The challenge of supplementary feeding: Can geometric analysis help save the kakapo?

DAVID RAUBENHEIMER
School of Biological Sciences, Ecology, Evolution & Behaviour Section, University of Auckland, Private Bag 92019, Auckland, New Zealand.
d.raubenheimer@auckland.ac.nz

STEPHEN J. SIMPSON
School of Biological Sciences, Heydon-Laurence Building, A08, University of Sydney, Sydney, New South Wales, Australia.
stephen.simpson@bio.usyd.edu.au

Abstract Foraging deficiencies and supplementary feeding play critical roles in kakapo (*Strigops habroptilus*) breeding biology and conservation. We present a framework for the analysis of complex nutritional data (called the geometric framework – GF) which may contribute further understanding of the relationships between natural foods, supplementary feeding and kakapo reproduction. We outline the basic concepts of the approach, and illustrate its application using data for the protein, lipid and calcium content of a natural food (green fruits of rimu *Dacrydium cupressinum*) and a supplementary feed ("muesli"). We provide some pointers for the broader application of GF to the problem of kakapo supplementary feeding, and close with a brief review of a literature which suggests that calcium might be a key limiting factor in kakapo reproduction. We hypothesise that supplementary foods with low macronutrient:calcium ratios are likely to be most effective in supporting increased reproduction.

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INTRODUCTION

Added to its role in testing ecological theory (e.g., Webb *et al*. 2005), food supplementation is increasingly used in the management of endangered species (Robertson *et al.* 2006). An important distinction in this regard is whether the target populations are limited by food availability per-se (quantitative limitation), or by specific food components (qualitative limitation). In making this distinction, Castro *et al.* (2003), for example, demonstrated that reproductive success of translocated hihi (*Notiomnystis cincta*) was contingent on supplementation with nutritionally complete foods, but not the carbohydrate component alone. In contrast, Kitaysky *et al.* (2006) have linked reduced recruitment in kittiwake (*Rissa tridactyla*) populations specifically to the proportion of lipids in the rearing diet of fledglings.

By far the simpler scenario is quantitative food limitation, since this can be remedied by supplementing with known suitable foods, thus avoiding the complex task of identifying the specific limiting component(s). Unfortunately, in some situations even quantitative food limitation assumes the complexity of qualitative limitation. One such case is where the suspected limiting foods are not available in sufficient quantities (due to seasonal, geographical, economic etc. constraints) to provide as a supplement to target populations. In this scenario, the management challenge is to identify or synthesise a substitute which has a similar effect on individual performance and demography as does the natural food. Given the tremendous chemical complexity of natural foods, it is a daunting - if not impossible - task to find an exact match, thus forcing decisions about the specific components which are likely to be the primary limiting factors i.e., the quantitative problem has become a qualitative one.

Such is the case for the critically endangered New Zealand flightless parrot, the kakapo (*Strigops habroptilus*). Kakapo are large (1.5 - 4 kg), herbivorous birds that breed only once every two - five years (Elliott *et al.* 2001). Once ubiquitous throughout New Zealand, habitat degradation and the impact of exotic predators have reduced the species to 86 known individuals, which as a part of an intensive management plan, have been translocated to three predator-free island refugia. In 1989, a programme of supplementary feeding was introduced in the hope that it would increase the worryingly low rate of reproduction in translocated populations (James *et al.* 1991; Powlesland & Lloyd 1994).

The choice of supplementary foods was guided by observations linking intermittent breeding with heavy fruiting years (mast years) of native podocarp plants, primarily rimu (*Dacrydium cupressinum*). Since podocarp fruits are not available in feasible quantities outside of the mast years, alternative supplementary feeds needed to be found, thus raising the question of which component(s) of the fruits might provide the relevant link to reproduction. Several lines of

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evidence pointed to protein: podocarp fruits were found in preliminary analyses to contain about twice the level of protein as other foods; the breeding season diet was found to contain about twice as much protein as the non-breeding season diet; and even this high level was low compared with the estimated protein requirements of some domesticated birds (Powlesland & Lloyd 1994). Added to this is the fact that egg production requires a substantial protein investment. Against a backdrop of strong emphasis in the ecological literature of the time on nitrogen as a major limiting factor (White 1983), this information quite reasonably led to the choice of protein-rich supplementary feeds.

While supplementary feeding has, arguably, had a positive impact on kakapo reproduction, to date the effects have been subtle: "The evidence that supplementary feeding improves breeding success in kakapo is not unequivocal, nor would one expect it to be with such small sample sizes" (Elliott *et al.* 2001). Some of the effects have, furthermore, been unanticipated, including an undesirable skew towards a male-biased sex ratio (Clout *et al.* 2002), which has subsequently been remedied (Robertson *et al.* 2006). Supplementary feeding has also been linked to kakapo becoming obese (Powlesland & Lloyd 1994; Bryant 2006).

A key factor motivating the writing of this paper is the "small sample sizes" to which Elliott *et al.* (2001) refer above. Not only does the dangerously low population elevate kakapo to one of the foremost conservation priorities, it also constrains research into the complex problem of devising effective supplementary feeds. In particular, many of the strengths of experimental biology cannot be deployed. For example, treatment manipulations which *a priori* might lead to less-than-optimal health outcomes should, arguably, be avoided (a situation reminiscent of research in human medicine), limited sample sizes reduce statistical power and may confound analyses, and invasive or intrusive techniques are precluded. Equally, the power of correlative analyses (e.g., matching reproductive outcomes to natural ecological variation or to management measures such as provision of supplementary feeds) is constrained by sample sizes, lack of statistically independent populations, and the extended duration of the reproductive cycle.

Given these constraints on experimentation and correlative analyses, the burden falls more strongly than is usual on deduction as a primary tool for exploring kakapo nutrition. In particular, a systematic approach is needed which can aid in wringing the most from existing data, identifying key gaps in those data, and directing future research on the problem of kakapo supplementary feeding. Our goal here is to introduce a framework based on the geometrical logic of state-space analysis which we hope might contribute towards an improved understanding of kakapo nutrition and to the ultimate goal of securing this precarious species.

