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Short note

No evidence that dietary nutrient deficiency is related to poor reproductive success of translocated takahe

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Abstract

The aim of the study was to identify from the existing literature the essential nutrients that are known to affect egg fertility and hatching success in birds, and compare the concentrations of these between source and translocated populations of the endangered New Zealand takahe. Takahe are herbivorous, and those that have been translocated to highly modified island sites with pasture grasses have higher rates of egg infertility and low hatching success compared to takahe that breed in native tussock grasslands in Fiordland. Nine essential nutrients were analysed from infertile eggs collected from Fiordland and four island sites over a 4-year period. Only manganese showed any evidence of being in low concentrations in island takahe relative to Fiordland takahe, a result consistent with earlier analysis that showed low concentrations of manganese in takahe plant-foods on islands. However, manganese was in lowest concentrations on Kapiti Island where takahe consistently have the highest reproductive success of the four island sites, with 10 of the 15 samples from the other three islands falling within the range of the Fiordland samples. Therefore neither manganese nor any of the other eight essential nutrients appear to be widely deficient in island birds. Based on the results of this study, a supplementary feeding programme to improve egg fertility in takahe is not recommended.

Keywords: Avian diet; Egg infertility; Nutrients; Porphyrio; Translocation

1. Introduction

Obtaining proper energy and nutritional needs is essential for survival and successful reproduction for birds living in the wild (Murphy, 1996). Procurement of food and energy requirements are not so much a problem for birds living in captivity, but nutrients essential for breeding can sometimes be lacking in artificial diets, thus prompting many zoos and conservation agencies involved in captive breeding programmes to include nutritionists on their staff. Conservation managers dealing with free-ranging populations can take some comfort in the fact that nutritional deficiencies of birds living in the wild are rare (Robbins, 1983; Klasing, 1998). This applies to birds living in their natural habitat, but what happens when an endangered species is translocated outside its historical range or to a refuge habitat that might be slightly different or modified relative to that of the source population? Whether the new environment can provide the essential dietary nutrients to allow the successful establishment of the new population is a relevant question in assessing translocation programmes, but is rarely investigated directly. This is partly because nutrient requirements and deficiency levels, although well researched in domestic birds (e.g., National Research Council, 1994), are generally unknown for free-living birds (Robbins, 1983; Klasing, 1998).

This study examines whether dietary nutrient deficiencies are related to reduced reproductive success in translocated populations of the highly endangered takahe (*Porphyrio hochstetteri*). Takahe are a large (up to 4 kg), flightless, herbivorous rail endemic to New Zealand (Bunin and Jamieson, 1995; Lee and Jamieson, 2001). Although once widespread throughout New Zealand, the only remaining natural population of takahe occurs in the South Island in mountainous Fiordland, where they feed primarily on highly nutritious native grasses and ferns (Mills et al., 1980, 1991). As part of a management strategy, Fiordland takahe

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were introduced to four offshore island reserves, which had been farmed in the past but are now mammal-free and consist of a mosaic of introduced pasture grasses and regenerating native forests (Crouchley, 1994). Although adult takahe living on these modified island refuges appear healthy (i.e. maintain weight, plumage condition, etc.) and have higher survival rates than takahe in Fiordland, island pairs have higher rates of egg failures due to egg infertility and embryo deaths (Bunin et al., 1997; Jamieson and Ryan, 2000, 2001). This poor reproductive success has been evident ever since the initial founding birds first started breeding on islands in 1986. Several factors, such as chemical toxins (Jamieson and Ryan, 1999), endophytic toxic fungi (Jamieson and Easton, 2002) or abnormal rates of water loss during incubation (Small, 1999), have been studied and eliminated as possible causes of the high rates of egg failure. Island takahe could be suffering from inbreeding depression (Jamieson and Ryan, 2000; Jamieson et al., 2003), but another possible explanation, dietary nutrient deficiencies, has not been eliminated. Indeed, genetic and environmental factors such as poor diet are not mutually exclusive and may work together to affect egg failure rates.

