source population from which they came.

Establishment of takahe on islands is uniquely different from the transfer of most other endangered New Zealand species on to offshore islands. Most endangered passerines that have been translocated have been moved from one forest habitat to another of a similar type (Innes & Hay 1991). Takahe, on the other hand, have come from an alpine-tussock habitat in the Fiordland mountains in the south of the South Island and have been transferred to lowland islands with introduced grasses and patches of native forest in the more temperate central and northern parts of the country (Fig. 1). We presume that the small, isolated population of takahe remaining in Fiordland has gone through a genetic bottleneck and is inbred. We also presume that takahe from the present Fiordland population are adapted to living and breeding in an alpine environment (Mills et al. 1984, 1988, 1991). It was initially predicted that takahe would establish well in lowland islands, since they were once found throughout New Zealand (Beachamp & Worthy 1988, Gray & Craig 1991). This prediction has, for the most part, been born out as adult takahe have increased

in numbers on islands (Ryan & Jamieson 1998) and have successfully bred and raised offspring (Clout & Craig 1995). However, low egg viability in translocated populations was not expected. We therefore hypothesise that the transfer of inbred takahe to a grassland habitat substantially different from the one in which it has had a long evolutionary history, may have induced the expression of inbreeding depression (in the form of high egg infertility) in the face of an environmental challenge (Keller et al. 1994).

We cannot test this hypothesis experimentally on takahe as there are restrictions on the movement and handling of this highly-endangered species, but we can derive predictions and examine these by two different means. First, we postulate that takahe translocated to islands should have higher rates of infertility than takahe living in their natural habitat on the mainland. We also postulate that infertility rates should be higher in island takahe than in other rare or endangered New Zealand birds that are from small inbred populations but have not been translocated to different habitat types.

The kakapo (*Strigops habroptilus*) population on Little Barrier Island has gone through a similar translocation process to that of takahe on islands. A remnant population of kakapo occurred on Stewart Island in the far south of the South Island of New Zealand. Because Stewart Island had feral cats, most of the remaining kakapo (approximately 40 adults) were transferred either to nearby Codfish Island, which was cat-free but similar in habitat to Stewart Island, or to Little Barrier Island, a large semi-tropical island off the north-eastern coast of the North Island (Clout & Merton 1998) (see Fig. 1). Codfish and Little Barrier islands have similar terrain but the vegetation and plant-food species are very dissimilar (Moorhouse & Powlesland 1991, Powlesland & Lloyd 1994). As with takahe, translocated adult kakapo have had very high survival rates and have bred on both islands (Clout & Craig 1995, Clout & Merton 1998). However, if infertility is relatively high for kakapo translocated to Little Barrier Island compared to those birds moved to nearby Codfish Island, then this would suggest that infertility is related to transferring birds to substantially different habitat type rather than the translocations per se.

We also analyse egg infertility rates and juvenile productivity of takahe among the translocated populations themselves. Specifically, we examine age, relatedness and origin (translocated versus island raised) of breeders to determine whether any of these factors are associated with, and thus potentially explain, the poor reproductive success among island takahe.

2.2 METHODS

2.2.1 Egg failure rates in takahe and other New Zealand birds

A questionnaire was sent to researchers studying other rare and (or) endangered non-passerine birds in New Zealand which are long-lived and have small clutch sizes (1–3 eggs) similar to that of takahe. Researchers were asked to provide, where possible, data on: 1) proportion of total eggs laid that failed to hatch, and 2) the proportion of failed eggs that were infertile, addled, contained a dead embryo, were predated, or failed for some other reason. Some researchers reported their data on a per year basis while others reported their data as a total over two or more years combined.

Addled eggs can result from either infertility or very early embryo death, making it difficult to determine true rates of infertility. In a detailed study of island takahe in 1996 and 1997, in which eggs were candled earlier than normal in the incubation period and removed from nests if they showed no sign of development, the percentage of addled eggs was reduced from an average of 25% in the five previous seasons to 7% (see Section 10). All eggs that were removed and examined at early stages of the incubation period were found to be infertile, suggesting that addled eggs are likely to be infertile, at least for takahe. For the purposes of this analysis and the one that follows, addled eggs are included with infertile eggs in all species to give a maximum estimate of infertility. For the comparative analysis we concentrate on hatching failure due to infertility and embryo death, as these parameters are assumed to be better indicators of inbreeding depression than, say, predation or nest desertion.

2.2.2 Infertility and juvenile production in island takahe

The mating system of takahe is presumed to be genetically monogamous (see Section 3). A 'SPARKS' database based on pedigree records was used to generate relatedness coefficients for pairs breeding on islands. Breeding pairs with a coefficient of relatedness (r) of 0.06 or greater (i.e. shared at least a set of grandparents) were considered closely related, and breeding pairs with $r < 0.06$ were considered unrelated. Data on the rates of infertility and juvenile production of takahe on Mana, Kapiti, Maud and Tiritiri Matangi Islands collected by the Department of Conservation between 1991 and 1997 formed the basis of the analysis. After nests were located each season, eggs were candled at approximately ten days of age to assess fertility and normally re-candled at least once more before they were due to hatch. When eggs were infertile or had died during incubation, they were removed and the nests were destroyed to promote re-nesting. The outcome of each egg in a clutch was categorised as either infertile, embryo death, did not hatch (unknown fertility), early chick death (up to four weeks), or fledged juveniles (survived over four weeks). Using these data, we examined two components of takahe reproductive success on islands: 1) number of fledged juveniles per breeding pair, and 2) number of infertile eggs per pair as a proportion of total eggs of known fertility.

2.3 RESULTS

2.3.1 Egg failure rates in takahe and other New Zealand birds

The percentage of total eggs that failed to hatch was high for most of this sample of rare and (or) endangered birds in New Zealand with the exception of yellow-eyed penguins (*Megadyptes antipodes*) in Otago (Table 1). However, the percentage of total eggs that were infertile was higher in island takahe (42%) and in kakapo on Little Barrier Island (62%) compared with their sister populations (19% and 27%, respectively), as well as other populations of endangered species throughout New Zealand (Table 1). Differences in the way proportional data were reported and summarised (see Methods and Table 1) precluded the different species from being compared statistically.

TABLE 1. COMPARISON OF PERCENTAGE OF INFERTILITY AND OTHER CAUSES OF EGG FAILURE IN ENDANGERED SPECIES IN NEW ZEALAND.

SPECIES (modal clutch size)	POPULATION	TOTAL NUMBER OF EGGS LAID	FAILED EGGS (% TOTAL EGGS LAID)	INFERTILE EGGS (% TOTAL EGGS LAID)	INFERTILE EGGS (% FAILED EGGS)	EMBRYO DEATH (% FAILED EGGS)	PREDATION (% FAILED EGGS)
Takahe (2) (*Porphyrio mantelli*)	Fiordland[2,3]	61	31	19	60	22	1
	Islands[3]	45	66	42	64	24	0
Kakapo (3) (*Strigops habroptilus*)[4]	Codfish I.[4]	36	47	27	58	25	17
	Little Barrier I.[4]	10	70	62	89	11	0
Yellow-eyed penguin (2) (*Megadyptes antipodes*)	Otago[3]	104	15	14	89	0	0
New Zealand pigeon (1) (*Hemiphaga novaeseelandiae*)	Pelorus Bridge[5]	45	67	4	7	0	33
Brown kiwi (1) (*Apteryx australis*)	Tangiterotia[5]	26	77	15	20	0	10
Great spotted kiwi (*A. haastii*)	Northwest Nelson[5]	19	63	16	25	0	17

1 Includes broken, disappeared, displaced and desertion
2 Excludes eggs that were reared under artificial conditions
3 Data are averages derived from several breeding seasons
4 Data from individual female kakapo were averaged across two or more breeding seasons
5 Data are totals from several breeding seasons combined
6 Recorded as missing but may have been due to predation

A high percentage of the failed eggs of yellow-eyed penguin were categorised as infertile (89%), but this represented only a small proportion of the total eggs laid (Table 1). Similarly, the high percentage of failed eggs that were infertile in Fiordland takahe and Codfish Island kakapo represented a smaller proportion of the total eggs laid than was the case for island takahe or Little Barrier Island kakapo (Table 1). In addition, egg infertility appears to be a greater problem than embryo death in takahe and kakapo. In both species of kiwi, egg failure was primarily due to desertion. Desertion was a consequence of disturbance by predators or researchers although a small (unspecified) number of 'rotten' eggs were deserted late in the incubation stage and may have been infertile (McLennan et al. 1996).

In summary, egg infertility rates in takahe are substantially higher in pairs translocated to islands than in pairs from the source population in Fiordland and are generally high compared to those of other endangered bird species in New Zealand with the exception of kakapo translocated to Little Barrier Island.

2.3.2 Patterns of infertility and juvenile production in island takahe

Infertility and juvenile productivity data from 26 males and 25 females in 34 pairings and 101 pair-years between 1991 and 1997 were analysed. There were no significant differences in the proportion of infertile eggs or the number of juveniles produced between years (Figs 2 and 3) and between the four islands (Figs 4 and 5) (Kruskal Wallis tests, h values ranged from 4.7 to 0.30, p values from 0.59 to 0.96), and data were pooled for some of the analyses below. The relatively high proportion of infertile eggs on Tiritiri Matangi Island (Fig. 4) was primarily due to two pairs never producing a fertile clutch of eggs.

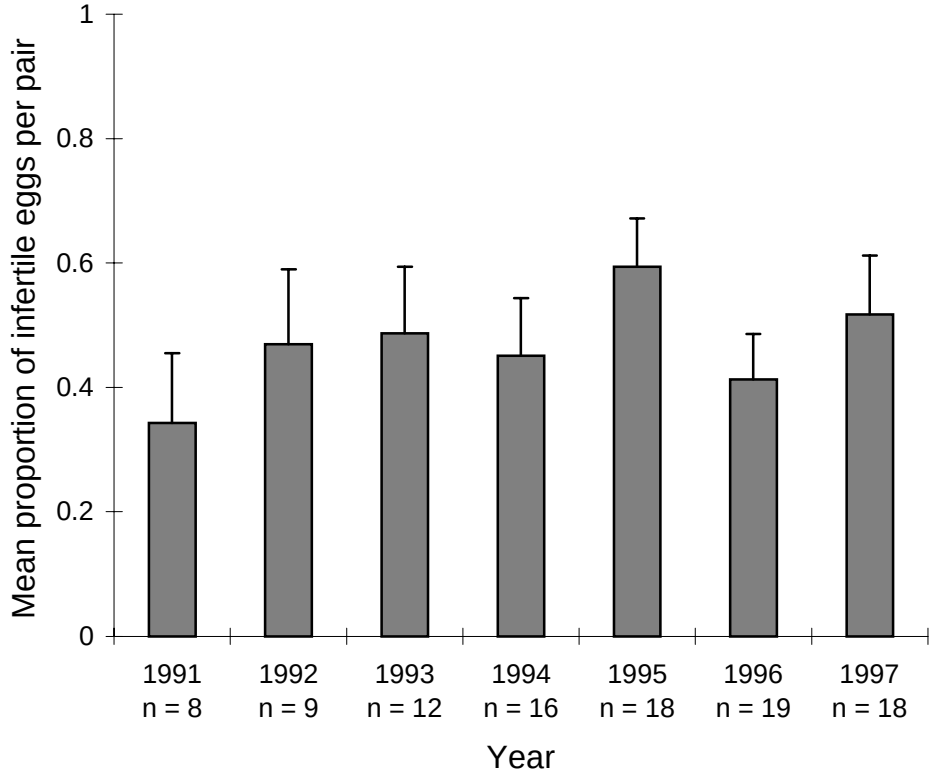


Figure 2. Mean proportion (+ SE) of infertile eggs to total eggs of known fertility per pair for island takahe between 1991 and 1997.

Figure 3. Mean number (+SE) of juveniles per pair for island takahe between 1991 and 1997.

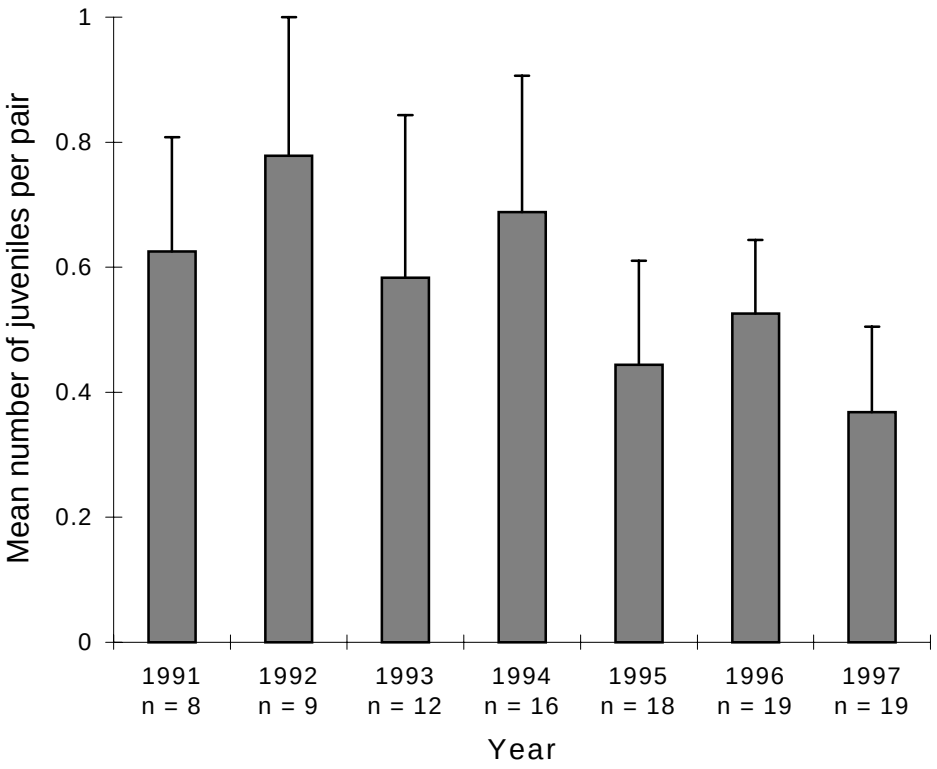
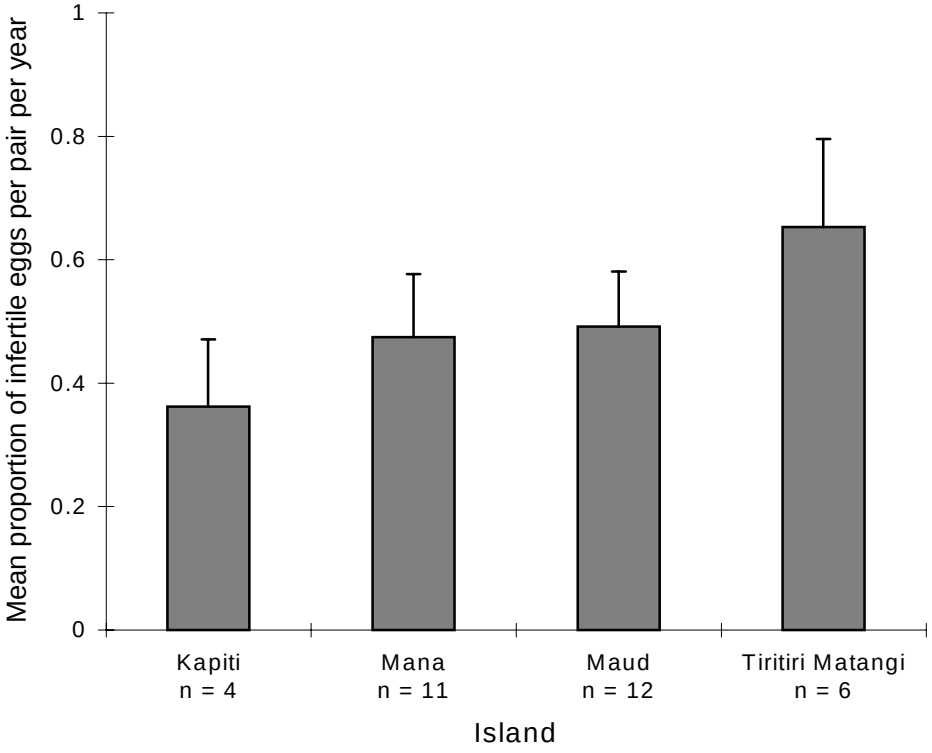


Figure 4. Mean proportion (+SE) of infertile eggs to total eggs of known fertility per pair per year on four offshore islands.