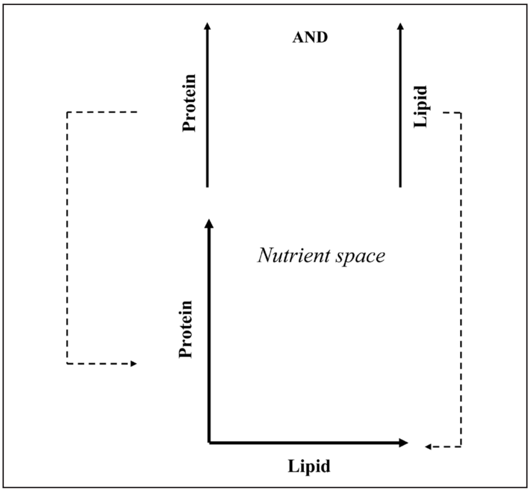

Figure 1 In geometrical analysis scales representing separate nutrients (here protein and lipids) are combined to form a *nutrient space*

We begin with an introduction to geometrical analysis, and then illustrate its use in the context of kakapo nutrition. Thereafter, we reflect more broadly on the directions that geometrical analysis highlights as priority areas for future research into kakapo nutrition, and close with a brief review of literature which suggests that the dietary balance of macronutrients vs. calcium might be a key factor in kakapo reproduction.

CONCEPTS IN GEOMETRIC ANALYSIS

The systematic use in nutritional studies of state-space geometry was first introduced by Raubenheimer & Simpson (1993) and its broader implications explored by Simpson & Raubenheimer (1993a). The geometric framework (GF) has since been applied in nutritional studies of insects (e.g., Simpson *et al.* 1995; Simpson & Raubenheimer 2000; Jones & Raubenheimer 2001; Raubenheimer & Simpson 2003; Lee *et al.* 2003), spiders (Mayntz *et al.* 2005), fish (Raubenheimer *et al.* 2005), frugivorous birds (Schaefer *et al.* 2003), chickens (Raubenheimer & Simpson 1997), rats (Simpson & Raubenheimer 1997), and humans (Simpson *et al.* 2003; Simpson & Raubenheimer 2005). In this section we introduce those basic concepts of GF that are germane to our exploration of kakapo nutrition.

GF has three principal aims: (i) to focus on the interactive effects of the multiple compounds that comprise foods; (ii) to integrate within a single model the key biological facets in the nutritional interactions that take place between consumers and their foods (nutrient requirements, foods, feeding etc.); and (iii) to integrate the various stages of food and nutrient processing (feeding, assimilation, utilisation and excretion).

Interactions of food components

At a superficial level, nutritional ecology is underpinned by the simple processes of finding, selecting, eating and utilising

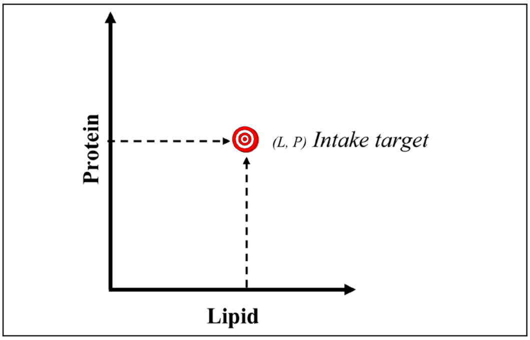

Figure 2 Nutrient requirements over a stated time period are represented by a point in nutrient space, an *intake target*, whose coordinates are given by the animal's separate requirements for the nutrients.

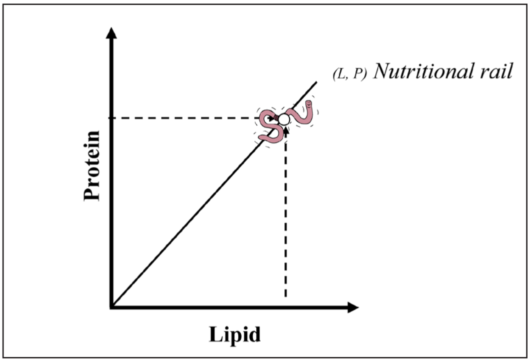

Figure 3 A stipulated quantity of food is represented by a point in nutrient space with coordinates defined by the amounts of each nutrient in the food. The balance of nutrients is represented by the slope of a line that joins the origin and the point representing a stated amount of the food, termed a *nutritional rail*.

foods. Foods, however, are not simple, unitary, resources, but complex mixtures of compounds each of which has its own relevance for the animal. Some are sought-after nutrients, and some are toxins which are best avoided. Among nutrients, some are needed in greater quantities than others, and in virtually all cases the animal's requirement changes with age, reproductive status, activity levels, recent feeding history, health, temperature etc. Furthermore, the dividing line between "toxin" and "nutrient" is not always distinct: some toxins are beneficial to health if taken in low levels (Calabrese & Baldwin 2003), while most nutrients are toxic if eaten in excess (Raubenheimer *et al.* 2005).

A fact that greatly complicates nutritional research is that the consequences for the animal of the levels in the food of any one component usually depend on the levels of other components i.e., their effects are interactive. A primary goal of geometrical analysis is to quantify these interactive effects. This is done by combining two or more food components in a model, where each is represented as an axis in a nutritional state-space ("nutrient space") (Fig. 1).

The facets of nutritional interactions

The nutrient space provides a framework for integrating the facets in nutritional interactions between consumers and foods within the context of the chosen food components. These facets include:

Nutrient requirements

The animal's nutrient requirements over a given time period are represented as a point, termed the "intake target", whose coordinates are given by the separate requirements for the two nutrients (Fig. 2).

Foods

By analogy with the intake target, a food item is represented as a point whose coordinates are defined by the constituent amounts of the two nutrients (Fig.3; hollow point represents the protein-lipid composition of the worm). A food item can also be characterised by the balance of the nutrients it contains. Since nutrient balance is a ratio, it is represented as the slope of a line that passes through the origin and the point representing the food item, termed a "nutritional rail" (Fig. 3; solid line). Nutrient balance provides a very powerful metric for representing foods, since it can generalise from individual food items to any quantity of that food type.