The nutrient content of avian eggs can be an excellent indicator of dietary inadequacies in laying females and also provides an indirect measure of nutrient availability in the environment (Robbins, 1983; Murphy, 1996; Klasing, 1998). A pilot study indicated that concentrations of the trace elements manganese and selenium could be detected in the contents of non-developing takahe eggs, which can be identified and collected at the nest by candling eggs during the incubation period (Jamieson and Ryan, 1999). In the present study, I first identified from the existing literature the specific minerals, vitamins and fatty acids which, when deficient in the diet, are known to affect fertility and hatching success in birds. The availability of these essential nutrients in the diet of laying female takahe was then estimated by measuring nutrient concentrations in their eggs. Because actual nutrient requirements and deficiency levels are generally unknown for wild birds and for takahe in particular, the mean concentrations of various dietary nutrients in undeveloped/infertile eggs collected from nesting takahe were compared between island populations and the Fiordland population. In using the Fiordland population as a reference group, I assume that eggs collected here were not infertile as a consequence of local nutrient deficiencies. Because fertile eggs could not be collected and sacrificed for nutrient analysis, this assumption could not be tested directly. However, it is likely to hold true because Fiordland, unlike the island populations, represents a natural population of takahe living in unmodified habitat. Eggs collected from the Department of Conservation's Takahe Captive-breeding Unit near Fiordland were also included in the analysis for comparative purposes. Here, takahe receive some supplementary feeding, but most food comes from native vegetation growing inside large enclosures.

With the above data, I tested the hypothesis that nutrient concentrations should be significantly lower in females breeding on islands if a deficiency in one or more nutrients was causing poor reproductive success. The ultimate management aim is to use this information to determine whether an experimental study involving supplementary feeding is necessary to try to improve breeding success of takahe on the islands.

2. Dietary nutrients and their affect on fertility and hatching in birds

There are 38 nutrients that are essential in the diet of birds: 14 inorganic elements or minerals, 13 vitamins, 10 amino acids, and one fatty acid. Deficiencies in any of these are common in birds raised and bred in captivity where food is supplied, but are much less common in wild birds (Klasing, 1998). An extensive review of the literature indicated that deficiencies in nine nutrients can cause egg infertility or poor hatching success (see below), and of these only selenium and calcium are likely to be limiting in a natural diet. It should be noted, however, that takahe are primarily herbivores (Lee and Jamieson, 2001), feeding only occasionally on insects and invertebrates which are key sources of nutrients for many birds (Robbins, 1983; Klasing, 1998). The following information on the nine essential minerals, vitamins, and a fatty acid known to affect fertility and hatching success in birds was taken from Robbins (1983), National Research Council (1994), Murphy (1996), and Klasing (1998).

Manganese is important in the activation of many enzymes and deficiencies can cause reduced fertility and poor hatchability in birds. It is readily available in plant matter but there is species and regional variability in bioavailability. Manganese content in island plant-foods (as a percentage of dry weight) was approximately half that of plant-foods found in Fiordland (for list of takahe plant-foods and their percentage content, see Jamieson and Ryan, 1999).

Selenium is important for its metabolic association with vitamin E (see below). It can be stored in tissues but levels in plants are extremely variable. Selenium content in island plant-foods, especially clover, was high relatively to Fiordland plant-foods (Jamieson and Ryan, 1999).

Zinc deficiencies can cause several metabolic problems including infertility and early embryo and chick deaths. High concentrations of zinc increase requirements for selenium, copper and iron. Zinc content in takahe plant-foods was highly variable but was twice the percentage dry weight in plant-foods on islands than in Fiordland (Jamieson and Ryan, 1999).

Copper deficiencies can result in infertility but produces other symptoms such as abnormal egg shells (which have not been noted in takahe). High levels of zinc can impair copper uptake. Copper may not be readily available in plants, but was found in higher concentrations in island versus Fiordland plant-foods (Jamieson and Ryan, 1999).