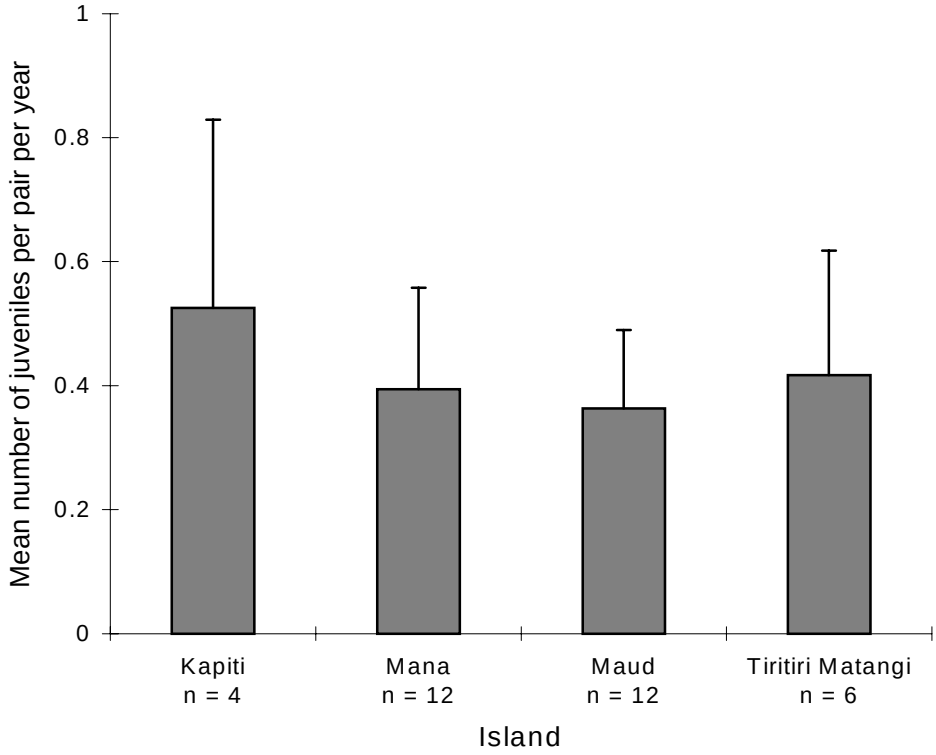


Figure 5. Mean number (+SE) of juveniles per pair per year for takahe on four offshore islands.

There were no significant differences between 22 unrelated pairs and the 9 closely related pairs in the mean (± se) proportion of infertile eggs laid (0.49 ± 0.06 and 0.52 ± 0.12, respectively) (Mann-Whitney test, w = 126.5, p = 0.92). Unrelated pairs produced more than twice the mean number of juveniles from related pairs (0.54 ± 0.11 versus 0.20 ± 0.12), but the difference was not significant (w = 109, p = 0.11).

Although inbreeding alone did not seem to explain the poor reproductive success of island takahe, other factors were associated with high infertility and low juvenile productivity. For example, infertility and juvenile productivity had not improved in the last three years of the study compared with the four previous years (Figs 2 and 3). This suggests that, as time goes on, island takahe are showing no signs of adapting to whatever is causing the problem of low reproductive success.

Reduced productivity may be a result of individuals in the population getting older. We divided all the males and females that bred in 1997 into three age categories: 2–5 years (n = 8 males, 9 females), 6–10 years (n = 5 males, 6 females), and 11+ years (n = 5 males, 2 females) and tested for differences in our measures of reproductive success. However, we found no significant differences among the age categories for males or females in either of the reproductive success measures (Kruskal Wallis tests, h values range from 3.8 to 0.09, p values from 0.15 to 0.96). The only trend in the data was that male infertility tended to increase with age. However, when we repeated the analysis for the 1996 season, the opposite trend was evident. We conclude that increase in the age of individuals does not explain the poor reproductive success in the latter years of the study.

We also asked whether birds that hatched and were raised on the islands (n = 20) had better reproductive success than birds that were originally translocated to the islands (n = 12). Again, we found no significant difference in

either the proportion of infertile eggs (0.48 ± 0.08 versus 0.50 ± 0.08) or in the number of juveniles produced (0.36 ± 0.11 versus 0.53 ± 0.14) (Mann-Whitney tests, w = 269.5 and 308.5, p values = 0.94 and 0.38, respectively).

Only 24% of the total number of juveniles produced between 1991 and 1997 (n = 54) came from the 165 first-clutch eggs, with 57% of juveniles coming from 116 second-clutch eggs and 19% of juveniles from 33 third-clutch eggs. Comparisons within years indicated that significantly more juveniles per egg came from second clutches (0.25 ± 0.029) than from first clutches (0.08 ± 0.019) (Wilcoxon paired-sample test, z = –2.4, N = 7 years, p = 0.02). On an individual basis, 17 males (65%) and 16 females (64%) had never produced a juvenile from a first clutch.

A high proportion of eggs in first clutches were infertile (53.6% ± 4.57, n = 7 years) or the embryos or chicks died during incubation or just after hatching (38.0% ± 3.61). Although these proportions were lower, on average, for second clutches (42.7% ± 5.20 and 33.1% ± 4.61, respectively), they were not significantly different when compared within years (Wilcoxon paired-sample test, z = –1.4, p = 0.17 for infertility and z = –0.84, p = 0.40 for embryo/chick death). Island females were laying their first clutches two months earlier on average (Sept.–Oct.) than Fiordland female takahe (Nov.–Dec.). It is therefore possible that island males were not producing adequate numbers of sperm early in the season, resulting in high rates of infertility. However, despite first clutches being laid over a 7–13 week period for the three years that were analysed, we found no significant relationship between date of laying and the proportion of infertile eggs, the relationship being negative (i.e. more infertility early in season) in two of the years (Spearman's rank correlation, 1997: rho = –0.40, p = 0.14, 1995: rho = –0.33, p = 0.25) and positive in the other (1996: rho = 0.34, p = 0.20).

We also compared the fertility of a pair's second clutch with respect to the fertility of its first clutch, combining results across pair-years. For those pairs for which their first clutch was completely infertile (n = 26 pair-years), 46% laid completely fertile second clutches while a further 18% laid partially fertile second clutches (Table 2). Of those pairs that laid partially fertile first clutches (n = 15), 40% laid completely fertile second clutches. Of those that laid fertile first clutches but subsequently lost their eggs or chicks (n = 10), most (60%) again laid fertile clutches, and only a small minority (10%) laid completely infertile second clutches (Table 2). From these results we conclude that fertility rates appear to improve with re-nesting and laying of a second clutch.

TABLE 2. PROPORTION OF ISLAND TAKAHE PAIRS THAT LAID 'FERTILE', 'PARTIALLY FERTILE' OR 'INFERTILE' SECOND CLUTCHES WITH RESPECT TO THE FERTILITY CATEGORY OF THEIR FIRST CLUTCHES BETWEEN 1991 AND 1997.

FERTILITY CATEGORY OF 1ST CLUTCH	FERTILITY OF 2ND CLUTCH			
	FERTILE	PARTIALLY FERTILE	INFERTILE	N
Fertile	0.60	0.30	0.10	10
Partially fertile	0.40	0.33	0.27	15
Infertile	0.46	0.19	0.35	26

2.4 DISCUSSION

Whether small, inbred populations of birds suffer from inbreeding depression has been a topic of debate in New Zealand over recent years (Craig 1991, Wallis 1994). In theory, a population that had gone through a genetic bottleneck and subsequently had a long history of inbreeding would have purged many of its deleterious alleles. As a consequence, it might not suffer from inbreeding depression, assuming the inbred individuals continued to be subjected to the similar environmental selection pressures (Dhondt 1996). However, when inbred groups are subjected to new environmental stresses, reproductive success and/or survival can decline markedly (e.g. Keller et al. 1994). That is, new environmental stresses can induce inbreeding depression.

Although predation is still the main factor limiting population growth in many populations of New Zealand birds, this does not mean that other factors such as high infertility are absent (Wallis 1994). Our analysis indicated that a large proportion of egg failure in translocated populations of kakapo on Little Barrier Island and takahe on four predator-free islands is attributable to infertility. Infertility rates were very high relative to Northern Hemisphere birds (10%, Koenig 1982), and also high relative to other endangered New Zealand species, including the source populations from where the translocated populations originated (Table 1). However, infertility rates were lower for kakapo translocated to nearby Codfish Island than those moved to Little Barrier Island, suggesting that infertility might be related to substantially different habitat type rather than the translocations per se.

The above results support the hypothesis that inbreeding depression in the form of high rates of egg infertility can be induced in an inbred population that is subjected to a change in its environment. Further analysis of island takahe revealed that breeding pairs that were unrelated (as determined by pedigrees) had just as high infertility rates as breeders that were closely related, which appears to be counter to the hypothesis. However, island takahe appear to be highly inbred and thus levels of genetic heterozygosity are similar between related and unrelated pairs (see Section 3). Inbreeding tends to affect fertility more in male birds than female birds (i.e. females still lay similar number of eggs) but inbred females have reduced hatchability of their eggs (Sittmann et al. 1966). Takahe exhibit both of these forms of reproductive failure and we are suggesting that translocating inbred birds to a different environment has exacerbated this problem. We acknowledge that we are unsure what exactly it is about islands that would induce some form of physiological stress in breeding takahe. Although the island environment does not appear to be climatically as harsh as that of Fiordland, both habitats are nevertheless substantially different, particularly in their plant-food species and in the density of birds (see below), and one or both of these may be the key.

Additional analyses were able to eliminate other possible factors that might be associated with infertility and low juvenile productivity of island takahe. For example, age of birds and whether they had been raised on islands or had been originally translocated were poor predictors of reproductive success. Furthermore, infertility and juvenile productivity did not differ significantly between islands or between years. If anything, productivity was slightly lower in the last three years of the study, suggesting that whatever the problem is, it

may not be getting better; nor are birds adapting, at least not over the short term. High infertility and low juvenile productivity were particularly evident in first clutches, although there was no relationship to laying date. It is possible that male and female reproductive systems are not synchronised for first clutches of the season but become so after one nesting attempt; why this should be the case (i.e. why females are ready to lay eggs but males are not ready to produce sperm) is not at all clear and would be unexpected.

Another possibility is that high levels of aggression early in the breeding season might be inhibiting sperm production. Takahe seem to favour certain areas and territories on islands, and managers have witnessed fights associated with territorial take-overs and / or mates, sometimes leading to serious injury or even death. Fighting is particularly prevalent in early spring when breeding territories and pair bonds are being established. If associated increases in stress levels were excessive then this might have a negative influence on reproductive performance in both males and females. Density of territorial birds in the Fiordland population is much lower and thus intense fighting among neighbouring birds of the nature seen on islands would be much less common. The failure of the takahe captive-breeding programme at the National Wildlife Centre at Mount Bruce in the 1960s and 1970s was thought to be due to the close proximity of breeding pairs (J. Craig, pers. comm.). However, only a small proportion of the total number of island pairs is involved in fights over territories in any one year. In addition, fertility rates and juvenile productivity are relatively low even in second clutches laid later in the season. Therefore high levels of territorial aggression is not completely satisfying as an explanation of the low reproductive success of island takahe, but it may be a contributing factor.

The results presented here are not meant to imply that the translocation of takahe to predator-free islands has been a failure, as the overall population has steadily increased despite the poor reproductive success of breeding pairs. Poor reproductive success in the form of high egg infertility and low hatching success is still an anomaly of translocated populations. If our hypothesis that this is due to inbreeding depression is true, then poor breeding success is something that managers may have to live with in the short term until there is local selection for better breeders. Whether birds should be transferred between islands to maximise outbreeding or be allowed to further inbreed within islands to promote locally adaptive gene complexes (Craig 1991), is an issue the Takahe Recovery Group still needs to address.

3. Mating system, inbreeding and genetic variation [1]

3.1 INTRODUCTION

From a conservation perspective, there are two central goals in the genetic management of endangered populations: (1) to maintain the genetic variation of the population in order to preserve its evolutionary potential, and (2) the prevention of excessive inbreeding in order to minimise potential negative effects of inbreeding depression (Simberloff 1988). Crucial to both goals is the need to elucidate the genetic mating system of the species under consideration and consider the associated consequences for management. Knowledge of the genetic mating system of a species allows pedigrees to be constructed, from which levels of inbreeding may be estimated. Pedigree records can also be used to manipulate matings in order to minimise or prevent inbreeding. Furthermore, a high level of undetected extra-pair paternity in a relatively small population will lead to offspring that are collectively less genetically variant since they are sired by fewer males.

The island populations of takahe have slowly expanded from 24 founding individuals to a current total of 56 birds of one year of age and older (Ryan & Jamieson 1998). The number of breeding pairs presently on each of the four islands varies: Tiritiri Matangi (5 pairs), Mana (6 pairs), Maud (6 pairs) and Kapiti (2 pairs). Current management of takahe assumes that extra-pair fertilisations (EPFs) do not occur and that pedigrees accurately represent parentage. This is based on observations of long-term pair bonding and defence of large, all-purpose territories by a flightless bird. However, given that copulation events are rarely observed, that some home ranges overlap, and that most genetic studies on socially monogamous birds reveal some degree of EPFs, the need to determine whether takahe do engage in EPFs is imperative. Furthermore, the sperm competition hypothesis (Birkhead 1996) predicts that the incident of EPFs should increase as a function of density. Therefore, as island populations increase, one might also expect to find EPFs.

The primary aim of this study is to test the assumption that island takahe are genetically monogamous, using DNA profiling. The second aim is to compare levels of genetic variation in takahe with those observed in other avian species, as reflected by minisatellite DNA.

3.2 METHODS

Forty island takahe 5 months of age or older were captured in April 1997 using a combination of hand nets and large mist nets set at ground level. Up to 5 ml of blood (part of which was used in the nutrient analysis, Section 5) was collected from each bird from the inter-tarsal vein, using a 26-gauge sterile butterfly

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needle fitted to a 5 ml disposable seringe. Samples were immediately transferred to liquid nitrogen and stored at –80°C until analysis. Twelve samples collected on previous occasions were also made available for analysis. In total, 37 offspring from 11 presumptive families were analysed for paternity. Of the 37 offspring that were sampled, 7 were approximately five-month old juveniles still on their parents' territory, 10 were non-territorial 1–2-year-olds, and the remaining 20 were breeding adults. Takahe pedigrees for each island were constructed from a Department of Conservation database.