Feeding

As an animal eats a food, it gains nutrients in the balance present in the food, such that its nutritional state changes by "moving" along the nutritional rail. The consequences for the animal depend on the slope of the rail in relation to the intake target. If a nutritional rail passes directly through the intake target, the animal can satisfy its nutrient requirements by eating this "nutritionally balanced" food (Fig. 4A). But it could not reach its target by eating a nutritionally imbalanced food (Fig. 4B), unless it supplemented its intake with a second imbalanced food whose rail falls on the opposite side of the target to the first (Fig. 4C). In this way it could "zigzag" its way to the target by eating "nutritionally complementary" foods.

Regulatory interactions

The problem with nutritionally imbalanced foods is that they place an animal in a predicament where it is forced to over-ingest some nutrients, and/or under-ingest others. Thus, an animal eating the nutritionally imbalanced food depicted in Figure 4b has three options, as illustrated in Figure 5. It could, firstly, eat until it reached point (i) on the nutritional rail, thereby satisfying its requirement for protein but not for lipids. At the other extreme, it could eat until it reached point (ii) where it would have gained the required amount of lipid, but only by eating an excess of protein. It could, thirdly, feed to an intermediate point (iii), where it suffers a shortage of lipid and also an excess of protein, but neither of which are as large as at the extremes. Since both nutrient excesses and deficits can impact on health (Raubenheimer *et al.* 2005), the decision of how much of the imbalanced food to eat is critical for the animal.

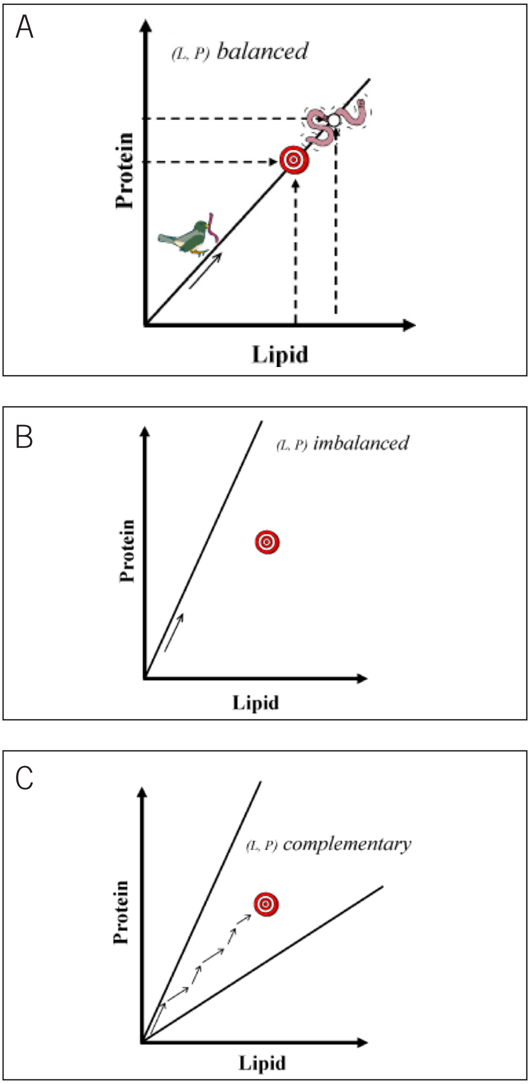

Figure 4 When an animal eats, it "moves" along the nutritional rail of the food: A. the rail of a *nutritionally balanced* food passes through the intake target; B. the rail of a *nutritionally imbalanced* food misses the intake target; C. by eating *nutritionally complementary* foods the animal can "zigzag" between rails towards the intake target.

Regulatory mechanisms for the intake of different food components thus evolve by natural selection to interact and, we believe, the details of these interactions should constitute an important component of nutritional studies .

Stages of nutrient processing

Nutrition can be viewed as a process in which chemicals and energy undergo a series of semi-serial transfers, each involving specific filters which have evolved in the context of the animal's phylogenetic constraints and nutritional ecology (Raubenheimer & Simpson 1998). To this point we have considered one of these, the transfer of nutriment from the external environment into the alimentary tract of the animal, where the filter involves food selection, feeding regulation and, in the case of kakapo, the egestion from the mouth of fibrous "chews" (Kirk *et al*. 1993). From the alimentary tract a component is transferred via digestion and absorption into the blood for further distribution, while the remainder is returned to the environment in the excreta. A component of absorbed nutrient is selectively allocated to various functions (growth, metabolism, reproduction etc.), and the balance is voided, principally via the faeces and urine.

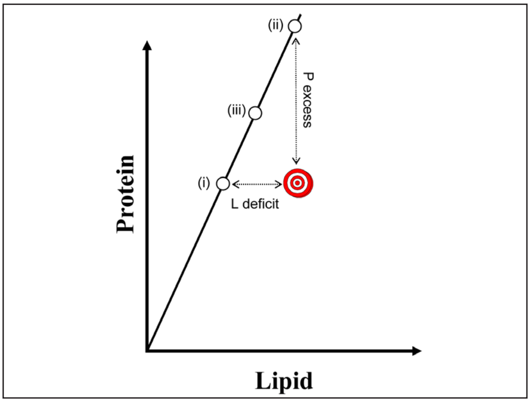

Figure 5 Three options for an animal eating a nutritionally imbalanced food containing excess protein relative to lipid.

The device for modelling these exchanges is the nutrient budget. At its simplest (Raubenheimer & Simpson 1994, 1995), a nutrient budget may be expressed as an equation:

$I_x = U_x + E_x$.....................equation 1

where I, U and E denote intake, utilisation and excretion respectively, and subscript x denotes the nutrient in question (e.g., p for protein).