Iodine is an integral part of the thyroid hormones. Deficiency levels of dietary iodine results in very low egg iodine levels causing poor hatchability and retards absorption of the yolk sac. Iodine is not distributed uniformly in the environment and its content in avian foods is extremely variable. Plants growing in regions with iodine deficient soils are deficient in iodine. Iodine concentrations were not measured in plant-foods of takahe.

Calcium is the most prevalent mineral in the body of birds and is required in the diet in greater amounts than any other mineral, although most birds that lay 1–2 eggs can use calcium from bone reserves. Calcium deficiencies in wild birds can be a major limitation to reproductive success, causing problems with shell formation, but is not known to affect hatching directly. Calcium was found in significantly higher concentrations in island than in Fiordland plant-foods (Jamieson and Ryan, 1999).

Vitamin E deficient males have reduced fertility because of abnormal and low numbers of sperm, and deficient females lay normal looking eggs that have low hatchability, especially late embryo death. Vitamin E is found in highest levels in food of plant origin but deficiencies of zinc, copper, iron, manganese and especially selenium increase demands for vitamin E.

Vitamin B_{12} deficiency causes poor hatchability and death at about 17 days incubation in chickens. It is required in very small amounts and found only in bacteria and food of animal origin; plants are devoid of vitamin B_{12} unless they are fermenting.

Vitamin A deficiencies affect spermatogenesis in males as well as developmental processes in embryos, which normally die within 48 h. Vitamin A is most likely to be deficient in wild birds that rely on agricultural grains during the winter, and is present in extremely variable levels in most plant foods. However, normal stores in the liver of a laying chicken fed a diet devoid of vitamin A can supply vitamin A to eggs for up to three months. Animals, especially insects, are good sources of vitamin A. Plants do not contain vitamin A itself, but do have a variety of carotenoids which are precursors for vitamin A. Overall, vitamin deficiencies of any kind occur rarely in free-living birds and few deficiencies have been reported in herbivores (Klasing, 1998).

Linoleic acid is the only essential fatty acid needed by birds. Linoleic acid deficiencies in poultry can cause impaired spermatogenesis in males and result in higher mortality of fertile eggs. They can also cause a decrease in egg size, but an earlier study found no link between egg size and hatching success in takahe (Small, 1999). Linoleic acid deficiencies in wild birds are uncommon as it is found in all natural foods, especially seeds.

Finally, problems with avian reproduction can also arise if nutrient concentrations are at toxic levels. However, such conditions are relatively rare in wild free-ranging birds, and concentrations need to reach extremely high levels (Klasing, 1998).

3. Methods

Takahe normally lay two large (up to 96 g) eggs. Monitored nests are checked by Department of Conservation staff once or twice during the incubation stage. The development stage of the embryo can be assessed by placing a small, bright torch/flashlight at one end of the egg (referred to as ‘candling’), and eggs that show no signs of development are removed (Jamieson and Ryan, 1999). Eggs that were removed were subsequently opened, the germinal disc closely examined and deemed to be infertile if no signs of development were evident. Only infertile eggs were used in the nutrient analysis to assure that the content of eggs from all sites was of a similar (non-development) status. All takahe eggs used in the analysis were collected from the 1997–1998 ($n=9$), 1998–1999 ($n=13$), 1999–2000 ($n=7$) and 2000–2001 ($n=11$) breeding seasons.

Egg contents (yolk plus albumin) were stored in containers and frozen until processing, when they were thawed and then homogenized. The analysis itself was contracted to two commercial laboratories: LABNET Invermay Ltd. conducted the selenium, manganese, zinc, copper, iodine, calcium and vitamin B_{12} analyses and Department of Human Nutrition, University of Otago conducted vitamin A and E and linoleic acid analyses (the latter included profiles of all fatty acids). Because of a failure to resolve peaks in the Vitamin A samples, no results were obtained.