DNA digested with either *Hae*III, *Alu*I, or *Hin*fI, was used to generate profiles with probes PV47-2, human probes 33.15 and 33.6, YNH24, and 3'HVR according to the methods and references in Lettink (1999).

For each family, DNA samples from the mother, offspring, and potential fathers (i.e. resident male and any other breeding male(s) present on the island and for which we had blood samples) were analysed in adjacent lanes on the same agarose gel. Where possible, all families from the same island were analysed on the same gel. In order to assign paternity, profiles were analysed for band matching, unattributable fragments, and band sharing between offspring and potential fathers. Further details of the profiling analysis can be found in Lettink (1999). It should be noted that since island populations of takahe were formed from translocations, and continue to be modified by them, there is effectively no 'natural' population structure.

3.3 RESULTS

What follows is a brief overall summary of the results; details of the DNA profiling and band sharing analyses can be found in Lettink (1999).

3.3.1 Paternity analysis

The paternity of 23 (62%, n = 37) offspring was assigned, and in all cases found to be consistent with the resident male. Failure to assign paternity to the remaining 14 (38%) offspring was due to an inability to exclude one or more potential males other than the resident male as a result of high levels of band sharing. For the three offspring from the two-female, one-male trio on Mana Island, neither maternity nor paternity could be resolved.

For the 14 offspring for which paternity could not be resolved, band sharing data combined with cuckoldry opportunities (based on the proximity of neighbouring territories) were considered. For two offspring, paternity was assigned based on high band sharing with the resident male (0.815, 0.870) and low band sharing with the only other non-resident male (0.308, 0.273). Furthermore, the two males also did not share adjacent territories. The paternities of two additional offspring were assigned solely according to territory isolation. In this case band sharing between resident and non-resident males were similar, but their territories were several kilometres apart. Therefore, combining the above criteria, we were able to assign paternity of 73% of offspring to the resident male, and unable to resolve the other 27%. Overall, we were unable to exclude the resident male as the possible father for any of the offspring.

3.3.2 Genetic variability analysis

Band sharing data obtained for takahe were compared with those reviewed by Papangelou et al. (1998) for DNA fingerprint variation in 70 avian species. Papangelou et al. (1998) concluded that, in general, obtaining mean band sharing values of 0.50–0.65 (or greater) for unrelated individuals from avian populations is indicative of a 'genetically depauparate' population. In contrast, for outbreeding populations, mean band sharing values are significantly different (0.20–0.30). Band sharing among unrelated individuals from inbred populations is similar to that reported for first order relatives from outbreeding populations (0.60).

Band sharing values for unrelated island takahe (0.66) are slightly higher than that of the small/inbred population category (0.50–0.65). Indeed, the band sharing value from a sample of the original island founders (0.57), falls above that indicative of small/inbred populations.

3.4 DISCUSSION

The paternity of over a quarter of all chicks could not be resolved using DNA profiling with probes pV47-2 and 33.6, due to the low levels of minisatellite variation recorded. However, paternity in all the other cases that were resolved was assigned to the resident male. Moreover, the resident male could not be excluded as the father in any of the cases. The results obtained are therefore consistent with the notion that island takahe are genetically monogamous. It can be concluded that if extra-pair fertilisations do occur, they are not likely to be frequent events, although mating patterns may not remain constant as island populations of takahe increase. For the time being, though, it would seem reasonable to assume that island takahe pedigrees constructed from observational records reflect the true relationships of breeders.

DNA profiling also revealed that island takahe exhibit relatively low levels of genetic variation according to the classification generalisations proposed by Papangelou et al. (1998). Genetically depauparate populations may result from a number of processes acting either in combination or single-handedly. These include inbreeding, recent bottlenecks or founder effects, and influence of genetic drift due to small population sizes.

Given what is known about takahe, it is not surprising to find low DNA minisatellite variation. Subfossil evidence indicates that takahe were once widespread throughout New Zealand (Beachamp & Worthy 1988), although there appears to have been two different species on the North and South Island, according to morphometric and genetic data from sub-fossil remains (Trewick 1996b, 1997). It is thought that numbers of both species declined dramatically after the arrival of humans, and that takahe had become quite rare by the time of European colonisation (Beachamp & Worthy 1988). Takahe are thought to have persisted in Fiordland because of the area's remoteness from activities associated with human colonisation, such as deforestation, hunting and the introduction of mammalian predators (Bunin & Jamieson 1995). Although this population has been protected since its discovery, the decline in numbers has continued to the present day.

The low levels of DNA minisatellite variation observed in island takahe profiles are potentially indicative of translocation-induced bottlenecks and/or persistence in a single small population in Fiordland over time. The ability to discriminate between these two possibilities will necessitate a comparison of genetic variation between island and Fiordland takahe, but the high band sharing values of the original island founders is consistent with the latter view. Ardern & Lambert, (1997) have recently shown that the extremely low levels of genetic variation observed in Chatham Island black robins are a consequence of the species' persistence as a small single population over the last ~100 years, rather than the extreme bottleneck experienced in during the late 1970s, at which time just five individuals remained.

At present, there is little consensus over how much genetic variation a given population or species requires in order to ensure survival over the long term. Caughley & Gunn (1996) distilled the genetic variation dilemma down to three questions. Firstly, is the population of interest characterised by reduced variation? Secondly, if this is the case, is there any cause for concern? And lastly, can the population be managed in a way that minimises or mitigates the perceived impact of low genetic diversity? The second question may be the hardest to answer, due to the difficulty in pin-pointing a decline in 'fitness' in a population over time. Fitness has many components, and their expression is confounded through environmental factors and fluctuations resulting from random events such as storms. Takahe and kakapo have high egg infertility and low hatching success that may be a consequence of inbred birds being subjected to new environmental stresses upon being transferred to new habitats (Section 2).

In summary, we cannot conclude that low genetic variation per se, as reflected by minisatellite DNA, is a cause of concern for island populations of takahe. Further research is needed to determine whether or not genetic variation is indeed declining in takahe. However, by assuming takahe are genetically monogamous, we can proceed to use pedigree data to evaluate the fitness consequences of close inbreeding of takahe on island refuges (Lettink 1999).

4. Fertility comparison with pukeko

4.1 INTRODUCTION

Three of the islands to which takahe were introduced were sheep and cattle farms for many years before they were taken over by DOC and turned into wildlife sanctuaries. Low fertility of takahe eggs on these islands may result from toxic pesticides and herbicides used in these farming operations. Soil samples had been previously tested in 1994 for levels of several pesticides on Mana and Maud Islands (R. Empsom, pers. comm.). Most of those samples gave very low readings, except for a small localised area on Mana Island where sheep had been dipped. If pesticide residues were still present in the environment and were at a level that could affect fertility of takahe, we might expect them to have a similar effect on egg fertility in pukeko, which nest on Mana Island and share the same habitats and eat similar plant foods to those eaten by takahe (Trewick 1996a).

4.2 METHODS

Pukeko nests were located on Mana Island and eggs candled to assess fertility. In most cases eggs were fresh when found, so their progress could be checked two or three times during their incubation.

4.3 RESULTS

Five pukeko nests were found during two fortnight stays on Mana Island between 18 September and 28 October 1996. Of 24 eggs found, two were infertile and one was of unknown fertility. Three embryos died at some point during development and the remainder were either seen to hatch or were developing normally at the last candling. Average infertility of these five clutches ranged between 9 and 13%, depending on the fertility of the unknown egg. By comparison, the infertility rate per pair for takahe on Mana for the same period was twice as high (22%).

4.4 DISCUSSION

Toxins have never been implicated in any of the post-mortems of embryos or chicks that have been conducted over the years and were detected in only trace amounts in tests of soil samples on Mana and Maud Islands. If toxins had been affecting fertility, we might have expected pukeko and takahe to have similar levels of infertility given that they live in the same habitats and eat similar foods (Bunin 1995, Trewick 1996a). The rate of infertility in clutches of pukeko living

on Mana Island was low relative to takahe but higher than that found in a mainland group of pukeko living near Dunedin (average 5% over 5 years; I. Jamieson, unpubl. data). Although the conclusions that can be drawn are limited by the small sample size, the results suggest that herbicides and pesticides are probably not adversely affecting fertility in takahe. It might be useful to confirm the above suggestion by subjecting some infertile takahe and pukeko eggs to chemical analysis.

Finally, pukeko are sometimes regarded as pests by managers and are often culled on Mana and Maud Islands. Although numbers may have to be controlled from time to time, this study and others we are conducting show that pukeko are a valuable comparative model for takahe research and it would be unwise to eradicate them completely from the islands.

5. Dietary trace elements and nutrition

5.1 INTRODUCTION

Past concerns over nutritional deficiencies causing high levels of infertility and embryo death in island takahe prompted the initiation of a supplementary feeding programme in the 1994/95 breeding season. No significant improvement in productivity was seen in that season compared with the previous breeding season (I. Jamieson, unpubl. data). A comparison of the diet and nutritional status of takahe on islands with takahe in Fiordland might show whether nutritional deficiencies had been causing low productivity in island birds. Our objectives were: 1) to compare the trace element composition of takahe plant food on islands with that in Fiordland and identify possible deficiencies, and 2) to assess whether any deficiencies in plant foods were reflected in trace element concentrations in the blood and eggs of takahe, and, if so, whether they were of biological significance.

5.2 METHODS

5.2.1 Vegetation analysis

Plants eaten by takahe on Mana, Maud and Tiritiri Matangi Islands were identified from observations of feeding birds and examination of piles of grass blades left behind after feeding. Five main food species were identified on Mana and Maud Islands, and four on Tiritiri Matangi (see Appendix 1). One sample of each food species was taken from 3–5 territories on each island between 21 October and 9 November 1996. Samples of three species of tussock and *Celmisia petriei*, which are the main food species of Fiordland takahe (B. Lee, pers. comm.) were collected from the Murchison Mountains on 16 November 1996 (Appendix 1).

Samples were sent to Southern Chemical Consultants Ltd., Invercargill, and the concentrations of 19 trace elements (see Appendices 2–4) were measured using spectrometer analysis. Statistical comparisons using ANOVA were carried out in two different ways: plant species were either combined and compared between the islands and Fiordland or were divided into two broad groups (grasses/ tussocks and non-grasses) and compared between the two sites.

5.2.2 Blood and egg analysis

Levels of trace elements in plants do not necessarily reflect the actual levels in the birds themselves, which could accumulate trace elements over time. Unfortunately, large quantities of blood are required to examine all 19 trace elements analysed in the vegetation analysis. Therefore we concentrated on two trace elements (selenium and manganese) that were found in significantly higher concentrations in the Murchison vegetation samples (see Results) and, more importantly, are known to play an important role in fertility (Hurley &

Keen 1986, Levander 1986). Not all samples could be tested for manganese due to the large volume of blood needed to detect the naturally low concentrations of this trace element.

Blood sampling was done in conjunction with two other projects that required the capture and handling of birds on each of the four offshore islands as well as at Burwood Bush Captive Rearing Unit and in the Murchison Mountains. Sampling was carried out on islands in April 1998 and at Burwood Bush and in the Murchison Mountains in May 1998.

Takahe were captured by hand and hand-nets or by chasing individuals into a fenced-off area, and sampling was carried out in the field at the site of capture. Blood samples were taken from the brachial or inter-tarsal vein using a 23-gauge butterfly syringe. A maximum of 5 ml of blood was collected from each bird with 1–2 ml used in this study and the remainder used for the paternity analysis (Section 3). A full physical examination of the bird, including heart rate and respiratory rate, was carried out following sampling. The bird was then released.

Blood samples were stored as whole blood in heparin or EDTA tubes in a refrigerator until sent to Foodlab South, Invermay, for selenium analysis, or Hill Laboratories, Hamilton, for manganese analysis. Both elements were measured using atomic absorption spectrophotometry.

We were subsequently told that larger quantities of blood from each bird would be needed if we wanted to test for both selenium and manganese in each sample. Therefore as a pilot study we collected and froze the contents of failed eggs from island birds (and one from Burwood Bush) and sent them to Foodlab South for atomic absorption spectrophotometry analysis.

5.3 RESULTS

5.3.1 Vegetation analysis

When all vegetation types were pooled together, the level of all trace elements except silicon and chromium differed significantly between samples from the islands and those from the Murchison Mountains (see Appendix 2). In most cases, concentrations were higher in the islands, except for manganese, molybdenum, selenium and titanium. When vegetation types were analysed separately, similar results emerged; all elements except iron, aluminium and silicon were found to differ significantly, but only manganese and selenium were found in significantly higher concentrations in Fiordland tussocks than in island grasses (Appendix 3). Manganese, molybdenum and titanium were in significantly higher concentrations in *Celmisia petriei* from the Murchisons than in clover from islands, but all other elements except sodium, zinc, chromium, selenium and strontium were significantly higher in clover (Appendix 4).

5.3.2 Blood and egg analysis

Selenium levels were measured for 31 takahe on four islands, at Burwood Bush and in the Murchison Mountains (see Appendix 5). Levels ranged between 65 and 472 µg/kg for all birds and were similar to the range (148–332 µg/kg, n = 9) found

in blood samples taken in 1992 from islands and Burwood (D. Eason, unpubl. data). As no effect of gender on mean selenium levels of island takahe was found ($f = 0.002$, $p > 0.05$, $n = 21$), both sexes were combined for further analysis. As significant differences in mean selenium levels were found between island populations ($f = 3.9$, $p < 0.05$, $n = 22$), islands were compared individually with Burwood and Murchison Mountains populations. Mean selenium levels varied significantly between these populations ($f = 5.0$, $p < 0.01$, $n = 31$), but surprisingly, they were generally higher in island birds (Fig. 6). Highly variable samples from Maud Island resulted in very large confidence intervals, but removing Maud from the analysis did not change the overall results. Mean selenium levels for birds on Tiritiri Matangi were significantly higher than for birds found on Mana, at Burwood and in the Murchison Mountains (Fisher's PLSD, p values range from 0.001 to 0.01). Mean selenium levels on Kapiti were significantly higher than in the Murchison Mountains ($p = 0.02$) (see Fig. 6).

Mean manganese levels were tested in nine takahe from islands and five from the Murchison Mountains (see Appendix 5). Levels ranged between < 15 µg/kg (below detection levels) and 44 µg/kg. No significant differences were found between islands (Fig. 7) so their samples were combined because of small sample sizes. Manganese levels were higher in the Murchison Mountains than on islands, but differences were not statistically significant ($t = 2.0$, $p = 0.07$, $n = 14$).

Detection levels in blood were quite low for manganese and additional samples difficult to obtain, so we made further analyses using contents of infertile takahe eggs (yolk + albumin) collected from the islands. Mean selenium levels (230 µg/kg) were slightly higher than that reported for poultry eggs (161 µg/kg), but mean manganese levels (1.2 µg/g) were much lower than the adequate level reported for poultry eggs (33 µg/g) and fall within the deficiency range. However, takahe eggs from Fiordland have yet to be analysed for comparative purposes.