Natural selection ensures that the three terms in this equation are tightly integrated so that the animal achieves a beneficial outcome from its foraging. In general this amounts to maintaining as stable as possible the quantity of the nutrient in question that is utilised for growth, reproduction, enzyme synthesis etc. (i.e., U_x). This involves the animal, as a first step, regulating I_x to the target level (in Fig. 6, for example, the target level for the protein budget would be achieved by feeding to point (i) on Food 1 or point (vi) on Food 2). However, depending on the interactions with other nutrients (e.g., lipid in Fig. 6) the target intake for protein might not be achievable. Thus, if feeding on Food 1 (Fig. 6) the animal might ingest a moderate (point (iii)) or extreme (ii) surplus of protein, and if feeding on Food 2 it might suffer a moderate (v) or extreme (iv) deficit of protein. To offset the discrepancy between target and actual intake, many animals have

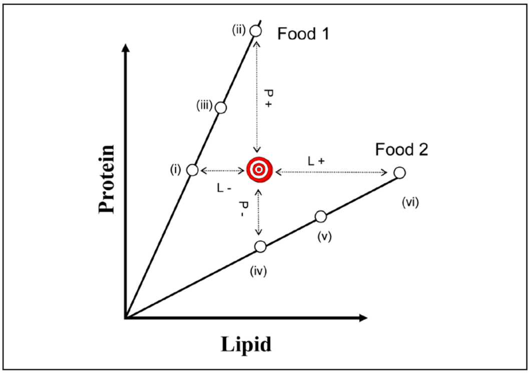

Figure 6 Consequences of regulatory interactions for a hypothetical animal eating either imbalanced Food 1 or imbalanced Food 2. It can satisfy its protein requirements by feeding to point (i), where it incurs a lipid deficit of L- or by feeding to point (vi), where it incurs a lipid excess of L+. At any other point it will incur a moderate (point iii) or extreme (ii) excess or a moderate (v) or extreme (iv) deficit of protein.

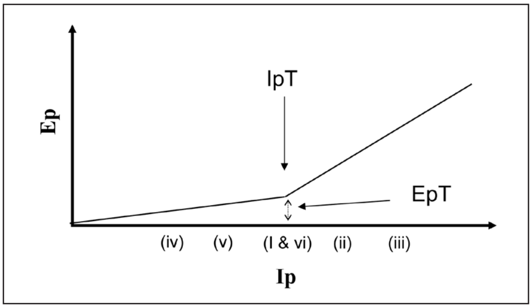

Figure 7 Utilisation plot showing the relationship between the level of protein eaten (Ip) and the amounts excreted (Ep). IpT represents the intake target coordinate for protein, and EpT the amount of protein excreted when the animal eats that amount of protein (i.e. when it reaches points i or vi, Fig. 6). When it eats less than the target level of protein, excretion decreases in proportion to the deficit (points iv and v) and when it exceeds the target it increases in proportion to the excess (ii and iii).

evolved post-ingestive homeostatic filters which modulate the levels of the nutrient excreted (e.g. E_p) (see López-Calleja *et al.* 2000 for an example from birds).

These relationships can be explored using plots of intake (e.g. I_p) against excretion (E_p), termed "utilisation plots" (Raubenheimer & Simpson 1994). The general expectation for a nutrient that is homeostatically regulated is illustrated in Figure 7. A certain level of excretion of the nutrient (E_pT) occurs at the target level of intake (I_pT), whereas at lower intakes (points (iv), (v)) the amount of the nutrient excreted decreases, and at higher intakes (points (ii), (iii)) the level excreted increases.

Such homeostatic responses provide a "regulatory signature" which can be very useful in elucidating the nutritional priorities and requirements of animals (see below), and in predicting the consequence of eating imbalanced foods (see Discussion).

MEASURING INTAKE TARGETS

Incorporating an estimate of intake targets greatly strengthens the power of a nutritional model. There are four approaches to deriving such estimates, which can be used singly or in concert depending on the system.

a) Performance. The most direct approach is to confine animals to one of a range of foods differing in the balance of nutrients (i.e., one of a range of nutritional rails). The rail which gives the best performance likely represents the optimal balance of nutrients, while the amount eaten of this food over the relevant period indicates the position of the intake target on the rail. Examples are given by Raubenheimer & Simpson (1997) and Simpson *et al.* (2004).

b) Pattern of intake. Evolutionary theory predicts that an animal, given the opportunity, will ingest the balance and amount of nutrients that is best for it i.e., it will achieve its intake target. It follows that when given an appropriate combination of complementary foods (Fig. 4C), the point of intake selected by the animal will serve as a good estimate of the intake target. This inference is particularly strong if it can be demonstrated that the animals *defend* the selected point i.e., when given different combinations of complementary foods nonetheless select the same point of nutrient intake (Raubenheimer & Simpson 1997; Simpson *et al.* 2004).

c) Pattern of utilisation. In the terms of the nutrient budget outlined in equation 1, the above method (b) amounts to measuring the nutrient selectivity of the first-stage filter, ingestive regulation. Applying the same logic to later-stage filters, the pattern of modulation of nutrient excretion (or its converse, utilisation) provides a guide to the target intake (the inflection point in Fig. 7).

d) Ecological criteria. Assessing the outputs of nutritional filters - as in b) and c) above - is particularly useful as a guide to intake targets for animals that live in complex nutritional worlds that require extensive filtering (i.e. feed on a range of foods with widely divergent rails). In simpler worlds, where the animal feeds predominately from one or a few food types that are similar and relatively invariant, the composition of the food can provide a direct estimate of nutrient requirements.

KAKAPO NUTRITION

In this section we illustrate how GF can provide fresh insights into the nutrient balance required in supplementary food provided to kakapo. For illustration we have chosen to focus on the October-November-December period, since it is at this stage in the annual cycle where nutrition is most likely to determine whether females are likely to attempt breeding in a given year. Thus, below a weight of 1.5 kg females do not breed (Elliott *et al.* 2001), and nutrition during the early breeding season is critical for exceeding this weight. During this period the major component of the kakapo diet is green fruits of rimu, or of other podocarps.