For manganese, copper and zinc, samples were mineralized by wet combustion in perchoric/nitric acid and then concentrations determined by atomic absorption spectrophotometry. Selenium was prepared as above except the sample solution was diluted with 6 mol/l hydrochloric acid to prevent inter-element interference, and then the concentration determined by hydride vapour generation atomic absorption spectrophotometry. Calcium samples were dried, organic matter combusted by acid, residue taken up in dilute hydrochloric acid, and lanthanum oxide added as a releasing agent. Concentration was determined by atomic absorption spectrophotometry. For linoleic acid, samples were extracted in cholorform/methanol to take up lipids. Lipid extract is dried under nitrogen, then methylated with methanolic sulphuric acid. The sample was reconstituted in hexane, then analysed by gas chromatography. Linoleic peaks were identified by retention time matching to a known standard. For iodine, samples were digested with aqueous TMAH at 90 °C. The concentration is then determined by ICP–MS (inductively coupled plasma–mass spectometer). For vitamin B_{12}, samples were diluted in water and extracted from protein complex by boiling at pH 4. A known amount of bound Co^{57}–B_{12} was measured using a gamma counter to determine concentration. Vitamin E extraction and analysis followed that of Thurnham et al. (1988).

The first batch of eggs was analysed in 1999 and the nutrient concentrations measured included manganese, selenium, zinc, copper, vitamins E, and B_{12}, and linoleic acid. Iodine and calcium were added to this list for a second batch of eggs analysed in 2002. Preliminary examination of the dataset indicated two outliers for the vitamin B_{12} concentrations, which were omitted in the main analysis. Nutrient concentrations were normally distributed after log transformation. Differences among the years when the eggs were collected and between the two batch analyses were tested using analysis of variance and *t*-tests, respectively. For comparative purposes across sites, takahe reproductive success was estimated by calculating the annual number of independent juveniles produced over the total eggs laid and averaged over the four seasons that eggs were collected. Differences in nutrient concentrations and reproductive success among locations were tested with one-way analysis of variance followed by Tukey–Kramer multiple comparisons tests. Pooled island versus Fiordland comparisons were tested with *t*-tests. Bonferroni adjustments were performed on the *P* values because of the large number of statistical tests carried out for each analysis. All statistical analyses were carried out on JMP version 3.2 (SAS Institute, 1997).

4. Results

Takahe on Maud and Tiritiri islands had significantly lower reproductive success than takahe in Fiordland (bottom-right graph, Fig. 1). Mean reproductive success of birds on Mana was intermediate between these extremes while reproductive success for birds on Kapiti was comparable to that of Fiordland and Burwood birds (Fig. 1).

Eggs were collected from six sites, over four breeding seasons and analysed in two batches. None of the means of the nine nutrients measured varied significantly among years (one-way ANOVAs, all P values >0.10), and only one varied significantly between the two different batch analyses (*t*-tests with sequential Bonferroni adjustment, manganese: $P=0.045$; all other P values >0.10). Consequently, and because samples sizes were small, we pooled eggs across breeding seasons and batch analyses when testing for differences among the six sites.

Of the nine nutrients analysed, two showed a significant difference among the six sites in their mean concentrations (Fig. 1). Linoleic acid concentrations were significantly higher for Tiritiri than Fiordland (Fig. 1), which was probably related to the relatively high levels of total fatty acid found in eggs from Tiritiri (data not shown). For only one nutrient (manganese) was the mean level lower on the islands compared to Fiordland, significantly so on Kapiti Island (Fig. 1). Because the sample sizes for individual islands were small, we pooled the nutrient measurements of the three island sites that had relatively low reproductive success (i.e. Maud, Tiritiri and Mana) and compared this mean to that of the Fiordland samples. Again, only manganese showed any trend toward lower concentrations on the island sites, but it was not significant (*t*-test with sequential Bonferroni adjustment: $t=2.94$, $P=0.063$). Overall, 10 of the 15 manganese samples from these three islands fell within the range of concentrations found in the Fiordland samples.