5.4 DISCUSSION

Takahe plant foods on islands and in the Murchison Mountains show distinct differences in trace element composition. This is not surprising given the differences in soil and vegetation. What is surprising is that only 4 of the 19 trace elements tested were found in higher concentrations in the Murchison Mountains—manganese, selenium, molybdenum and titanium. Most research into the requirements of birds for these minerals has been done on poultry or game birds. Molybdenum and titanium are not considered essential nutrients, but manganese and selenium both have important biological roles (Combs & Combs 1986, Hurley & Keen 1986, Levander 1986). Selenium has a metabolic link to Vitamin E and deficiency can result in reduced egg production and poor embryonic survival (Combs & Combs 1986) and in impaired sperm function (Levander 1986). Manganese deficiencies in breeding birds can reduce egg production and reduce hatchability in incubated eggs. Absorption of these minerals from food is affected by several factors: gastro-intestinal absorption, levels of other minerals and vitamins (e.g. excess dietary calcium can inhibit the absorption of zinc and manganese), the form of the nutrient and the amount of food consumed (Hurley & Keen 1986, Fowler 1996). This makes it difficult to relate trace element levels in vegetation directly to the levels in the actual birds.

Figure 6. Mean concentration (±SE) of blood selenium in takahe on islands, at Burwood Rearing Unit and in the Murchison Mountains.

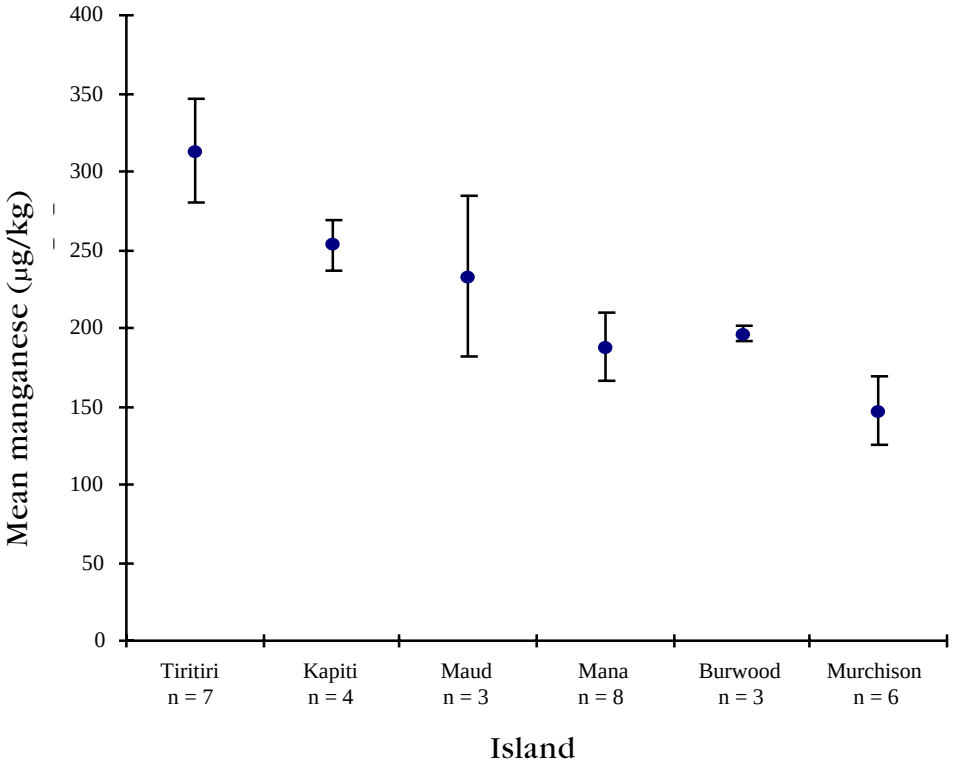
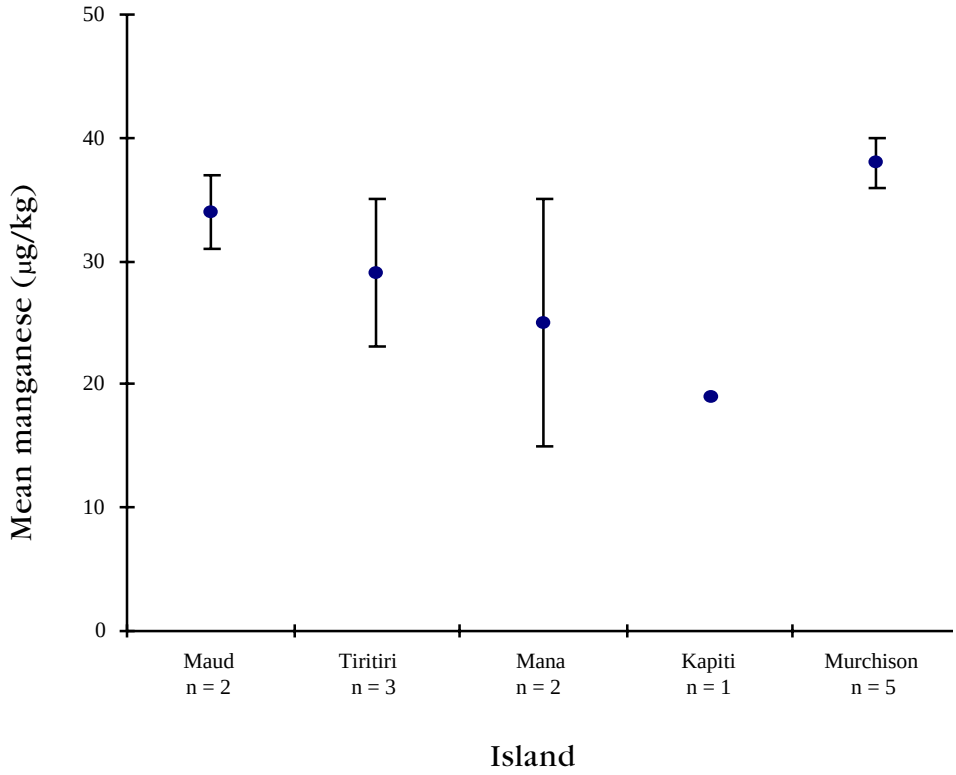


Figure 7. Mean concentration (±SE) of blood manganese in takahe on islands and in the Murchison Mountains.



The levels of selenium found in takahe blood from all locations appear to be at the low end of values reported in the literature on poultry (Combs & Combs 1986, Levander 1986). However, selenium levels from birds from Tiritiri Matangi and Kapiti Islands were significantly higher than in the Murchison Mountains population. This was unexpected given that the vegetation analysis indicated higher levels in the Murchison Mountains. Selenium concentrations were particularly high in birds on Tiritiri Matangi (Fig. 6), which might be a

consequence of takahe regularly receiving poultry pellets from the managers to reduce the frequency of territorial disputes (B. Walter, pers. comm.). Nevertheless, birds on Tiritiri Matangi have similar levels of high egg infertility and low juvenile productivity to takahe on the rest of the islands. It is interesting to note the comparatively low levels of selenium on Mana Island (Fig. 6). This island had drought conditions over the summer prior to sampling and the birds were being fed supplementary food for several months. Supplementary feeding was halted approximately one month before sampling took place. We might have expected selenium levels on Mana to be higher than they were given that the pellets had adequate levels of selenium. However, in a study of chickens, egg selenium levels returned to pre-supplementary levels within eight days of withdrawing supplemented selenium (Levander 1986).

It is also possible that the mean selenium levels in the birds from the Murchison Mountains is lower because samples were mostly taken from juvenile birds. This seems unlikely because the selenium level of the one adult from the Murchison Mountains fell in the middle of the range of juvenile values.

Manganese levels were lower on average in island birds, but not significantly so, although sample sizes were small and variable. Four of five island birds had levels deemed to be in the deficiency range for poultry, the remaining bird falling in the marginal range for poultry; although wild birds may have lower requirements than farmed birds (Hurley & Keen 1986; (B. Hananeia, pers. comm.). All Fiordland birds fell within the marginal range.

Results from testing egg samples indicated that there was enough material present for detecting both selenium and manganese in single eggs, which therefore would be suitable for further comparative analyses. The levels recorded for both trace elements were slightly lower than those recorded in chicken eggs, although deficiency levels for chicken eggs were not reported (Hurley & Keen 1986, Levander 1986).

In conclusion, nutrient deficiencies could be causing poor reproductive success of island takahe but are unlikely for 3 reasons. (1) According to the literature, nutrient deficiencies in wild birds are very uncommon. This coupled with the fact that closely-related pukeko living on the same islands as takahe and feeding on similar plant foods do not suffer from high egg infertility and low hatching success (Section 4), makes a general nutrient deficiency on islands unlikely. However, the specific diet of the two species may be different enough to result in variation in nutrient up-take. (2) It is difficult to explain how a nutrient deficiency would have a strong effect on first clutches but less of an effect for second and third replacement clutches (Section 2), which must be more energetically demanding on breeders. And (3), a one-year supplementary feeding programme in 1994/95 did not significantly improve reproductive success and produced no strong trends. In addition, captive breeders at Burwood Bush are supplementary-fed with a specially modified turkey feed but some also suffer from high levels of egg infertility and low hatching success. Overall, there is a lack of information and expertise on nutritional requirements of wild birds in New Zealand. Experts in the field need to be consulted before further analysis or the possibility of a supplementary feeding experiment is undertaken.

6. Water loss rates and egg morphology [2]

6.1 INTRODUCTION

High rates of egg failure in island populations of takahe, particularly deaths of embryos or chicks at or soon after hatching, may be related to translocated Takahe laying an egg that has evolved under very different environmental and climatic conditions in Fiordland. Although sub-fossil evidence indicates that the takahe were once found throughout New Zealand (Mills et al. 1984), recent analyses of osteometric data suggest that two species of takahe may have existed—a North Island species (*Porphyrio mantelli* (Owen)) and a South Island species (*P. hochstetteri* (Meyer)) (Trewick 1996b). The surviving South Island takahe has presumably been present in an alpine-like environment for many thousands of years, and may not have an eggshell that can cope with nesting at sea level in drier and warmer conditions on the more northern islands. For example, if pore size and density have been selected for the Fiordland environment to Bird eggs normally lose water during incubation and the rate of water loss can affect normal embryonic development and hatching success (Walsberg 1980, Ar & Rahn 1980). The factors influencing the rate of water loss during egg incubation are the conductance of the eggshell to water vapour and the water potential gradient (driving force) between the eggs and its environment (Ar et al. 1974). Eggshell conductance is influenced by pore width, length (or shell thickness) and density, and the diffusion coefficient of water vapour in air.

Size (mass and/or volume) is another variable feature of the avian egg that can potentially affect hatchability and early chick survival (Williams 1994, Carey 1996). Although it has been postulated that larger egg size fosters successful development at higher altitudes (because of lower temperature and barometric pressure), experimental evidence in support of a causal relationship between these factors is lacking (Carey 1996)

The aim of this study was to investigate whether Takahe introduced onto lowland islands might have experienced changes in rates of egg water loss and egg morphology relative to birds in the natural population in Fiordland. Along with daily water / weight loss, we compared eggshell thickness, pore width, pore density and egg size (length × width) between Fiordland and island Takahe, the latter treated as one population due to the small number of birds on each island. A further sample of eggs was obtained for comparative purposes from the Department of Conservation's Takahe captive breeding unit at Burwood Bush. These eggs were from resident Takahe that breed at Burwood Bush Reserve.

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6.2 METHODS

6.2.1 General nesting behaviour

The modal clutch size is two eggs, although clutches of one or three eggs are occasionally found. Eggs are incubated by both parents and nests are left unattended for only short periods of time (Ryan 1997). The incubation period lasts approximately 30 days. Takahe in the Murchison Mountains establish their breeding territories in the alpine zone (1100–1400 m a.s.l.) and build nests at the base of snow tussock. Each nest consists of a small nest bowl lined with tussock tillers. Takahe on islands breed close to sea level and nest in a variety of habitats, but normally under some form of vegetation that conceals the incubating bird from the surrounding area. The nesting season on the islands starts in early September. Failure of first and sometimes second clutches is high, however, and thus birds can lay one or two replacement clutches and continue to nest into January. The nesting season in the Murchison Mountains is shorter, starting in October and finishing in December. Birds in the Murchison Mountains normally lay only one clutch, although replacement clutches can occur when the weather permits.

6.2.2 Estimation of daily water loss

Field data on eggs from island populations were collected from September to January 1997 and 1998. Data for the Murchison Mountains were obtained from Department of Conservation field records. Field records date from 1984 to 1997, but most of the data used to calculate water loss was gathered in 1984 as part of the research programme to set up the artificial incubation unit in Burwood Bush. Measurements recorded in the field were egg length, maximum breadth, weight, developmental stage (determined by candling) and, when known, outcome (hatched or failed). Eggs were removed by hand and were measured with vernier calipers precise to 0.02 mm. Weight was measured to the nearest 0.5 g with Pesola spring balances. Because nearly all mass loss can be attributed to water vapour loss (Drent 1970, Rahn & Ar 1974), the daily rate of mass loss was also referred to as daily rate of water loss (MH_20, in g/day$^-$). Daily rates of mass loss from eggs were determined by marking each egg, weighing it at various intervals throughout incubation, and dividing the difference in weight by the number of days between weighings. Where more than one egg was in a clutch, a mean value was calculated. Visits to Takahe nests on islands normally occurred at least seven days apart. Visits to nests in the Murchison Mountains were less frequent and more sporadic due to the difficulty of access.

6.2.3 Eggshell thickness and pore counts

Samples of eggshells from Takahe eggs from the Murchison Mountains, Burwood Bush and island populations were collected between 1991 and 1998. Shells from hatched eggs were collected at the nest site, whereas shells from failed eggs were obtained after the egg had been collected from nests. Contents of eggs were removed and shells allowed to air dry. Eggshell fragments were taken from random sections of the eggs. Preparation methods were similar to those used by Ar & Rahn (1985). Eggshell fragments were boiled in 5.0% NaOH for 5 minutes to remove proteinaceous fibres from the inner surface of the shell and the cuticle on the outer surface of the shell. Ten measurements of shell

thickness were taken from each fragment of egg using callipers. These measurements were averaged to give a mean shell thickness of each fragment for each egg. After shell thickness measurements were taken, the eggshell fragments were immersed for 30 seconds in concentrated nitric acid to enlarge the pores, and then dipped in distilled water to stop the reaction. After drying, the fragments were placed under a dissecting microscope at 40 × magnification and the pores were counted using a grid square (25 mm^2) in the eyepiece. An average was taken from 30 random counts to give an overall pore density for each egg.

6.2.4 Eggshell description and pore width

Pieces of eggshell fragments from the Murchison Mountains and from the islands were scoured with boiling 5% NaOH and mounted on aluminium stubs using silver conducting paint, sputter coated with gold and examined with a Philips scanning electron microscope (S.E.M) at varying accelerating voltages (15–30 kW) (Board et al. 1977). Fragments were mounted so as to view the outer surface, the inner surface and the cut edge (radial surface) of each egg. Minimum pore width on the inner surface of eggs was measured on the S.E.M for a random sample of fragments.

6.2.5 Size index of eggs

A size index (length × width) was calculated for eggs from the Murchison Mountains and the islands and were compared for three separate breeding seasons (1995/96, 1996/97 and 1997/98). Data from each year were compared separately because some pairs bred over more than one season but individuals could not be identified in the Murchison Mountains population because many were not banded. An average size index was calculated for clutches with more than one egg. Analysis was conducted on first clutch eggs only to remove the confounding affects of decreasing egg size over progressive clutches (Williams 1994). Island female Takahe were further separated into founder (introduced onto islands) versus island-raised for additional analyses.