We note, however, that even if nutrition in the early breeding season is the critical factor in reproduction, it might not at this stage be limiting to female kakapo. Rather, the foods eaten by the female early in the season might provide a cue forecasting the suitability of nutrition at a later stage in the season, for example for rearing chicks (Elliott *et al.* 2001). In this case, the challenge of supplementary feeding is to emulate such food-borne cues, as well as provide foods which have the nutritional qualities forecast by the cue (i.e., are suitable for chick rearing). Plausibly, the mother's prediction might be based on availability during the early season of the same nutrient that is limiting to chick rearing.

The data

The following analysis is based on the extensive unpublished data set, compiled by D.K. Eason and colleagues, which consists of comprehensive chemical analyses of 14 natural kakapo foods and nine foods (including proprietary pellets) that have been provided as supplements to kakapo.

Model selection

An exhaustive analysis of these data would involve a staggeringly high number of interactions. Instead, we have used existing knowledge and intuition to focus the initial analysis, narrowing the options to those foods and nutrients we suspect *a priori* to be the most relevant to kakapo reproduction. We have focussed on the main foods of this period (green rimu fruits), and a supplementary-fed "muesli". The latter is a non-proprietary mix of nuts, fruits, honey and other supplements which has been fed to kakapo in recent years. The nutrients on which we have focussed are the macronutrients protein and lipid (measures of carbohydrate in supplementary feeds were unavailable), and the mineral calcium.

The decision to target macronutrients in the first instance is based partly on a body of theory and data spanning a wide range of taxa, which suggest that these major food components often play a central role in regulatory interactions, and also on the observation that most of the supplementary feeds are notably high in energy and protein. Our interest in calcium stems from its key role in the reproduction of many bird species, being essential for eggshell formation as well as skeletal growth of nestlings (Tordoff 2001). Many carnivorous and omnivorous birds meet this requirement by specifically targeting inorganic calcium sources, such as mollusc shells and bones, during the breeding season (Graveland 1996), an option apparently not available to strict herbivores such as kakapo. Further, a preliminary look at Eason's data revealed that rimu fruits are notably high in calcium compared with the supplementary food, which further suggested a possible link between the onset of reproduction and the availability of this mineral in the diet.

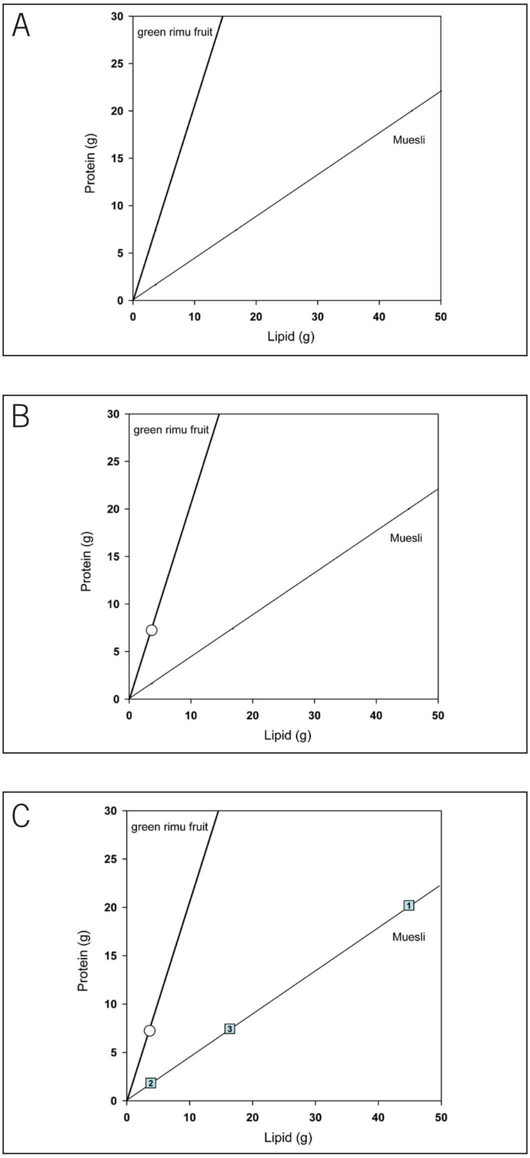

Figure 8 Geometrical models of protein-lipid interactions for kakapo eating green rimu fruit or muesli: A. nutritional rails showing the protein-lipid composition of the two foods; B. the protein-lipid intake (circle) achieved by eating 100g of green rimu fruit (the assumed intake target); C. points achieved by eating (1) 100g of muesli, (2) until lipid requirements are satisfied, and (3) until protein requirements are satisfied.

Model 1: Protein vs. lipid

Comparison of the foods

Figure 8A shows the protein:lipid nutritional rail is much steeper for green rimu fruits than for muesli, indicating a lower proportion of protein relative to lipid in the supplementary feed (7.4:3.7 vs. 20.0:45.3 for rimu and muesli, respectively).

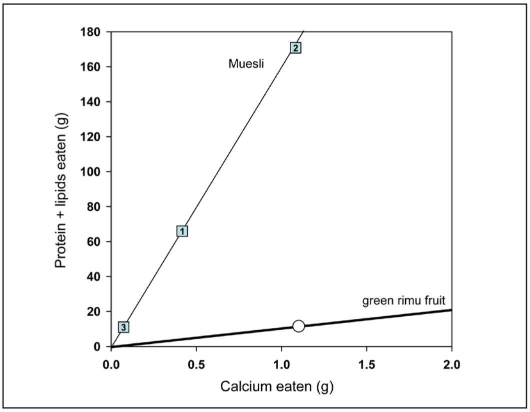

Figure 9 Geometrical model of macronutrient-calcium interactions for kakapo eating green rimu fruit or muesli. The circle denotes the intake achieved by eating 100g of green rimu fruit (the assumed intake target). The squares denote the intake achieved by eating (1) 100g of muesli, (2) until calcium requirements are satisfied, and (3) until protein requirements are satisfied.

Where is the protein:lipid intake target?

Information on ingestive and post-ingestive regulation of protein and lipid by kakapo are unavailable for estimating the position of the intake target (methods b and c above). We do, nonetheless, have some guidance from methods a) (performance) and d) (ecological criteria): the performance measure that needs to be emulated is reproduction, and the diet that is associated with this contains a high proportion of green rimu fruits. The composition of green rimu fruits can thus be used as an estimate of the target ratio of P vs. L (i.e., the nutritional rail on which the target lies) for potentially reproducing kakapo.