5. Discussion

One of the most time consuming aspects of this study was reviewing the literature to determine which nutrients needed to be analysed. Birds require 38 essential nutrients in their diet but analysing all 38 would have been prohibitively expensive. Therefore I identified nine nutrients that are known to specifically affect egg fertility and hatching success. This mini-review in itself could be useful to other avian conservation managers who suspect that nutrient deficiencies might be affecting locally threatened species.

Assuming that measuring the concentration of nutrients in infertile eggs of laying females is a reliable indicator of their availability in the environment and diet of takahe, then only manganese showed any evidence of being in low concentrations in island takahe relative to Fiordland takahe. This result is consistent with earlier analyses. Manganese was found in significantly lower concentrations in plant-foods of takahe living on islands (i.e. introduced pasture grasses and clover) compared with plant-foods of takahe in Fiordland [tussock grasses (*Chionochloa* spp.) and alpine daisies (*Celmesia petriei*)] (Jamieson and Ryan, 1999). There was also a strong trend ($P=0.07$) for manganese to be in lower concentrations in blood samples taken of island birds (Jamieson and Ryan, 1999). Manganese is important in the activation of many enzymes and deficiencies can cause reduced fertility and poor hatchability in birds

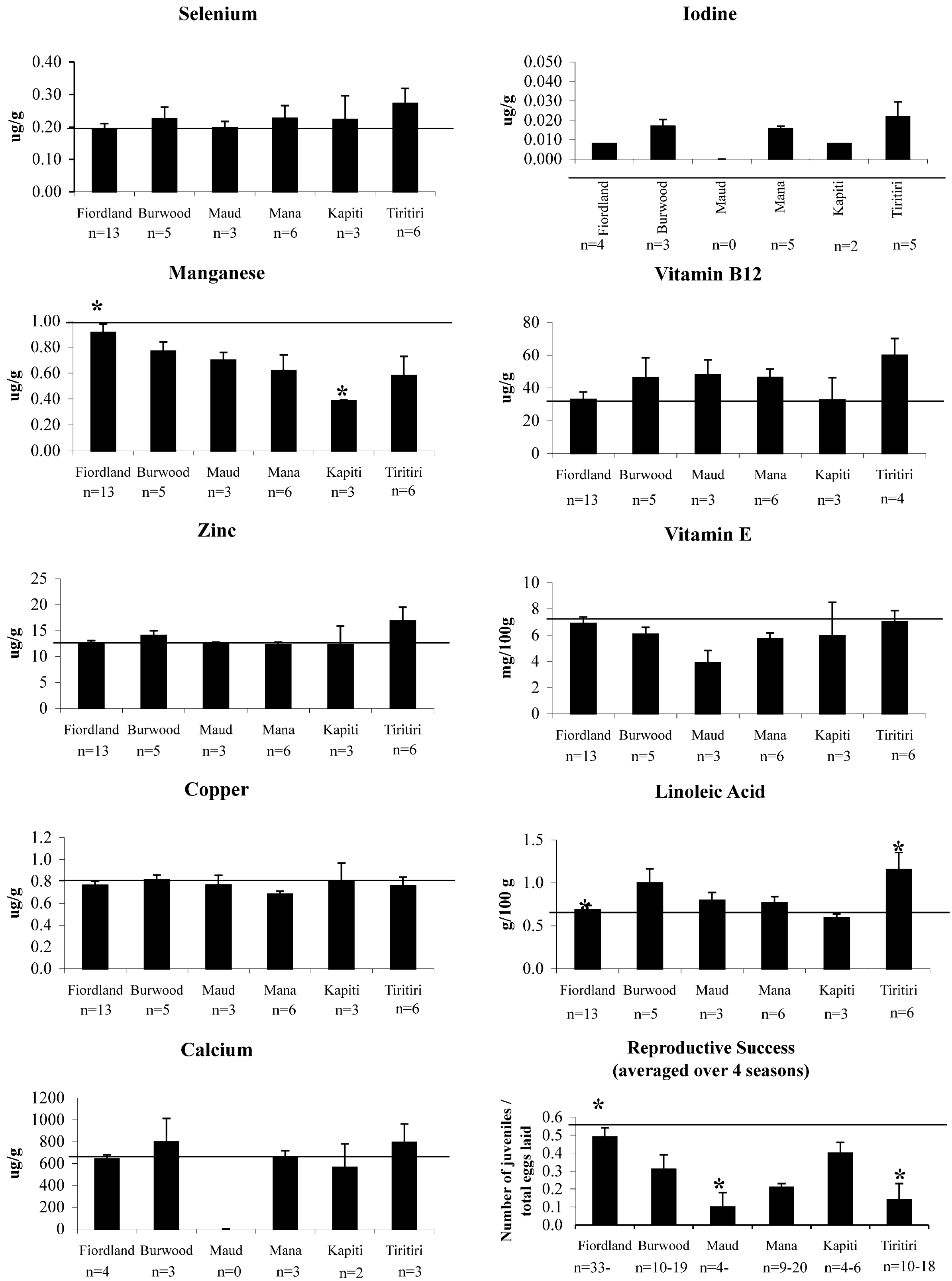

Fig. 1. The mean concentration (±S.E.) of nine essential nutrients found in takahe eggs collected at six different sites: Fiordland (natural population), Burwood (captive population), and Maud, Mana, Kapiti and Tiritiri Matangi islands (translocated populations). The number of egg is shown at the bottom of each graph. The graph in the bottom right corner shows an estimate of annual reproductive success (number of independent juveniles produced over the total eggs laid) at each site averaged over the 4 years that eggs were collected (±S.E.). The range in annual number of eggs laid over the four breeding seasons is given at the bottom of the graph. The horizontal line on each graph represent the mean value for Fiordland birds and asterisks indicate means that were significantly different from each other at the $P < 0.05$, as determined by Tukey–Kramer multiple comparison tests.