6.2.6 Statistical analyses

Daily water loss did not differ significantly between consecutive recordings during the incubation period ($t_8 = 0.14$, $p = 0.90$), therefore daily water loss data taken from individual nests were combined and averaged in the analysis. Because most data on daily water loss in eggs from the Murchison Mountains were from 1984 (see above) and because there were no significant differences in daily water loss across years ($f_{5,52} = 0.25$, $p = 0.94$), we used only daily water loss values from 1984 to avoid including measurements taken from the same breeding pairs over consecutive years; these data were compared to daily water loss values for island birds in 1997 and 1998. For island birds only, daily rates of water loss were compared among eggs that hatched, eggs in which embryos had died and infertile eggs using a one-way ANOVA. Differences between years (1997 and 1998) in the rate of daily water loss of individual (banded) pairs on islands was compared using a paired t-test. Differences in daily water loss between eggs from the Murchison Mountains (1984) and from islands (1997 and 1998) were compared using a t-test.

Differences in pore counts and shell thickness were analysed using a General Linear Model (GLM) with site (Murchison Mountains, islands, Burwood Bush) and outcome (hatched versus failed) as fixed and random factors respectively. Measurements of pore width, which were carried out on a different sample of eggs to that of the pore count and shell thickness data set, were taken from shell fragments collected from Murchison Mountain and island nests only. Therefore differences in pore width between these two sites were analysed separately using a t-test.

Egg sizes were compared between Murchison Mountains and island pairs for each year using t-tests; again, years were analysed separately to avoid including data from the same individual pairs laying over consecutive years. To determine whether egg size had an effect on hatching success in island pairs, sizes of fertile eggs that hatched and those that failed were compared using a t-test. Further analysis examined differences in egg size over years between founder females and island-raised females using a GLM with year as a class variable, female (founder versus island-raised) as a fixed factor and number of previous breeding attempts as a co-variate.

Size data were transformed using Y' = log (Y + 1), as suggested by Zar (1996). All values reported in the results are means ± S.E.

6.3 RESULTS

For island Takahe eggs, there were no significant differences in daily water loss among fertile eggs that hatched, fertile eggs that did not hatch and infertile eggs in 1997 ($f_{2,17}$ = 0.46, p= 0.64) or 1998 ($f_{2,7}$ = 0.07, p = 0.94), therefore all eggs were combined to increase sample sizes. Furthermore, daily water loss did not differ significantly between years for individual pairs (t_8 = 0.67, p = 0.55). Overall, there was no significant difference in daily water loss (MH_20, in g day^{-1}) between the Murchison Mountain estimates (0.553 ± 0.026, n = 26) and island estimates for either 1997 (0.509 ± 0.027, n = 20) (t_{34} = 0.96, p = 0.35) or 1998 (0.580 ± 0.027, n = 10) (t_{24} = 0.73, p = 0.47).

TABLE 3. MEAN ± S.E. PORE COUNTS AND SHELL THICKNESSES FOR HATCHED EGGS, FAILED EGGS, AND HATCHED AND FAILED EGGS COMBINED OF TAKAHE NESTING IN THE MURCHISON MOUNTAINS, ISLANDS AND BURWOOD BUSH CAPTIVE BREEDING UNIT (BRACKETS INDICATE SAMPLE SIZES).

EGG FEATURE	MURCHISON MTS	ISLANDS	BURWOOD BUSH
Pore counts (per 25 mm^2)			
Hatched	16.3 ± 0.2 (10)	16.9 ± 0.2 (9)	17.5 ± 0.1 (6)
Failed	17.1 ± 0.2 (10)	16.6 ± 0.2 (20)	16.3 ± 0.3 (8)
Combined	16.7 ± 0.2 (20)	16.7 ± 0.2 (29)	16.8 ± 0.2 (14)
Shell thickness (mm)			
Hatched	0.336 ± 0.008 (9)	0.343 ± 0.005 (10)	0.341 ± 0.004 (6)
Failed	0.340 ± 0.004 (22)	0.348 ± 0.006 (20)	0.361 ± 0.014 (8)
Combined	0.339 ± 0.003 (31)	0.346 ± 0.004 (30)	0.353 ± 0.009 (14)

Pore counts did not differ significantly among sites (Murchison Mountains, islands and Burwood Bush) ($f_{2,2}$ = 0.01, p = 0.99) nor between hatched and failed eggs ($f_{1,57}$ = 1.04, p = 0.31) (Table 3). There was a significant interaction between site and hatching success ($f_{2,57}$ = 7.86, p = 0.001), with failed eggs having a slightly higher mean pore count than hatched eggs for the Murchison Mountains, but just the opposite pattern for the other two sites. However, the maximum difference between any site and egg category was only one pore per 25 mm^2 of shell, and the combined totals of hatched and failed eggs were very similar among all three sites (Table 3). Shell thickness also did not differ significantly among sites ($f_{2,2}$ = 4.53, p = 0.18) nor between hatched and failed eggs ($f_{1,68}$ = 1.40, p= 0.24), with no significant interaction effect ($f_{2,68}$ = 0.22, p = 0.80) (Table 3).

Takahe eggshells have pores that are unbranched and have occluded outer orifices (Board et al. 1977). There were no obvious differences in eggshell structure between eggs from the Murchison Mountains and islands as judged from the SEM scans. Furthermore, there was no significant difference in the width of pores as measured from the scans for Murchison Mountain (23.2 ± 0.7 µm, n = 10) and island (23.9 ± 0.7 µm, n = 10) eggs (t_{17} = 0.63, p = 0.53).

For each year compared and for both types of egg categories, mean egg size was smaller on islands than in the Murchison Mountains, although the differences were only significant in the 1996/97 breeding season for all eggs combined (hatched and failed) (Table 4). Given that a greater proportion of island eggs fail to hatch (relative to Fiordland eggs) and island eggs tended to be smaller, we first examined whether fertile eggs that failed were smaller than those that hatched from first clutches of island birds for each of the years examined. However, there were no significant differences between the two groups of eggs for the 1995/96 (t_9 = 0.56, p = 0.59), 1996/97 (t_3 = 0.37, p = 0.74) or 1997/98 (t_{10} = –0.03, p = 0.98) breeding seasons.

We then examined whether females that had been raised on islands and subsequently bred had smaller eggs than females that were originally introduced onto the islands from the Fiordland population. However, we found the opposite

TABLE 4. MEAN ± S.E. SIZE INDEX OF HATCHED EGGS, AND HATCHED AND FAILED EGGS COMBINED FROM FIRST CLUTCHES OF TAKAHE NESTING IN THE MURCHISON MOUNTAINS AND ISLAND POPULATIONS FOR THREE DIFFERENT BREEDING SEASONS.

BREEDING SEASON	SITES	HATCHED EGGS			HATCHED AND FAILED EGGS COMBINED		
		SIZE INDEX (mm^2)	N	STATISTICS	SIZE INDEX (mm^2)	N	STATISTIC
1995/96	Murchison Mts	3508 ± 55	13	t_8 = 0.63	3586 ± 32	26	t_{32} = 1.76
	Islands	3439 ± 96	6	P = 0.55	3485 ± 47	18	P = 0.09
1996/97	Murchison Mts	3624 ± 52	16	t_6 = 2.16	3588 ± 41	25	t_{22} = 2.60
	Islands	3389 ± 96	5	P = 0.07	3415 ±52	11	P = 0.02
1997/98	Murchison Mts	3534 ±167	5	t_4 = 0.29	3590 ±61	15	t_{23} = 1.65
	Islands	3484 ± 46	7	P = 0.79	3471 ± 38	17	P = 0.11

pattern. Eggs from founder females (averaged across years, 3346.4 ± 49.2 mm^2, n = 57) were significantly smaller than those of island-raised females (3533.4 ± 50.9 mm^2, n = 34) ($f_{1,44}$ = 5.34, p = 0.03) with both groups of females showing a significant decline in mean egg size over time ($f_{7,44}$ = 4.06, p = 0.001), and at a similar rate of change ($f_{5,44}$ = 0.30, p = 0.92). This comparison only goes back to 1992 when there were sufficient numbers of island-raised females to compare with founder females, some of which had been breeding since 1986. We therefore decided to control for any differences between the two groups of island females in the number of previous breeding attempts (i.e. number of clutches laid). When the number of previous breeding attempts by each female was included in the analysis, the significant difference in mean egg size between founder and island-raised females disappeared ($f_{1,44}$ = 0.02, p = 0.88). Thus in both groups, egg size declined with number of breeding attempts.

Unfortunately, similar data from wild Fiordland females are not available, although the number of breeding attempts per female per season and thus the total number of breeding attempts over a female's lifetime would be much fewer (J. Maxwell, pers. comm.). When the average egg size of a sample of Fiordland females (3547.4 ± 27.8 mm^2, n = 22) was compared with that of a sample of island females taken from 1992 to 1995, and for which individual females had only laid two to six clutches (3556.2 ± 55.0 mm^2, n = 16), no significant difference in egg size was found between the two groups (t_{22} = –0.14, p = 0.89).

6.4 DISCUSSION

We hypothesised that the high rates of embryo and early-chick mortality might be associated with departures from 'normal' rates of water loss, i.e. those found in eggs from nests in the natural population in the mountainous region of Fiordland. However, the mean rate of daily water loss for takahe eggs in the Murchison Mountains was not significantly different from that of eggs from island populations. Furthermore, pore density, width and shell thickness—features known to affect the rate of water loss in eggs, did not differ significantly between sites.

Given that there was no difference in water loss between the two populations, and no differences in pore density, width and shell thickness, it would appear that any differences between the sites are either not great enough to affect water loss or the immediate nest environment is being moderated by the incubating bird. Regulation of egg water loss during incubation could consist solely of a female producing an eggshell with a porosity such that shell conductance is appropriate for the normal nest characteristics of the individual or species and the normal pattern of the parents' nest attentiveness (Walsberg 1980). When averaged over the incubation period, changes in ambient humidity or variations in parental physiology or behaviour that affect nest humidity are unlikely to drive egg water loss to deleterious levels. This conclusion is further supported by a study in which chickens hatched at sea level and transported to 2800 m a.s.l., produced eggs which had similar levels of water loss to eggs of control birds at sea level (Leon-Velarde et al. 1984). Since considerable variation in water loss exists in wild populations of birds at any altitude (Carey et al. 1983), it is likely that movement and successful breeding of a population up or down an altitudinal gradient is not necessarily limited by shell structure.

Daily water loss, eggshell porosity and thickness were also not significantly different between hatched and failed takahe eggs. This may be because a substantial number of failed eggs are actually infertile and infertile eggs may have normal egg morphology and physiology. For example, Campo & Ruano (1995) found that fertile eggs that hatched had significantly smaller weight loss than those that did not hatch, but weight loss of infertile eggs did not differ from that of fertile hatched eggs. For island birds, there was no significant difference in water loss among fertile eggs that hatched, fertile eggs that failed to hatch, and infertile eggs.

One difference that was evident between the natural alpine population and translocated island populations was egg size. For the last three years of the study, eggs from island Takahe were consistently smaller than eggs from Murchison Mountain birds, although only significantly so for the 1996/97 season. Up until 1995, average egg size of island females was similar to that of Fiordland females, but since then egg size for island females has got progressively smaller. We know that, on average, island Takahe produce a higher number of eggs per female per season (3.40 ± 1.50) than Murchison Mountain females (1.90 ± 0.50), due to the higher rate of clutch failure (Bunin et al. 1997). We therefore speculate that the smaller egg size is related to the increased production of eggs of individual females as their number of breeding attempts increase. Egg size in many species of birds increases with age to a certain point and then declines, possibly due to senescence and its accompanying effects of deterioration of physiological capacities (Saether 1990). With respect to this hypothesis, a larger proportion of island females may be reaching senescence earlier than their same age counterparts in the Murchison Mountains, resulting in the production of smaller eggs. However, two crucial points need to be kept in mind; (1) the reduction in egg size appears to be a consequence of a high rate of clutch failure not the cause of it, and (2) we found no significant difference in size between hatched and failed eggs for island females. Reproductive senescence can affect egg production in other ways such as delaying onset of laying, reducing clutch size and inhibiting re-nesting (Wiebe & Martin 1994), but analysis of these factors is beyond the scope of the present study.

In summary, we found no evidence that the eggs of takahe living on lowland temperate islands show deviations in their rates of daily water loss or other egg morphological features that would have been the result of translocating birds to an environment with climatic conditions substantially different from the source population.

7. Incubation behaviour

7.1 INTRODUCTION

Developing embryos can cope with a range of temperature and humidity, but extremes of either may affect the viability of the embryo (Romanoff 1949). Temperature and humidity are both affected by the behaviour of takahe while they are incubating their eggs. This study aimed to monitor takahe behaviour during incubation and record the humidity in the nest in order to determine whether egg failure is related to these factors.

7.2 METHODS

An infra-red sensitive camera was hidden in vegetation 50 cm from each monitored nest and connected by a 5 m long cable to a battery-powered time-lapse video recorder. An Orion Tiny Logger for measuring relative humidity was enclosed within a painted plaster dummy egg with holes drilled in it for air to flow. The system was left at the nest for a minimum of 48 h.

Video tapes were analysed for turning frequency, changeover times and time eggs were left exposed. The correlation of turning events with changes in humidity levels was assessed.

7.3 RESULTS

Five nests were monitored using the video set-up for one 48–60 hour period at various stages of the incubation period. Due to the limited time available, the time taken to recharge batteries and the timing of each takahe pair's nesting, no more than one video recording during the same incubation period was able to be carried out. Behavioural characteristics of each pair are summarised in Table 5, but no obvious differences or unusual patterns were evident. Males and females generally spent similar times incubating; males at night and females during the day. Eggs were turned approximately half hourly on average. Eggs were left exposed for approximately 2 minutes, on average, but the maximum time they were left uncovered ranged from 3 to 12 minutes.

Relative humidity was recorded in four of the five nests listed in Table 5. In addition, relative humidity was also recorded in Lucky and Tilly's nest from when the chick was through the air cell and vocalising but had not yet pipped, until it hatched. The data logger was found to be inaccurate but precise and the fluctuations recorded appeared to reflect the situation in the nest. Each time a bird left the nest, the relative humidity dropped by an average of 6.5% over the four nests (range 5.3–7.1%). Turning events were often reflected in a drop in relative humidity, but these changes were generally too small and for too short a duration to be considered significant.

Hatching success in the five monitored nests was high, with only one infertile egg and five chicks hatched from seven fertile eggs.

7.4 DISCUSSION

Relative humidity changes inversely with temperature, thus the changes in relative humidity seen in the nest reflect temperature change also. Some of this change would be due to changes in the ambient temperature, but most can be attributed to the behaviour of the birds while on the nest. The most dramatic changes in relative humidity were seen when birds changed over incubation with their partner, although these were usually fairly brief. Although the surface temperature of the egg may change a little when exposed for a few minutes, the internal temperature of the egg is not likely to be affected until about 30 minutes have passed (D. Eason, pers. comm.). The hatching success of the monitored nests was high (71%), and presumably this reflects the normal behaviour observed. It is possible that some aberrant behaviour still exists in the population and it is not known how consistent the behaviour of a pair is over time.