Having estimated the target nutritional rail from the balance of the two nutrients in rimu fruits, we seek to establish the target intake point along that rail. This is determined by the quantity of rimu fruit eaten daily by kakapo, data which are not currently available. Fortunately, however, the points we wish to illustrate apply whatever the actual daily intake of rimu fruit, so we can assume an arbitrary value. For simplicity, we will take this to be 100 g per day. This intake corresponds with a presumed target intake of 7.4 g protein: 3.7 g lipid (Fig. 8B).

The consequences of eating muesli

In Figure 8C we plot three scenarios. If the kakapo was to eat the same quantity of muesli as the amount of rimu that is required to reach the intake target (100g), it would end up at point 1. This would involve ingesting 2.7-fold excess of protein (20 vs. 7.4 g), and a massive 12.2-fold excess of lipid (45.3 vs. 3.7 g). Alternatively, the kakapo could eat an amount of muesli that gives it the required intake of lipid (3.7 g), but to do so would gain only 22% of the required level of protein (1.63 vs. 7.4 g) (point 2). Finally, it could eat to point 3, where it gains the target level of protein (7.4 g), but only by eating 16.7 g of lipid (a 220% excess). Thus, in terms of macronutrient balance muesli differs quite substantially from the natural food.

Model 2: Protein + lipid vs. calcium

In Figure 9 the nutritional rails of protein + lipid (P + L) vs. calcium for green rimu fruits and muesli are plotted (see Raubenheimer and Simpson 1999 for discussion of alternative methods for three- and higher-dimensional analyses). Also plotted is the amount of P + L and calcium that would be ingested if the kakapo ate 100 g of green rimu fruit (i.e., the assumed position of the intake target, represented by the hollow circle). As above, we have plotted the point on the muesli rail corresponding with an intake of 100 g of the supplementary feed (point 1). With this intake, a kakapo would eat 5.8 times the required level of macronutrient (65.3 vs. 11.1 g), and obtain only 38% of the required daily calcium intake (0.42 vs. 1.1 g). To ingest the target level of calcium, it would need to eat a spectacular 171 g of P + L (point 2), this being 15 times the target level. Given the strong influence that macronutrients (especially protein) can exert on food intake (Simpson & Raubenheimer 2000, 2005), the more likely outcome is that a kakapo would stop eating muesli when it reached the target level for P + L (point 3). In so doing, it would obtain only 6.3% of the required level of calcium (0.07 vs. 1.1 g). Muesli thus differs substantially from the natural diet in terms of the balance and levels of nutrients made available to kakapo.

A different supplementary food

In light of the limited success of the supplementary feeding program, kakapo managers have implemented a new feeding regime for 2006, by providing the proprietary Harrison's HPC pellets (R. Moorhouse pers. comm.). Here, we evaluate these pellets using the same models as for muesli above.

Figure 10A shows that the protein:lipid ratio in Harrison's HPC pellets is considerably more similar to green rimu fruits than is muesli. If a kakapo fed to point 3 (Fig. 10A) on this food, it would achieve the target level of protein intake and ingest only a relatively modest excess of 1.6 times the required level of lipid. If it fed to point 2 (the required level of lipid), it would obtain 59% the required level of protein. If it ate a daily intake of 100 g Harrison's HPC pellets, it would end up at point 1 with an intake of protein that exceeds the target by a factor of 2.4 (18 vs. 7.4 g) and an intake of lipid that exceeds the target by a factor of 4 (15 vs. 3.7 g). Overall, this suggests that Harrison's HPC pellets are near-balanced with respect to protein and lipid, but contain both of these nutrients at higher concentrations than do green rimu fruits. Thus, were protein and lipid the two nutrients constraining reproduction, the birds could come close to achieving the intake by eating a smaller amount of HPC pellets than of green rimu fruit.

What are the implications of Harrison's HPC pellets for kakapo calcium nutrition? This question is explored in Figure 10B, again showing for comparison the same analysis for muesli. For all three intake scenarios, the outcome on Harrison's HPC pellets in appreciably better than for muesli. If 100 g of the pellets was eaten (point 1), the kakapo would exceed its P + L target by a factor of 3 (33 vs. 11.1 g), compared with a 5.8-fold excess for muesli (above). If the target intake of P + L was achieved (point 3), the animal would get 27% of the required calcium intake, compared with 6.3% for muesli. Finally, to achieve the required calcium intake (point 2), it would exceed the macronutrient target by a factor of 3.6 (compared with 15 for muesli). Harrison's HPC thus represents an appreciably closer match to green rimu fruit than does muesli - but, by this analysis, there remains room for improvement.

DISCUSSION

We have demonstrated how geometrical analysis can assist with devising an effective supplementary food for kakapo. The analyses presented here are based on an initial sweep through a rich dataset. We are aware, however, of a wealth of additional information on nutritional and other aspects of kakapo biology, some of it presented in this volume, which have a bearing on selecting, developing, interpreting and testing further geometrical models. In the meantime, we highlight the following key questions which could form the focus of future analyses.

Which nutritional interactions?

This question is best approached by combining biological knowledge with considerations of the patterns that emerge from preliminary analyses of food composition data. We selected, for our illustrations, calcium and the macronutrients lipid and proteins as focal nutrients. Below, we review further data supporting our initial hypothesis that these nutrients might play a crucial role in devising an appropriate supplementary food for kakapo.

Where is the intake target?

The most direct approach to identifying the intake target is through measures of the nutrient composition of the natural diets eaten by foraging kakapo. In our illustrative analyses we used the composition of the major food in the early reproductive period (rimu fruits) as an estimate of target intake. While a useful first step, this is only a crude estimate for two reasons. First, although the majority item, green rimu is not the only food eaten by kakapo in the early breeding season (Wilson *et al.* 2006), and depending on their composition the ingestion of other foods might significantly influence the position reached in the relevant nutrient space. Second, individual fruits might differ in composition and the samples chemically analysed might not be representative of those eaten by kakapo.