(Robbins, 1983; National Research Council, 1994; Klasing, 1998). Manganese is readily available in plant matter but our earlier study indicated that manganese content in takahe plant-foods on islands was half that of plant-foods in Fiordland (Jamieson and Ryan, 1999). However, in the present study, manganese was in lowest concentrations in eggs collected from Kapiti Island where takahe consistently have the highest reproductive success of all the island birds (Fig. 1 and I. Jamieson, unpublished data). Although manganese concentration was lower *on average* in the combined Maud, Tiritiri, and Mana dataset, 10 of the 15 samples were within the overall range of all Fiordland samples. Therefore there does not appear to be strong evidence that manganese is widely deficient in island birds.

According to the literature, nutrient deficiencies in wild birds are uncommon. This, coupled with the fact that closely related pukeko (*Porphyrio porphyrio*) living on the same islands as takahe and feeding on similar plant-foods do not suffer from high egg infertility and low hatching success (Jamieson and Ryan, 1999), makes widespread nutrient deficiencies on islands unlikely. Nutrient levels are bound to vary from site to site, but gross deficiencies in one or more nutrients were not evident on modified island habitats where takahe had been introduced.

Given that this study found little evidence of widespread nutrient deficiencies, a supplementary feeding experiment to improve takahe breeding performance is not recommended. Instead, I suggest that new genetic stock from Fiordland be introduced to each island to try to counteract the effects of further inbreeding (Jamieson et al., 2003). In conclusion, this study could serve as a model for other investigations where dietary nutrient deficiencies are hypothesized to contribute to the poor breeding success of an endangered bird, especially ones that have been translocated outside their natural range or to a habitat that has been highly modified.

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