TABLE 5. SUMMARY OF TIME BUDGET OF INCUBATING TAKAHE PAIRS DETERMINED FROM TIME-LAPSE VIDEO RECOEDING OVER AN APPROXIMATELY 48-HOUR PERIOD.

BREEDING PAIR	AVERAGE NUMBER OF TURNING EVENTS PER HOUR (RANGE)	CHANGEOVER TIMES		AVERAGE TIME EGGS LEFT UNCOVERED (RANGE)
		MORNING	EVENING	
Ernie and Terri	1.7 (0–4)	5:03, 5:27	5:39	1 min 56 s (31 s–5 min 21 s)
Lucky, Toni and Tilly	1.8 (0–4)	5.29	6.27	2 min 59 s (49 s–7 min 19 s)
Tuarua and Toro	2.1 (0–5)	6:42	6:04, 6:39	3 min 23 s (1 min 06 s–12 min 23 s)
Selwyn and Vicki	2.3 (1–5)	5:43, 6:22	8:08, 8:58	1 min 15 s (29 s–3 min 47 s)
Snow and Mataku	2.4 (0–5)	5:47, 6:18*	6:05, 6:27*	1 min 33 s (16 s–9 min 18 s)
Mean	2.1 (every 29 min)	5:54	6:41	2 min 13 s

* Snow also incubated for about an hour during the middle of the day on both days that the video was recording.

8. Sperm collection techniques

8.1 INTRODUCTION

One of the possible causes of infertility in birds is low quality or quantity of sperm. Collecting semen from males identified as either 'high fertility' or 'low fertility' and examining it for characteristics such as sperm counts, ratio of live to dead sperm and abnormal sperm could give us an indication of whether males are responsible for high rates of egg infertility in island populations. The objective of this study was to collect and analyse semen from male takahe in order to assess the role of males in infertility.

8.2 METHODS

Three methods were proposed to obtain semen from male takahe: 1) human-imprinted males would be masturbated by hand; 2) a model papier maché takahe in a receptive posture would be presented to takahe that were showing signs of breeding activity; 3) takahe would be captured and restrained and their cloaca protuberance massaged to express semen. Capture would be by hand or using a net fence, and birds would have a dark bag over their head to minimise stress. None of these methods had been attempted on takahe before, thus methodology was developed as the work progressed. However, it was subsequently decided that Method 3 would not be pursued because handling the birds might disrupt their breeding attempts. In addition, it was felt that cloaca massage was a technique requiring more experience and expertise than we had at the time. A related method using electro-ejaculation is a very invasive technique involving complete anaesthetising of the individual bird and using an electrical probe in its cloaca to induce ejaculation (Samour 1986). This is likely to be quite traumatic for both birds and handlers and is not recommended (K. Rose, pers. comm.).

8.3 RESULTS

Method 1 could only be used with takahe that were human-imprinted and thus had limited potential. It was attempted with several takahe on islands (Mr Blue, Tussock, Greg) and one at Burwood (Green). The only success was achieved with Mr Blue, who willingly copulated on a hand. Analysis of his semen failed to show the presence of any sperm and given his past breeding record (no fertile eggs) he is probably sterile.

For Method 2 a steel-framed papier maché model was constructed, painted and introduced to males on Tiritiri Matangi (n = 5), Mana (n = 3) and at Burwood (n = 1). A typical reaction to the model was initially either wary curiosity or an unwillingness to approach it. Once the model had been left in place for several minutes, most birds ignored it. One unpaired male (Alec) was shown the model, but his reaction was limited curiosity at best.

A protocol for collection of sperm was developed (see Appendix 6) in consultation with Dr Patrick Casey, National Women's Hospital, Auckland.

8.4 DISCUSSION

Although the hand-reared birds used in Method 1 were very friendly towards humans, easy to approach and could be touched without showing fear, only one ejaculated after hand manipulation.

Models have been used successfully with bird species overseas, although usually with domesticated or hand-reared birds (Saint Jalme et al. 1994, Birkhead & Petrie 1995). Method 2 could be pursued further with a more realistic model (such as a taxidermy mount of a female takahe in a receptive posture), but two aspects of takahe behaviour suggest that any type of model might have limited success. First, takahe are highly territorial during the breeding season and second, they tend to form strong pair-bonds involving extended contact and courtship behaviours. It therefore seems unlikely that a strange 'bird' introduced briefly to a male in an already established pair would elicit any response except aggression. Although most takahe were not overtly aggressive, their reactions did not indicate any willingness to accept the model to a degree that would elicit copulation behaviour. However, a more realistic model may be the key and a pilot study testing a realistic stuffed bird could be conducted at Burwood Bush.

9. Detecting sperm on the perivitelline membranes of eggs [3]

9.1 INTRODUCTION

The ovum of birds is covered by the inner perivitelline membrane after its release from the ovary. Within minutes of fertilisation, a second layer, called the outer perivitelline membrane, is laid down on the ovum, trapping any spermatozoa present between the two membranes. The outer perivitelline membrane is thought to act as a barrier to excessive polyspermy and to retain the integrity of the ovum already penetrated by some spermatozoa (Bakst & Howarth 1977, Howarth 1984). The number of spermatozoa trapped on the outer perivitelline layer reflects the numbers of spermatozoa inseminated and present near the site of fertilisation (Wishart 1987, Birkhead et al. 1994).

A method whereby the sperm nuclei on the perivitelline membrane are stained with a fluorescent dye and counted under a photomicroscope has been used in poultry to show a relationship between the numbers of sperm and whether or not the egg was successfully fertilised (Wishart 1987). The usefulness of this method has also been highlighted in studies of sperm competition in birds (Birkhead et al. 1994, Birkhead 1996). Applying it to takahe eggs laid on islands could provide an explanation for the high rate of infertility. If successful, the method could also be used more widely as a non-invasive technique for assessing possible causes of low hatching success in other endangered species.

Counting sperm on the perivitelline membrane has only been used on relatively fresh unincubated eggs up to four days old (Birkhead et al. 1994, Wishart 1987). Takahe start to incubate their eggs soon after laying but managers do not normally candle eggs until after 10 days of incubation when normal development can be easily assessed, although lack of development of an embryo can be detected as early as six days (C. Ryan, unpublished data). If the perivitelline membrane of takahe eggs that had been incubated for several days was examined, it would not be clear whether a lack of sperm was due to few sperm reaching the tract or to sperm breaking down and deteriorating over time while the egg is warmed during incubation. Therefore, our immediate objective was to determine whether sperm are still present on the perivitelline membrane of eggs that had been incubated for at least seven days. We examined this problem by comparing sperm counts on the membrane of fresh versus incubated eggs using artificially inseminated domestic Turkey *Meleagris gallopavo.* We used turkey eggs because they were approximately the same size as takahe eggs and were readily available from two nearby hatcheries.

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9.2 METHODS

The first batch of eggs was obtained from Cust Turkey Hatchery, Christchurch, in late October 1997. Eggs were either fresh or had been artificially incubated for seven days. We obtained additional eggs from Crozier's Turkey Hatchery, Ashburton. These eggs were collected fresh in late January 1998 and returned to the University of Otago, where they were either examined fresh or artificially incubated under conditions similar to the Cust eggs (37.5°C and 80–90% humidity) in Rotarex incubators for seven days before being examined. We chose to incubate the eggs for seven days because this was close to the minimum period for which the development status of takahe embryos could be assessed.

Eggs were opened with scissors and the yolk and albumen separated. Each egg was categorised as fertile or infertile by the appearance of the germinal disk (Kosin 1945). Eggs were categorised into four different groups: (1) fresh—fertile, (2) fresh—infertile, (3) incubated—fertile, and (4) incubated—infertile. A 2–3 cm square was cut from around the germinal disk area and the perivitelline membrane was removed. The membrane was placed in a petri dish and rinsed in phosphate-buffered saline (PBS) to remove adherent yolk and albumen, and then placed on a glass microscope slide. The preparation was then stained at room temperature by adding two drops of 1 µg/ml solution of 4',6-diamidino-2-phenylindole (DAPI) (Sigma chemical Co., St Louis, U.S.A.) in PBS (Wishart 1987). A coverslip was placed over the entire piece of membrane, excess liquid was removed, and the preparation was stored in the freezer until required.

Slides were removed from the freezer and allowed to thaw. To estimate the numbers of sperm embedded in the membrane on the slide, a method originally developed by Wishart (1987) and modified by Birkhead et al. (1994) was utilised. Two parallel lines were drawn longitudinally 10 mm apart on the coverslip above the membrane with an indelible marker. The blue fluorescing, comma-shaped sperm nuclei were viewed with a Zeiss D-7082 reflected light fluorescence photomicroscope. Sperm were counted at 20–40 times magnification using the method of Wishart (1987). A defined area of membrane was assessed for the presence of spermatozoa by scanning perpendicularly between the two marker lines, focusing up and down to bring into view spermatozoa at different depths in the membrane. This gave a rectangular area of 8.05 mm^2. At least four scans were completed on all samples, and an average number of sperm was calculated for the area.

Membranes from eggs that were incubated were generally difficult to remove intact, and for one of the treatments (Crozier's Farm—incubated fertile eggs) no suitable samples were obtained for analysis. Therefore, we analysed sperm counts for fresh versus incubated eggs separately. Sperm counts with respect to fertility status for incubated eggs were not analysed statistically as it was clear they had no detectable sperm (see Results). Sperm counts for fresh eggs were analysed using a 2-factor (mixed model) General Linear Model with farm and fertility as fixed and random factors respectively. The data were transformed using $Y' = \log (Y + 1)$, as suggested by Zar (1996).

9.3 RESULTS

For fresh eggs, the differences in sperm counts between fertile and infertile eggs were highly significant ($f_{1,51} = 20.7$, $p < 0.001$). Fresh fertile eggs from both farms had more sperm than fresh infertile eggs (Table 6). There was no significant effect of farm ($f_{1,1} = 10.8$, $p > 0.19$), but the interaction between farm and fertility approached significance ($f_{1,51} = 3.4$, $p = 0.07$). The fresh fertile eggs from Cust hatchery had a much higher mean number of sperm than other groups of fresh eggs. By contrast, no sperm at all were seen on either fertile or infertile eggs that were incubated (Table 6), suggesting that sperm has broken down during the incubation stage.

9.4 DISCUSSION

The technique to detect sperm on the perivitelline membrane in eggs appears to work well for fresh unincubated turkey eggs. Although the differences were not significant, eggs from Cust's Hatchery tended to have greater numbers of sperm than those from Crozier's hatchery. This difference might be due to seminal fluid being diluted twice as much for artificial inseminations at Crozier's than at Cust's Hatchery (S. Shaw & P. Crozier, pers. comm.).

Fresh infertile eggs from both farms had fewer numbers of sperm on the perivitelline membranes than fresh fertile eggs, consistent with Wishart's (1987) finding with chickens. For incubated eggs, however, no sperm were detected in either infertile or fertile eggs, suggesting incubation over a period of time may affect the viability of sperm on the perivitelline membrane. This factor is important for assessing infertility in takahe and other species where managers remove failed (non-developing) eggs from the nest after a short period of incubation. Moreover, it appears that this technique of assessing fertility would not work, at least for takahe, due to the degradation of sperm after the eggs had been warmed for a period of time. We did examine several takahe eggs that had been incubated for 10 or more days but the perivitelline membranes were no longer intact. Takahe eggs could be candled at an earlier stage, but assessing whether or not the embryo is developing is extremely difficult before seven days of age and thus the risk of removing a fertile egg by mistake increases. This means we would need to remove a freshly laid egg from several pairs to assess whether egg failures were correlated with low sperm counts in sampled males.

TABLE 6. MEAN NUMBER (±S.E.) OF SPERM PER UNIT AREA (8.05 mm^2) ON THE PERIVITELLINE MEMBRANE OF TURKEY EGGS FROM TWO FARMS AND WHICH VARIED IN INCUBATION AND FERTILITY STATUS.

	CROZIER'S FARM	CUST FARM
Fresh infertile	0.2 ± 0.8 (n = 19)	2.1 ± 1.5 (n = 4)
Fresh fertile	2.3 ± 0.8 (n = 21)	30.5 ± 9.5 (n = 10)
Incubated infertile	0 ± 0 (n = 15)	0 ± 0 (n = 2)
Incubated fertile	*	0 ± 0 (n = 4)

* Not suitable for analysis because the membrane was not intact.

10. Improving the quality of reproductive data

10.1 INTRODUCTION

Past reporting of breeding outcomes of takahe by managers on islands has included large proportions of eggs of unknown fertility. These normally result from infertile or non-developing eggs becoming addled after prolonged incubation by birds, which makes true rates of infertility versus embryo death difficult to determine. In pinpointing the causes of low productivity in island takahe, it is important to distinguish between these two aspects of egg failure as their causes are potentially different. For example, infertility may be caused by genetic factors while embryo deaths may be caused by environmental conditions during incubation. It is also important to ensure that as much information as possible is gathered from failed eggs and chicks and that information collected is consistent between islands. Therefore, our objective was to identify ways in which the accuracy and amount of information gathered from failed eggs and chicks could be improved and to develop standard protocols to be used when dealing with eggs, embryos and chicks.

10.2 METHODS

There were three areas addressed over a three month period during the island breeding season: 1) training staff in candling techniques to reduce the number of eggs of unknown fertility, 2) improving existing resources on islands, and 3) reviewing the current situation (including 1 and 2 above) to determine where further improvements can be made.

Prior to the breeding season, time was spent at Burwood Captive Rearing Unit candling turkey eggs that were being incubated. This allowed us to become familiar with candling techniques and the normal developmental stages of eggs. In addition, a technique was developed using chicken eggs to determine whether failed eggs were infertile or fertile by examining the germinal disc area where the embryo begins to develop (see Appendix 7). Mana, Maud and Tiritiri Matangi Islands were visited as the first clutches of the season were laid, and eggs were candled with management staff. Kapiti Island was excluded due to time constraints and the small number of pairs currently living there. During this time we attempted to ensure that procedures being used were consistent between islands and also that any techniques we had learned over and above what staff already knew were passed on. In addition, the technique for determining fertility of failed eggs was demonstrated to staff.

To improve resources for island staff, we provided them with an account of the development of an embryo accompanied by diagrams of the developing egg every 2–3 days (like those used at Burwood). Prior to this, island staff only had photos taken at three stages during incubation. In addition, pukeko nests were located and eggs candled at various stages through development to assess whether they were suitable to use as a candling model for takahe development.

During visits to each island a review was undertaken of all aspects of collecting the information required to assist us in determining where productivity problems lie and ensuring that this information continues to be collected in the future. This included requirements of staff on islands, the resources island staff need to assist them in collecting quality information, and assistance needed from other organisations. In addition, an afternoon was spent with Professor Peter Stockdale (Veterinary Pathology, Massey University) dissecting chicks and getting advice on other aspects of low takahe productivity.

10.3 RESULTS

Our initial inquiries indicated that many island staff were not confident in their candling skills and some had misconceptions about developing eggs that resulted in incorrect assessments of fertility status. When island eggs were subsequently candled with the assistance of one of us (CR) who had gone through the intensive training programme at Burwood Bush, the proportion of addled eggs dropped dramatically from an average of 32% in the five previous seasons to 7% in 1996 and 0% in 1997 (Table 7). The vast majority of failed eggs were infertile (Section 2).

