



Original Article

Dietary protein selection in a free-ranging urban population of common myna birds

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Anthropogenic environments can offer rich sources of energy to urban wildlife, but little is known about how they impact on nutritional balance and food selection. Common mynas (*Sturnus tristis*) provide a powerful model system for testing the nutritional constraints and priorities of an invasive species that has successfully adapted to urban environments. Here, we use behavioral observations, field-based feeding trials, videography, and the right-angled mixture triangle model (RMT) to examine the macronutrient preferences of these invasive birds. Our behavioral observations showed that mynas consumed insects (40.6%), worms (33.2%), human discards (17.6%), and plants (8.6%). Our feeding trials using nutritionally defined foods showed that mynas had a clear preference for food dishes containing only high-protein (HP) pellets over high-lipid (HL) or high-carbohydrate (HC) pellets. In addition, mixed feeders were also presented in 2 combinations: 1) contained equal proportions of HP and HC pellets and 2) equal proportions of HP and HL pellets. HP pellets were selectively consumed from both mixed feeders, this involving an increase in feeding time. Overall, the RMT showed that mynas consumed a higher proportion of protein from the feeders than in their natural diet. Furthermore, the majority of our observations of birds feeding at the dishes containing HP foods ended in intraspecific aggression, suggesting that protein is a contestable resource. These results suggest that mynas at our urban study site are deficient in protein relative to fats and carbohydrates.

Key words: aggression, birds, invasive species, macronutrient selection, protein.

INTRODUCTION

Since the mid-1800s, naturalists, evolutionary biologists, and ecologists have drawn crucial insights from species invasions (Sax et al. 2007). These classic perceptions have fundamentally developed our views on many issues that underline animal behavior as an essential component of invasion biology. A suite of potential mechanisms have been suggested to contribute to invasiveness, including key traits of a species being preadapted to the environment, adaptability to novel environments, dispersal ability, reproduction, gregariousness, and omnivory (Holway and Suarez 1999; Kolar and Lodge 2001; Wright et al. 2010; Chapple et al. 2012). Most importantly, successful invasion has been associated with an ability to exploit different environments and use a broad range of foods (i.e., ecological generalism) (Levine 2008). The wide niche breadth

of generalists may predispose such species to utilizing new food sources and surviving novel environmental conditions, perhaps to a greater extent than more specialized species (Duncan et al. 2003; Romanuk et al. 2009).

During the invasion process, animals are likely to be confronted with unfamiliar foods (Hailey et al. 1998; Sol et al. 2012), as well as other challenges such as food dispersal and predator avoidance. The invasive success of vertebrates, which represent around 30% of the invasive species listed (Lowe et al. 2000), has been attributed to the ability to achieve their required energy intake by feeding on mixed diets (Hailey et al. 1998). Abundant evidence suggests, however, that the macronutrient composition of foods, rather than energy intake per se, is a major factor contributing to an optimal diet (Simpson and Raubenheimer 2012). The ability to subsist in different environments is thus linked to the challenges of ingesting, digesting, and assimilating a combination of foods that provide the required amounts and ratios of macronutrients (protein, lipid, and carbohydrates) (Simpson and Raubenheimer 2012). Dietary protein plays

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an important role given that it is used for lean growth and reproduction and, in many animals, also as a source of energy, whereas dietary lipid or carbohydrate alone cannot be used to increase the net amount of body protein (McWilliams 2011). Not surprisingly, many organisms, from slime moulds to humans, are known to strongly regulate their protein intake in order to meet their physiological requirements for this nutrient (Raubenheimer et al. 2009; Simpson and Raubenheimer 2012; Raubenheimer et al. 2012).