The most powerful approach would be to measure the nutrient composition of the actual foods eaten by a foraging individual. This could be done by combining observations of meal-taking with disappearance from pre-weighed feeders of foods of known composition. This would be especially useful if two or more complementary foods could be used (Fig. 4C). Another approach is demonstrated by Cottam *et al.* (2006), who sucked crop contents from recently-fed chicks and collected spilled food.

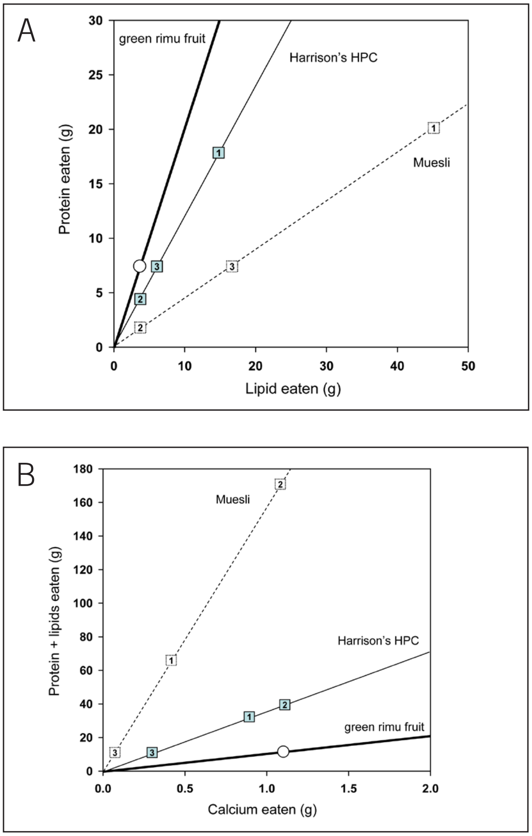

Figure 10 Geometrical models for kakapo eating green rimu fruit, muesli or Harrison's HPC pellets: A. protein-lipid interactions; B. macronutrient-calcium interactions. The circle denotes the intake target; the squares denote the amounts of nutrients eaten if kakapo ate (1) 100 g of green rimu fruit (the assumed intake target), (2) the amount needed to satisfy its need for the nutrient on the x-axis; (3). the amount needed to satisfy its need for the nutrient on the y-axis.

Such measures could be used to estimate not only the concentrations of the various nutrients in the diet, but also the absolute amounts of each nutrient eaten by foraging kakapo (concentration x amount eaten). This would provide an estimate of the target intake point as well as the target nutritional rail.

If similar measures were made of nutrients in the faeces (faecal composition x faecal mass), estimates of nutrient utilisation (equation 1) could be derived. This would enable an utilisation plot (Fig. 7) to be constructed, thus illuminating the post-ingestive regulatory priorities of kakapo for various nutrients. Strictly, this approach requires that the faeces in the analysis are matched to the foods in the analysis, a challenge that might only be met using captive or hand-reared birds.

Finally, existing data from other sources might indicate where the kakapo's intake target lies. Bryant (2006), for example, has used doubly-labelled water to obtain direct estimates of energy expenditure in free-ranging kakapo. Since kakapo appear not to modulate energy expenditure in response to variance in the energy content of foods (Bryant 2006), the observed level of energy expenditure when behaving *ad libitum* in the wild provides an estimate of natural energy requirements.

How do kakapo resolve regulatory interactions between nutrients?

Measures of the target intake point would enable estimates to be made of the ways that kakapo regulatory systems prioritise excesses vs. deficits of nutrients when eating imbalanced foods. For example, they would help determine whether kakapo feeding on different foods prioritise the mass of food eaten (point 1, Fig. 10A), or one or the other nutrient (points 2 or 3, Fig. 10A), or some compromise between these extremes (Simpson & Raubenheimer 1995; Raubenheimer & Simpson 1997, 2003). This could be determined using measures of intake rates of imbalanced foods of known composition. Such information, although secondary to identifying the relevant intake target coordinates, would be useful in predicting the consequences for kakapo of eating foods that differ in the balance of key nutrients, as modelled in Figures 8-10.

To what extent do kakapo regulate post-ingestive utilisation efficiencies?

Estimates of post-ingestive responses to excesses and deficits of various nutrients can provide a basis for predicting and interpreting the responses of kakapo to variable foods, both natural and supplementary. For example, if kakapo are not capable of voiding excess ingested energy through increased metabolic rate (Bryant 2006), reaching point 2 in Figure 9 would result in a dramatic accumulation of surplus energy. However, if they were able to increase the efficiency of calcium utilisation when eating calcium-deficient foods then points 1 or 3 in Figure 9 might be appropriate options for muesli-fed kakapo.

BIOLOGICAL POSTSCRIPT: THE GROWING CASE FOR CALCIUM, AND A RECOMMENDATION FOR SUPPLEMENTARY FEEDING

In the course of writing this paper, we encountered reason to believe that macronutrient-calcium interactions might be worthy of elevation from illustrative example to serious biological hypothesis.

Of particular interest is a paper titled "Constraints on reproduction by flying vertebrates: energy and calcium", in which Barclay (1994) proposed a physiological and evolutionary explanation for the characteristic small litter size of bats. Barclay noted that, owing to the biomechanical demands of flight, bats are unable to fly and forage for themselves until a relatively late stage of development, and so are dependent on maternal feeding for longer than are other mammals. The resulting foraging demands on the female are considerable, and impose a bottleneck on the number of young she can raise. The nutritional basis of the bottleneck, Barclay suggested, was calcium rather than the traditionally-believed energy, because of a mismatch between the high calcium demands of the growing skeleton and the low calcium/energy ratio of the foods of bats (insects, fruit and pollen). Data are presented suggesting that during mid-pregnancy a female of the insectivorous brown bat (*Eptesicus fuscus*) would need to eat two to four times her own body weight in insects to stay in calcium balance. In so doing, she would exceed her energy requirements by a factor of 3.5.