We also wanted to know whether the eggs of the closely related pukeko could serve as a suitable model of the various stages of development in the takahe. Five pukeko nests were located during two fortnight stays on Mana Island. Eggs were candled at several stages during development and compared with takahe eggs at similar stages. Although pukeko eggs followed the same general pattern during development as takahe eggs (and other bird eggs) the shape of the air-cell became much more slanted than in takahe eggs as development progressed. In addition, the candled image (i.e. light and dark patches in the egg) appeared to be somewhat different from takahe eggs. In many cases the shells were more darkly pigmented than takahe eggs, making candling more difficult.

10.4 DISCUSSION

When high rates of egg failure become a problem in a breeding population, it is good management practice to remove a non-developing egg / embryo as soon as possible so that it can be opened and examined to assess whether the egg was

YEAR	ADDLED EGGS (%)	N
1997	0	40
1996	7	46
1995	28	50
1994	54	46
1993	43	21
1992	11	19
1991	22	18

TABLE 7. PROPORTION OF EGGS OF UNKNOWN FERTILITY ON MANA, MAUD AND TIRITIRI MATANGI ISLANDS 1991–1997.

fertilised or whether the embryo died at an earlier stage of development. However, making the decision to remove an egg of an endangered species entails a large amount of responsibility, given that the fate of individual eggs has a relatively large impact on the population as a whole. It also requires a number of skills, confidence in those skills, and good resources to draw on for assistance. Island staff candle only a relatively small number of eggs each year, at only two or three stages during incubation. They have little background knowledge of the processes occurring during development of an egg and currently have few resources to draw on. These factors all contribute to a limited knowledge and lack of confidence regarding decision-making on the developmental status of takahe eggs.

Although some improvements were made during the course of this research contract in terms of staff ability and resources, there are additional steps that could be taken to improve the situation further. Protocols have been developed for various procedures to ensure that information is collected in a consistent way between islands (see Appendix 7)—these should be reviewed by the Takahe Recovery Group (TRG) and distributed to islands. Some procedures (e.g. collecting shells, egg contents, embryos, etc.) will obviously only need to be followed where that particular information is required.

Standard procedures will help to improve the present situation. However, the importance of understanding the principles behind the various techniques used in determining the status and age of a takahe egg (such as candling, age calculations, size of latrine) cannot, in our opinion, be understated. It is in this area that we feel staff would benefit from further training. This would be best achieved through a workshop where staff can have hands-on practice of techniques with eggs from a non-endangered species and have the opportunity to gain background information where they feel it is lacking. This kind of workshop would benefit not only island staff, but staff who are involved with candling takahe eggs in the Murchison Mountains and at Burwood. An opportunity already exists when staff gather to attend Takahe Recovery Group meetings—an extra day or half-day yearly devoted to a practical workshop would give new staff an opportunity to learn the basics and existing staff an opportunity to refresh their knowledge and learn about new techniques or developments that may have taken place since the last workshop.

Resources on islands could be further improved to assist staff in interpreting what they see during candling. For example, although they have pictures and some photographs of correctly developing eggs, there is little to help them recognise an incorrectly developing egg. We believe a more extensive photographic library of candled images of both correctly developing and failed eggs would be a valuable resource for all staff involved with the Takahe Programme. The Burwood Captive Rearing Unit is the obvious place to develop this resource base.

Discussion with Professor Stockdale of Massey University's Department of Veterinary Pathology highlighted a number of issues regarding specimens sent to him for analysis. First, the quality of analysis that can be provided depends on how well the specimen is preserved in the first place. At present, specimens are preserved whole in formalin. This is successful in preserving outer layers of a chick-sized specimen, but unless some dissection is carried out, the inner organs will not be preserved. Most island staff were not familiar with

techniques for dissecting specimens. In addition, the longer a specimen is left, the less it will be preserved. A workshop such as the one proposed above would be an ideal opportunity to pass on the skills necessary to prepare a specimen for preservation.

Second, some causes of chick death—such as those caused by bacteria—cannot be determined once a specimen has been preserved in formalin,. It may be necessary to get specific advice regarding taking bacterial swabs on dead chicks before preserving them so that causes of death such as yolk-sac infections can be assessed.

Third, there are limits to what vets can tell us with some specimens, particularly when they die before hatching. For example, of a sample sent in from one of the islands of a squashed egg with a well-developed embryo inside, Professor Stockdale could tell no more than what was obvious—it had been squashed by something. Again, it may be necessary to get specific advice from a vet regarding their limitations with respect to analysis of specimens.

Fourth, Professor Stockdale reiterated that eggs must be handled extremely delicately while candling as any undue shaking or excessive rotation can cause embryo displacement or death. Of course, his comments are common sense but we did notice varying degrees of care with which island staff handled eggs. In addition, we suggest eggs that are removed from under an incubating bird for candling, should be kept warm with a wool hat or jumper or by placing it in a box with a covered hot water bottle until candling is complete and the egg is replaced back into the nest. The risk of yolk-sac infections in eggs (which in turn cause deaths of very young chicks) could be minimised by staff wearing sterile surgical gloves during candling to prevent bacteria from being transmitted to eggs.

Professor Stockdale's opinion on the incidence of high infertility and a number of broken and squashed eggs was that both mating and incubation are very sensitive stages for birds in general. A certain level of intermittent disturbance is necessary for monitoring birds and to give sufficient information on fertility rates and hatching success but nest checks during incubation should be minimised. We agree with this comment and add that researchers and managers need to consult closely to identify pairs that tend to be aggressive or are easily scared off while on the nest so that caution can be taken when approaching these nests. Furthermore, we suggest that areas where pairs nest close to a track should be closed off to the public or sign-posted until eggs have hatched.

11. Takahe bibliography

A takahe bibliography was compiled; it currently contains 240 entries and consists of any literature directly or indirectly related to takahe. The bibliography is to be updated annually and copies can be obtained by contacting Ian Jamieson, the Department of Zoology, University of Otago.

12. Overview and recommendations

12.1 OVERVIEW

A comparative analysis indicated that takahe translocated to four offshore islands and kakapo translocated to Little Barrier Island are unusual among New Zealand endangered birds in showing high levels of egg infertility (Section 2). Further analysis within islands failed to identify any associated factors (relatedness, age, origin of breeders) except that fertility seemed to improve with the laying of second clutches (Section 2). Most pairs on most islands in most years appeared to be affected and the situation did not appear to be getting better. Relatively high egg fertility in pukeko living on the same islands suggests that toxins are unlikely to be causing a problem (Section 4).

There are a number of other possibilities that could explain these patterns. First, takahe in general could be highly inbred and thus high egg infertility rates and low juvenile productivity could be a result of inbreeding depression induced by a change in their environment upon being transferred from alpine habitat of Fiordland to more northerly lowland islands (Section 2). Although island takahe appeared to be genetically monogamous, they exhibit low levels of genetic variation as indicated by DNA profiling (Section 3). Thus transferring birds between islands to promote outbreeding may have little affect on the present problem **(Recommendation 1)**.

Second, the problem may be more directly a result of differences in the environment and habitat between Fiordland and the islands. The most obvious difference is in the introduced species of grasses on the islands upon which takahe feed. Our analysis of two trace elements (selenium and manganese) known to be important for reproduction, did not show any alarming differences when blood samples were analysed (Section 5). However, there was a trend for the manganese levels to be lower on islands, but sample sizes were small. Analysis of essential nutrients from infertile eggs rather than blood may be a more effective way of testing for differences in trace elements between Fiordland and the island populations **(Recommendation 2)**.

Other possible causes of high infertility, especially in first clutches of the season, such as low sperm production, are more difficult to investigate and to remedy even if evidence could be found to support them. First and second clutches of individual pairs could be compared by counting sperm numbers on the perivitelline membrane of freshly laid eggs (Section 9). Unfortunately, this method would require that some fertilised eggs be sacrificed to determine whether the above pattern exists. A pilot study using this method is presently being carried out on a small number of takahe eggs to determine the suitability of this approach **(Recommendation 3).** Other methods of collecting sperm samples from males, such as hand ejaculation and using a papier maché model, were unsuccessful (Section 8). Trialling a more realistic taxidermy model at Burwood Bush may be worth pursuing **(Recommendation 4).** However, even if support was found for the hypothesis that low sperm production was due to

raised testosterone levels in males fighting for breeding territories, it is not clear what management procedures could be put in place to prevent birds from doing what they do naturally. As long as fighting between birds declines as the breeding season progresses and pairs produce viable second clutches, then perhaps this is a problem that managers (and takahe) will have to live with.

No evidence was found that island takahe have difficulty regulating water loss inside the egg (Section 6) or show unusual patterns of incubation (Section 7), factors that could potentially contribute to poor hatching success. Eggs on islands tended to be smaller than eggs from Fiordland, which appeared to be related to the increased production of eggs of individual females as their number of breeding attempts increases (Section 6). A larger proportion of island females may be reaching senescence earlier than their same age counterparts in the Murchison Mountains, resulting in the production of smaller eggs. However, the reduction in egg size appeared to be a consequence of the high rate of clutch failure, and not the cause of it.

Some of the research in this report has relied on comparative data from pukeko living in the same island habitat as takahe. To date, pukeko on islands with takahe have been treated as pests and, at least on Mana, have been culled on a regular basis. While the numbers of pukeko on some islands may have to be controlled from time to time, the management reasons and effectiveness of shooting need to be reviewed and weighed against the benefits of having a closely related (native) species to the takahe available for research purposes (**Recommendation 5)**.

High proportions of eggs of unknown fertility have been a feature of breeding reports from islands in the past, making it difficult to determine whether infertility or other problems causing death after fertilisation are causing high egg failure (Section 10). This was due to non-developing eggs being left in the nest and incubated for a considerable period of time and thus becoming 'addled', and to managers not being properly trained in techniques to assess fertility if eggs had been removed after candling. A review of procedures used by island staff to monitor nests, candle eggs, examine removed eggs, store failed eggs and prepare dead chicks for post mortem revealed a number of steps that could be taken to improve the information and data collected during the breeding season. The Takahe Recovery Group should also ensure that island staff receive training on a regular basis, are aware of what samples are required each season for research purposes, and are given guidelines and protocols so that methods are standardised across all islands and that nest monitoring data are collected in a consistent way (**Recommendation 6**; and see Appendix 7).

One of the most crucial components of nest monitoring is determining when a pair has initiated egg laying. Yet this is also the most difficult and most time-consuming aspect of nest monitoring and the one that island managers have the least time to devote to. With the exception of Tiritiri Matangi, managers on the other islands are simply too busy with other duties to spend the necessary hours finding nests, especially those pairs nesting away from the manager's house. However, once the nests are found, managers are able to schedule regular nest checks and monitoring of chicks. Over the past several years, Christine Ryan and Angus Small from Otago University have assisted managers with finding nests, especially on Mana and Maud Islands, but at the time of publication, no arrangements had been confirmed for this takahe research on these islands over

1999/2000 and subsequently. The authors emphasise the need for DOC to create a short-term or part-time position for a field worker with knowledge of takahe nesting behaviour to monitor breeding pairs and find nests between mid-August and mid-December on Mana, Maud, and Kapiti islands (as is being done on Tiritiri Matangi). **(Recommendation 7)**.

12.2 RECOMMENDATIONS

In summary of the above, the authors recommend:

1. The policy of transferring birds between islands to promote outbreeding should be reviewed after the results of the genetic study are made available (1999 Takahe Recovery Group meeting)
2. Discussion of whether a supplementary feeding programme needs to be implemented should wait until the analysis of selenium and manganese concentrations in failed eggs from islands, Burwood Bush and Fiordland is completed (in progress) and further expert advice is sought.
3. A small sample of freshly laid eggs from first clutches should be removed and examined for sperm counts on the perivitelline membrane to confirm that infertility (and possible sterility of certain males) is related to absence/low sperm numbers.
4. Trialing of sperm collection methods could continue using captive pairs at Burwood Bush. A stuffed female takahe in a receptive posture could be placed in an enclosure with a pair in which the female has just laid eggs and commenced incubating. This should ensure that the male is still sexually active but that his mate is occupied on the nest, thus increasing the chance that he might attempt to copulate with a strange female.
5. The Takahe Recovery Group should seek input to any future management plans to cull pukeko on islands that contain takahe, so that their removal does not impinge on on-going or proposed comparative research on pukeko and takahe. At the very least, island managers should inform the group of any impending plans to cull pukeko.
6. A series of recommendations arising from the section on improving data collection and management procedures are listed below:

   a) Establish a yearly workshop for all staff (particularly new staff) involved in takahe work, covering techniques used in candling, opening eggs to determine fertility, preparation of specimens for analysis and any recent developments in technique or protocol. This would be most effectively run in association with the Takahe Recovery Group meeting, when many people involved in takahe work are present. Chicken or turkey eggs could be used for practising candling techniques. One such workshop has already been held—at the 1997 Takahe Recovery Group meeting—and was deemed very useful by the participants.

   b) Enlarge the existing photographic library of candled images of correctly developing eggs but include failed eggs as well. This would be most easily achieved at Burwood Bush.

c) The island co-ordinator should ensure that each island receives written guidelines and protocols for collecting, examining and storing eggs, embryo and chick specimens (some of these are given in Appendix 7).

d) The Takahe Recovery Group should decide before each breeding season whether they want material (i.e. eggs, embryos, and chicks) collected for research purposes and whether there are any requirements beyond the standard protocol for collection or storage. Island managers should be advised of the decision in writing.

e) A balance needs to be struck between monitoring nests frequently enough to record nest failures or successes and to detect problems or improvements in the population and minimising nest checks to reduce risk of disturbance to breeding birds. Except where specific research has been approved, nests should be checked between Days 3–5 to record number of eggs laid, between Days 10–13 to candle eggs to determine development status, and at approximately Day 30 to record hatching outcome. In addition, sections of track should be closed to the public where birds are nesting close by and are likely to be disturbed, until after eggs have hatched.

f) Staff should wear sterile surgical gloves while candling takahe eggs, and should change gloves between nests to reduce the risk of bacteria being transmitted to eggs causing yolk-sac infections. Removed eggs should also be kept warm.

g) The island co-ordinator needs to ensure that nest data from each island is properly recorded and entered into a computer database at the end of each breeding season (as is already done for the Fiordland population). These data should be summarised and compared with previous years (or made available to a research scientists to do so) well before the annual Takahe Recovery Group meeting.

7. The authors recommend that the Wellington and Marlborough Conservancies budget for a part-time or short-term contract for an experienced fieldworker to locate takahe nests on Mana, Maud and Kapiti Islands between mid-August and approximately mid-December of each year.

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Appendix 1

List of takahe plant-food species collected from four sample locations.

LOCATION	SPECIES COLLECTED	PART COLLECTED	NUMBER OF REPLICATES
Mana Island	Clover	Stem and leaf	3
	Cocksfoot	Stem	5
	Yorkshire fog	Stem	4
	Prairie grass	Stem	5
	Sedge	Stem	5
Maud Island	Clover	Stem and leaf	5
	Cocksfoot	Stem	5
	Yorkshire fog	Stem	5
	Phalaris aquatica	Stem	4
	Mixed pasture species	leaf	5
Tiritiri Matangi Island	Clover	Stem and leaf	5
	Cocksfoot	Stem	5
	Yorkshire fog	Stem	5
	Perennial ryegrass	Stem	5
Murchison Mts	*Chionochloa pallens*	Stem and leaf	4
	C. crassiuscula	Stem and leaf	4
	C. rigida	Stem and leaf	4
	Celmisia petriei	Stem and leaf	4

Appendix 2

Amount of trace elements in takahe plant-food species (given as mean percentage of dry weight) collected from four sample locations.