Urbanization is characterized by the substitution of natural vegetation by man-made structures that may alter the abundance and species richness of native insects (Rickman and Connor 2003). Urban environments provide access to artificial breeding sites and anthropogenic foods, which support a variety of native and invasive birds (Weiser and Powell 2010). These ecosystems are also known to contain a wide range of macronutrient combinations that are influenced by human activities (Kaye et al. 2006). In urban environments, the abundance of human discards likely raises the levels of lipids and carbohydrates (Eagle and Pelton 1983; Pierotti and Annett 1987). In combination with a potential reduction in the availability of natural foods such as insects, urban birds may experience a mismatch between protein demand and its availability. In turn, that mismatch may go on to increase the intensity of competition over protein leading toward agonistic behaviors (Murphy 1993).

Common mynas (*Sturnus tristis*, formerly *Acridotheres tristis*; hereafter mynas) are passerine members of the *Sturnidae* family, which provide a powerful model system for testing nutritional preferences in the diet of an invasive species that has successfully adapted to urban environments. This species evolved in open woodland habitats in India (Sengupta 1976), and has since invaded all continents except Antarctica and South America—it is now considered among the “100 World’s worst invaders” (Lowe et al. 2000). In Australia, mynas were introduced into Melbourne and Sydney in 1862, as a predator to control the abundance of grasshoppers (Hone 1978). They have since become established as a major pest species in New South Wales, Australian Capital Territory, Victoria, and Queensland, and are predicted to expand their range (Grarock et al. 2013). Mynas are known for their ability to adapt to a wide range of urban and semirural habitats (Pell and Tidemann 1997; Lowe et al. 2011), and for their impact on fruit and vegetable crops (Feare and Craig 1998; Kaur and Dhanju 2013). Mynas are also believed to compete with native species over cavity-nesting sites posing a major threat to native wildlife (Lowe et al. 2011; Grarock et al. 2012, 2014) and are further suspected of spreading disease (Reed and Fatunmbi 1993; Ishtiaq et al. 2006). These successful invaders are generalist omnivores that feed on a wide range of foods including invertebrates, plants, fruits, and human discards, with insects being their preferred dietary choice (Moeed 1975, 1976; Sengupta 1976; Pell and Tidemann 1997).

Here, we combine the use of behavioral observations, feeding trials, videography, and nutritional geometry (right-angled mixture triangle nutritional models [RMT]) to examine the macronutrient preferences of Common myna birds at an urban field site. In particular, we addressed 3 questions to test the prediction that mynas select specific macronutrients over energy: 1) do common mynas select high-protein (HP), high-lipid (HL), and high-carbohydrate (HC) pellets from the feeders in nonrandom proportions? 2) Do common mynas show greater agonistic behaviors over access to certain foods than others? 3) Do Mynas selectively target specific foods, despite having to invest increased foraging effort to do so? We predict that urban mynas will show a strong preference for a

diet with HP content. Furthermore, if protein is deficient in their natural environment, we predict that mynas will display agonistic behaviors and/or increase their foraging effort to attain protein. These findings aid us in understanding the nutritional priorities that govern foraging behavior in a highly successful invasive species.

MATERIALS AND METHODS

Behavioral observations in opportunistic feeding events

Prey capture success during feeding events was assessed during the nonbreeding season from March to September 2014 in urban areas around Sydney, New South Wales, Australia. Observations with the aid of Nikon 8x42 Monarch binoculars were opportunistic and made over a large geographic area to minimize the likelihood of repeating the measurements from the same bird. Given the challenges of quantifying foods taken in the field, especially for insectivorous birds (Remsen and Robinson 1990), we used the very broad food categories of Sengupta (1976): insects, worms, plants, fruits, and human discards, which we considered to provide a good compromise between resolution and reliability. We were confident about food identification in 85% of observations, and omitted from analysis any cases where there was uncertainty over which category of food was being eaten. Following Greenberg (1984), prey capture was considered successful when prey was observed in the beak of the bird and consumption was confirmed when birds wiped its bill after a prey attack.