Barclay (*loc. cit.*) suggested that the same might apply to volant (flighted) birds, particularly those with long periods of parental care and where the mother alone provides the energy and nutrients to the young (i.e., polygynous species). As a test of the widespread belief that the density of bird bones (and hence their calcium requirements) is low, Barclay supplemented the data of Prange *et al.* (1979) to demonstrate that there is no overall difference in this respect between bats, other mammals, and volant birds. Pneumatisation results in some bones in birds (e.g., wing bones) being lighter than those in mammals, but this is offset by increased mass of others. In particular, the leg bones of birds are more robust than mammals, probably to meet the extra mechanical demands of bipedal vs. quadrupedal weight-bearing and possibly also the mechanical demands of landing (Prange *et al.* 1979). Added to the calcium cost of bird bones is the cost of producing eggshells, which consist of up to 98% calcium hydroxyapatite (Reynolds *et al.* 2004).

The high calcium demand of birds during growth was measured directly by Bilby & Widdowson (1971), who found that in the two weeks from hatching to fledging the nitrogen concentration of the body doubled while the calcium concentration increased seven to eight-fold, representing a 100-fold increase in the amount of calcium in the body. Reynolds *et al.* (2004) review studies suggesting that calcium limitation is widespread among birds, and can frequently be lifted using calcium-rich supplementary feeds.

Although flightless, many of the arguments for the calcium-constraint hypothesis apply equally to the kakapo. For example:

a) High calcium demand. Indirect evidence suggests that kakapo skeletons are unusually large and dense, and hence calcium-demanding. Firstly, kakapo are the largest of the parrots, and among both birds and mammals, skeletal mass is proportionately larger for larger animals (Prange *et al.* 1979). Secondly, flightless birds tend to have larger and denser leg bones than volant ones, but reduced wing bones (Iwaniuk *et al.* 2004). Kakapo have enlarged legs and pelvis, which enable them to walk long distances and to climb, but also have retained relatively large wings (James *et al.* 1991; Livezey 1992).

b) Females bear the burden of calcium acquisition. Kakapo are polygynous, and so the female alone provisions the developing chicks with food. There is, furthermore, a long nestling period (72 days) during which the chick is solely dependent on the mother, and a protracted fledgling period of 246 days (Eason *et al.* 2006).

c) Selective feeding on calcium rich foods. The high calcium content of podocarp fruits and their prevalence in the diet suggests that kakapo might target calcium-rich foods. Also in line with expectations, podocarp fruits feature more heavily in the breeding season, in breeding years as opposed to non-breeding years, and in the diets of females than of males (Wilson *et al.* 2006).

d) Calcium constraints and life history. Barclay (1994) predicted that birds that have evolved under calcium constraint should have smaller clutch sizes. Kakapo have a small mean clutch size of 2.5 eggs, each of which is smaller than would be predicted by their body size (Eason *et al.* 2006). They also breed infrequently.

It could be argued, furthermore, that some of Barclay's premises apply more strongly to kakapo than to volant birds. For example, kakapo appear to have unusually large skeletons (and hence a high calcium demand), and being flightless the mother's ability to forage selectively for calcium-rich foods is limited compared with volant species. Also, being strict herbivores, it is unlikely that kakapo take calcium-rich items derived from animals, such as bones or shells. Together, these data suggest that calcium might well play a role in limiting reproduction by kakapo.

If low calcium was the only aspect of supplementary food that differed from rimu fruits, then plausibly kakapo could simply eat more of the foods to satisfy their calcium demand. However, there are strong indications that calcium might interact with the relatively high levels of macronutrients in supplementary food to prevent this, as modelled in Figures 9 and 10B. In particular, kakapo have an exceptionally low metabolic rate and daily energy expenditure (Bryant 2006), indicating a low demand for energetic macronutrients. They also have a limited capacity to void energy which is ingested in excess of their requirement. The route for excretion of excess energy in many animals is through an increase in metabolic rate which is dissociated from physical activity (Stock 1999; Zanotto *et al.* 1997; Trier 2003), a phenomenon known as diet-induced thermogenesis. Kakapo, however, do not alter their metabolic rate in response to the energetic content of their foods, but rather accumulate ingested excesses as lipid, leading to obesity (Bryant 2006). Limited capacity for voiding excesses of a nutrient (e.g., energetic macronutrients) usually limit the amount of deficient nutrients (e.g., calcium) that can be obtained from imbalanced diets (Raubenheimer & Simpson 1997). It is thus highly unlikely that kakapo could feed to point 2 in Figure 9.

The macronutrient-calcium hypothesis thus seems worthy of further investigation in kakapo. The shift, in 2006, to using Harrison's HPC pellets, with their better macronutrient:calcium balance (Fig. 10B), provides an initial "experiment" of sorts. If the balance of macronutrients: calcium does turn out to be important, then how might this be implemented in the supplementary feeding programme? We see three possibilities: i) provide a complementary food that is high in calcium; ii) raise the calcium levels in the supplementary food; and iii) reduce the macronutrient content of the supplementary food.

The first possibility would enable kakapo to mix an intake that is more optimal with respect to their requirements than a single imbalanced food (Fig. 4C). Although many other bird species do mix their foods in this way (Reynolds *et al.* 2004), it is uncertain whether kakapo have the ability to specifically regulate their intake of calcium – their selection of green rimu fruits might be based on other stimuli. Even if they do show a calcium-specific appetite, it is uncertain whether they would recognise many high-calcium supplements (e.g. shells or bones) as being edible. Nutrient-specific appetites are usually expressed when an animal is deficient in the nutrient in question (Simpson & Raubenheimer 1993b), which would not be the case if calcium serves as a cue to females in the early breeding season rather than being a limiting factor in this period. The second possibility, raising the calcium content of the supplementary food, bypasses these problems, but might introduce another. The food would continue to have levels of macronutrients that are appreciably higher than green rimu fruits, and by the same logic developed here for calcium this might result in limited intake of other nutrients. We would, therefore, recommend the third alternative: to lower the macronutrient content of the supplementary feeds, bringing the overall balance better into line with the composition of the natural foods.

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