ELEMENT	MEAN PERCENTAGE (DRY WEIGHT)				
	MANA I.	MAUD I.	TIRITIRI MATANGI I.	MURCHISON MTS	P
Sodium	0.3447 ± 0.2064	0.1804 ± 0.1238	0.1397 ± 0.0501	0.1184 ± 0.0808	***
Magnesium	0.2024 ± 0.0795	0.2438 ± 0.0600	0.2640 ± 0.1194	0.1066 ± 0.0536	***
Phosphorus	0.3922 ± 0.0987	0.3733 ± 0.0875	0.3648 ± 0.0428	0.0956 ± 0.0577	***
Sulphur	0.2010 ± 0.0597	0.2498 ± 0.0730	0.2293 ± 0.0528	0.1152 ± 0.0271	***
Potassium	3.1551 ± 0.6878	3.3614 ± 0.3922	2.8658 ± 0.5471	1.1391 ± 0.4725	***
Calcium	0.2358 ± 0.2432	0.3777 ± 0.2870	0.3921 ± 0.4154	0.2020 ± 0.2148	*
Manganese	0.0198 ± 0.0169	0.0163 ± 0.0138	0.0136 ± 0.0046	0.0370 ± 0.0129	***
Iron	0.0063 ± 0.0066	0.0094 ± 0.0084	0.0100 ± 0.0101	0.0041 ± 0.0025	**
Copper	0.0008 ± 0.0002	0.0010 ± 0.0004	0.0008 ± 0.0003	0.0005 ± 0.0001	***
Zinc	0.0027 ± 0.0008	0.0042 ± 0.0031	0.0029 ± 0.0012	0.0019 ± 0.0007	***
Molybdenum	0.0001 ± 0.0000	0.0001 ± 0.0000	0.0001 ± 0.0000	0.0002 ± 0.0000	***
Aluminium	0.0053 ± 0.0051	0.0078 ± 0.0092	0.0059 ± 0.0055	0.0034 ± 0.0024	*
Silicon	0.4610 ± 0.2364	0.4619 ± 0.2510	0.7705 ± 0.4025	0.4798 ± 0.3113	N.S.
Chlorine	2.2197 ± 0.7468	2.0601 ± 0.6940	1.6454 ± 0.6801	0.5818 ± 0.3020	***
Titanium	0.0004 ± 0.0005	0.0009 ± 0.0009	0.0014 ± 0.0016	0.0941 ± 0.2960	*
Chromium	0.0002 ± 0.0001	0.0002 ± 0.0001	0.0002 ± 0.0001	0.0315 ± 0.1767	N.S.
Selenium	0.00005 ± 0.00002	0.00005± 0.00004	0.00006 ± 0.00003	0.00007 ± 0.00003	*
Strontium	0.0018 ± 0.0007	0.0026 ± 0.0011	0.0023 ± 0.0011	0.0014 ± 0.0015	**
Nitrogen	1.9291± 1.2418	2.9775 ± 1.5402	2.4505 ± 1.4972	0.6916 ± 0.2784	***

P values refer to ANOVA comparing the trace element levels in all samples from the three islands pooled to all samples from the Murchison Mountains.

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, N.S. = not significant

Appendix 3

Amount of trace elements (as mean percentage of dry weight) in grass samples from Mana, Maud and Tiritiri Matangi islands compared with tussock samples from the Murchison Mountains.

ELEMENT	MEAN PERCENTAGE (DRY WEIGHT)		
	GRASSES (N = 53)	TUSSOCKS (N = 24)	P
Sodium	0.2200 ± 0.1519	0.1271 ± 0.0901	***
Magnesium	0.1997 ± 0.0425	0.0854 ± 0.0358	***
Phosphorus	0.3750 ± 0.0845	0.1026 ± 0.0654	***
Sulphur	0.2107 ± 0.0609	0.1296 ± 0.0092	***
Potassium	3.2269 ± 0.6106	1.0949 ± 0.49799	***
Calcium	0.1928 ± 0.1100	0.0810 ± 0.0190	***
Manganese	0.0182 ± 0.0141	0.0333 ± 0.0098	***
Iron	0.0059 ± 0.0059	0.0044 ± 0.0024	N.S.
Copper	0.0008 ± 0.0003	0.0005 ± 0.0001	***
Zinc	0.0031 ± 0.0019	0.0017 ± 0.0007	***
Molybdenum	0.00009 ± 0.00007	0.00021 ± 0.00005	***
Aluminium	0.0049 ± 0.0061	0.0032 ± 0.0018	N.S.
Silicon	0.6617 ± 0.2747	0.6340 ± 0.1773	N.S.
Chlorine	2.2387 ± 0.5772	0.5905 ± 0.3150	***
Titanium	0.0006 ± 0.0006	0.0003 ± 0.0002	*
Chromium	0.00020 ± 0.00010	0.00035 ± 0.00011	***
Selenium	0.000048 ± 0.000026	0.000069 ± 0.000024	**
Strontium	0.00182 ± 0.00056	0.00057 ± 0.00026	***
Nitrogen	1.8504 ± 0.8650	0.7817 ± 0.2547	***

P values refer to ANOVA.

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, N.S. = not significant

Appendix 4

Amount of trace elements (as mean percentage of dry weight) in clover samples from Mana, Maud and Tiritiri Matangi islands compared with *Celmisia petriei* samples from the Murchison Mountains.

ELEMENT	MEAN PERCENTAGE (DRY WEIGHT)		
	CLOVER (N = 13)	*Celmisia petriei* (N = 8)	P
Sodium	0.2343 ± 0.2239	0.0921 ± 0.0347	N.S.
Magnesium	0.3845 ± 0.0804	0.1704 ± 0.0480	***
Phosphorus	0.3852 ± 0.0653	0.0748 ± 0.0067	***
Sulphur	0.2952 ± 0.0289	0.0719 ± 0.0102	***
Potassium	2.7978 ± 0.2106	1.2716 ± 0.3840	***
Calcium	0.9137 ± 0.2389	0.5651 ± 0.0473	***
Manganese	0.0105 ± 0.0038	0.0480 ± 0.0153	***
Iron	0.0192 ± 0.0088	0.0033 ± 0.0028	***
Copper	0.0011 ± 0.0001	0.0006 ± 0.0000	***
Zinc	0.0042 ± 0.0027	0.0026 ± 0.0002	N.S.
Molybdenum	0.00013 ± 0.00003	0.00018 ± 0.00003	**
Aluminium	0.0123 ± 0.0072	0.0048 ± 0.0034	*
Silicon	0.1204 ± 0.0637	0.0175 ± 0.0147	***
Chlorine	0.9638 ± 0.2794	0.5556 ± 0.2771	**
Titanium	0.0021 ± 0.0018	0.3752 ± 0.5174	*
Chromium	0.00028 ± 0.00007	0.12512 ± 0.35350	N.S.
Selenium	0.000091 ± 0.000023	0.000081 ± 0.000035	N.S.
Strontium	0.0039 ± 0.0008	0.0038 ± 0.0005	N.S.
Nitrogen	4.9877 ± 0.2309	0.4213 ± 0.1372	***

P values refer to ANOVA.

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, N.S. = not significant

Appendix 5

Concentrations of selenium and manganese in blood samples of individual takahe from six locations.

LOCATION	NAME OF TAKAHE	SEX	AGE	SAMPLE ID	SELENIUM (ug/kg)	MANGANESE (ug/g)
Tiritiri Matangi I.	Aroha	F	A	2	331.5	
Tiritiri Matangi I.	Manuiti	F	A	8	241.7	
Tiritiri Matangi I.	Pounamu	F	A	9	364.6	0.030
Tiritiri Matangi I.	Glencoe	F	A	13	237.0	
Tiritiri Matangi I.	Blossom	M	A	14	237.0	0.018
Tiritiri Matangi I.	Kowhatu	M	A	15	471.7	
Tiritiri Matangi I.	Whakama	M	A	16	305.5	0.039
Mana I.	Puffin	F	A	17	97.6	0.044
Mana I.	Bunchy	?	A	19	198.4	<0.015
Mana I.	Selwyn	M	A	20	160.6	
Mana I.	Tebee	M	A	21	222.0	
Mana I.	Mowai	M	A	27	215.0	<0.015
Mana I.	Tilly	F	A	28	254.3	
Mana I.	Lucky	M	A	29	252.8	
Mana I.	Kris	F	A	30	104.7	
Mana I.	Terri	F	A	26	unsuitable	
Kapiti Is.	Taku	M	A	31	214.2	
Kapiti Is.	Squeak	F	A	33	258.3	
Kapiti Is.	Iti	F	A	35	292.9	
Kapiti Is.	Beaker	F	A	37	246.5	0.019
Maud Is.	Hilda	F	A	38	320.5	0.037
Maud Is.	Maud	F	A	39	233.1	0.032
Maud Is.	Eric	M	A	40	144.1	
Burwood Bush	Houdini	?	A	42	191.3	
Burwood Bush	Owen	M	A	45	205.5	
Burwood Bush	Dana	F	A	46	191.3	
Murchison Mts.	unnamed	?	J	47	215.7	
Murchison Mts.	unnamed	?	J	49	133.1	
Murchison Mts.	Simon	M	A	52	123.6	
Murchison Mts.	unnamed	?	J	53	65.4	
Murchison Mts.	unnamed	?	J	54	189.0	
Murchison Mts.	Quan's chick	?	J	55211	152.8	
Murchison Mts.	R34969/C50	?	A	59	insufficient	
Murchison Mts.	Clyde	M	A	50	-	0.034
Murchison Mts.	Trapper	?	A	51	-	0.042
Murchison Mts.	Nick	M	A	55	-	0.042
Murchison Mts.	Guide	?	A	57	-	0.034
Murchison Mts.	Skimmer	M	A	58	-	0.039

Appendix 6

Protocol for collection of semen

1. Collect semen and immediately transfer it to a small vial. Try to keep it at blood temperature while the specimens are prepared—the best way to do this is to keep it in your pocket.
2. Using a micropipette, measure 10 µl of semen and deposit it on to one end of a microscope slide. Using the edge of a second slide, smear the drop of semen across the slide by pulling the drop back slightly towards the closest end, then pushing it to the other end of the slide, resulting in a thin layer of semen spread across the centre of the slide. Repeat this for 2–3 slides.
3. The remaining semen should be diluted: 10 µl of semen agitated with 0.5 ml of 10% formalin in an epindorf tube.

Appendix 7

Protocol for opening eggs, storing shells, contents, and embryos

A: Determining whether an egg is infertile or an early-death embryo

A tiny embryo will become visible in a fertilised egg after 2–3 days of development. However, for candling purposes, it is not until after six days of incubation that development of an embryo is apparent. When you first candle an egg at 7–10 days, wearing sterile surgical gloves to minimise the risk of bacterial infection, you should be able to tell whether it is developing or whether it either never started developing (i.e. it was infertile) or it stopped developing in the first few days. If you know what day the egg was laid (plus or minus 1–2 days), then you should know what the embryo will look like, using the development chart. Weighing the egg and calculating the age of the egg (using the formula in the Takahe Recovery Plan) should give you further information on whether the egg is developing correctly. If you know that an egg is at least 8 days old, but the calculated age is only 3 days, it is most likely the embryo has stopped developing. Key things that indicate an infertile egg or early death embryo are an aircell that is beginning to take up the width of the large end of the egg along with a yolk that is still round and sitting in the centre (when looked at in profile at about 7–10 days). If an egg is any older than 10 days, it should be immediately obvious if it is infertile as it will have a large air cell but a fresh-looking yolk (Figure A7.1). If the yolk has flattened and is touching the air cell, then development is occurring.

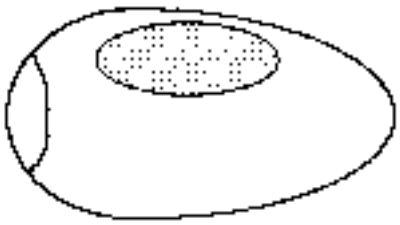

Figure A7.1. An infertile egg at approximately ten days old—note the fresh yolk but large aircell.

Once you have decided that an egg is no longer developing, it can be examined more closely. Remove the egg from the nest. Have on hand some small pointed scissors and some containers to empty the egg contents into when you have opened it up. Begin by recandling the egg to make sure the yolk is sitting on top. Carefully remove yourself and the egg from the candling bag, keeping the egg in the same position. Take your scissors and cut an oval-shaped hole around the top of the egg, being careful not to push the scissors in too far below the surface of the shell so as not to pierce the yolk sac. Lift off the lid and observe carefully **before** you tip the contents out of the egg. The centre of the yolk is where the embryo, if the egg is fertilised, will develop. If the egg is infertile, you should see a small group of white cells 2–3 mm across. If the egg was fertilised but died at one day of age, this group of cells will be a 'donut' shaped, clear in the centre with a ring of white cells 4–5 mm across. If the embryo died after 2–3 days of

development then you may be able to see a tiny (5–10 mm) bean-shaped embryo. Distinguishing these structures takes practice and is also dependent on the state of the egg and how long it has been incubated. It is possible to see the white cells on an infertile egg after about 16 days of incubation. Beyond this, the egg rapidly breaks down and the yolk goes rotten (i.e. addled). If you have a sloppy egg it is a good idea to break it open into a dish and poke around in the yolk to see if you can find any evidence of an embryo.

B: Storing the contents of failed eggs

If the contents of the egg are to be stored for analysis, tip them into a small plastic container and label with the following details before freezing:

- island,
- parents of egg,
- clutch number,
- date laid,
- date removed,
- status of egg when removed (e.g. infertile, dead embryo x days etc.).

C: Storing egg shells

Rinse the inside of the shells in tap water and air dry in a warm, dry place. Shells prepared in this way do not need to be frozen or refrigerated and can be stored at room temperature. Labels with all the details above should be written on the shell with pencil.

D: Preserving embryos and chicks in formalin

Larger embryos and dead chicks to be stored for analysis should be preserved in 10% formalin. The volume of the container should ideally be ten times that of the specimen. Formalin will only penetrate the first few millimeters of the specimen so, for a thorough preservation, some dissection should be carried out. Before dissecting a dead chick, examine all openings (i.e. eyes, beak, ears) for sign of any discharge (e.g. blood, pus) and record any that you see. Also record the weight of the chick.

Lay the chick on its back and make an incision from the keel to the vent (cloaca) (Cut 1, Fig. A7.2) and a second cut across the chick underneath the ribs (Cut 2, Fig. A7.2), then peel back the skin. Reach in behind the abdomen and lift the organs up to break the mesentery (glad-wrap like membrane). This is so that the formalin can get in and around all the organs. Make another incision through the skin from the top of the head to the bottom of the skull (Cut 3, Fig. A7.2) and peel back the skin. Make more cuts in the skull itself (it is a similar texture to fingernails) and carefully peel it back to expose the brain. Make a fourth incision down the centre of the neck (Cut 4, Fig. A7.2) to expose the windpipe, being careful not to cut it.

Figure A7.2. Incisions to make when preparing a chick for preservation in formalin.