Diets

A total of 3 diets were formulated based on corn gluten, meat and bone meal, powdered cellulose, soy protein concentrate, cornstarch, and poultry fat. These ingredients were selected over semisynthetic constituents to reflect nutrient sources that may be available to myna birds in the wild, that is, mixtures of animal protein sources, human discards, and arable crops. All diets were formulated to be isoenergetic (2600 kcal/kg) and were balanced for all macrominerals and microminerals, including digestible phosphorus, calcium, sodium, potassium, and chloride to minimize confounding effects between diets. The common energy density was achieved using powdered cellulose as indigestible filler. The HP diet contained 42% crude protein, 7% lipid, traces amount of starch and was formulated with all amino acids balanced relative to digestible lysine (putatively the first limiting amino acid for myna birds based on observations from other avian species) (Table 1). The HC diet contained trace amounts of crude protein (nitrogen from nonproteinaceous sources), 12.3% lipid, and 42% starch (Table 1). The HL diet contained trace amounts of nitrogen from nonproteinaceous sources, 26.7% lipid (the highest that could be achieved without introducing an energy density confound), and trace amounts of starch (Table 1). The 3 pellet types were subtly but discernibly different in coloration, with the high-protein content of the HP food imparting a slightly darker hue than the other 2 pellet types (the bowl in the foreground of the photo in Figure 1). The difference was substantially less than the range of colors within food categories encountered in the urban environment (e.g., different grains or different types of bread), but nonetheless enabled us to distinguish the pellet types to measure their relative consumption from mixed feeding bowls (see below). All diets were cold pelleted at 60 °C and offered in a pelleted form (4-mm diameter and 3-mm length) and average weight 0.1 g (range 0.09–0.11 g).

Table 1
Ingredients and calculated nutrient composition of the 3 foods provided during the field-based experimental trials

Ingredient (%)	HP	HC	HL
Corn starch	—	42.00	—
Corn gluten meal	40.00	—	—
Meat and bone meal	10.00	—	—
Poultry by-product meal	20.00	—	—
Powdered cellulose	23.96	39.26	63.52
Soy protein concentrate	1.28	—	—
Poultry fat	—	—	15.61
Soy oil	2.62	—	15.00
Sodium chloride	—	0.30	0.37
Sodium bicarbonate	0.16	0.27	0.28
Potassium bicarbonate	0.60	1.28	1.04
Potassium sulphate	0.50	0.50	0.75
DL methionine	—	—	—
Lysine HCL	—	—	—
Threonine	—	—	—
Limestone	0.38	0.91	0.95
Monocalcium phosphate	—	2.00	1.98
Vitamin/mineral premix ^a	0.50	0.50	0.50
Metabolizable energy, kcal/kg	2600.00	2600.00	2600.00
Crude protein	42.00	0.42	0.21
Total calcium	0.90	0.90	0.90
Total phosphorus	0.53	0.43	0.42
Digestible phosphorus	0.40	0.40	0.40
Digestible Lys	1.27	0.00	0.00
Digestible Met+Cys	1.36	0.00	0.00
Digestible Thr	1.34	0.00	0.00
Sodium	0.22	0.22	0.22
Potassium	0.58	0.58	0.58
Chloride	0.23	0.23	0.23

^aDosed to supply per kilogram: ethoxyquin, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 mg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl- α -tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 mg; Zn, 60 mg.

Feeding stations

The study was conducted between July and August 2014 at Waverley Park (33°53'59"S, 151°15'34"E), Sydney, New South Wales, Australia. This area was selected as mynas are well known to be present during nonbreeding season. The cafeteria experimental design comprised a food station of plastic containers (67 × 15 × 20 cm³) with 4 circular holes that contained individual dishes each filled with 200 g of dry food pellets of predefined macronutrient composition (HP, HL and HC; Figure 1a). As part of the field setup, 5 black pegs were located within 20 cm of the food station to facilitate the behavioral analysis of the birds (Figure 1b). The food station was set up for 4 h during the morning, and the position of each dish within the station was varied daily. With the goal of testing foraging effort between protein source mixed with a nonprotein source, we additionally created 2 different mixes of foods: mix 1 contained equal amounts of HP and HC pellets whereas mix 2 contained equal amounts of HP and HL. Food dishes were individually weighed at the end of each day to determine the mass of food consumed. For dishes containing a mix of pellets, the different pellet types were carefully separated based on subtle differences in coloration (see above) and weighed.

To collect behavioral observations from the feeding stations, we used customized video camera (AU USB Flash Drive DVR Camera DV) with a 36° lens angle and sensor resolution of 720 × 480 HD at 30 frames/s with a MicroSD 64 GB storage capability. The unit was powered by a lithium polymer battery that enabled 10 h of continuous video recording. All components were encapsulated in a waterproof housing. To standardize the field of view, the camera units were placed at a 45° angle attached to a small bamboo stake (60 × 1.2 × 1.2 cm³) at a 1-m distance from the feeders (Figure 1a).

Behavioral analysis from feeding stations

From a total of 90 h of video footage, we assessed foraging effort and total macronutrient consumption by recording the total number of birds and time spent feeding at dishes containing the



Figure 1

Experimental setup of the cafeteria design for myna birds. (a) Feeding station charged with nutritionally defined foods with HP, HL, and HC content. Note the position of the video camera (left side) in relation to the feeding station. (b) Myna birds consuming different macronutrient specific foods within the 20-cm area delimited by the 5 black pegs.

different foods provided each day (i.e., giving us the total amount of foraging effort spent at a dish on an experimental day). To assess aggressive encounters at the feeding dishes, for each individual feeding at a dish we recorded whether the feeding event was terminated spontaneously or by the individual being displaced by conspecific interference; that is, by a conspecific flying or running directly at the feeding individual, following [Lowe et al. \(2011\)](#). Data extracted from the video footage were analyzed frame-by-frame using the open source software program Avidemux 2.6. Mynas are known for their ability to learn aversions to an environment in which they observe conspecifics encounter dangerous events such as capture and predatory attacks ([Griffin and Boyce 2009](#)). For this reason, birds were not captured and marked for individual recognition. Neither was it possible to distinguish between individuals on the basis of their features, given that mynas are monomorphic birds ([Feare and Craig 1998](#)). Therefore, here we have assumed that each time a bird is observed feeding it is a unique animal. This assumption may perhaps caveat our results. However, we have 2 reasons to assume that our true sample size (i.e., of truly unique animals) is above the median for behavioral field studies on birds ([Taborsky 2010](#)). Firstly, each “feeding event” was characterized by a large group of unique individuals (typically ranged from 5 to 40). Secondly, we gathered data from several different groups of birds.

Statistical analyses

Prey composition of diets

For statistical comparisons, frequencies of prey species successfully captured were tested against an expected equal distribution using chi-square tests (χ^2).

Consumption of different macronutrients from feeding stations

To assess differences in the consumption of food from each dish, we used linear mixed models (LMMs) implemented with the `lmer` function in the package `lme4` ([Bates et al. 2014](#)). In these models, the response was the amount of food eaten from a dish at the end of each day, measured in grams and log transformed (+0.5; [Yamamura 1999](#)). The predictor was a 5-level categorical variable giving the dietary content of the dish (HP, HC, HL, HP+HC, or HP+HL, with HP as the intercept). As described above, dishes contained different diets in different combinations at each feeding station on each day, therefore we also included a random factor giving a code denoting the feeder setup, correcting for the fact that the combination in which dishes of different diets were offered may influence consumption. We used the same LMM method to assess whether birds selectively consumed HP over HC or HL (i.e., nonprotein) constituents of mixed foods.

Aggression over nutrient access at feeding stations

To assess differences in aggression over macronutrients, we used a generalized linear mixed model (GLMM) implemented using the `glmer` function in the `R` package `lme4` ([Bates et al. 2014](#)), specified with a logit-link function (family = binomial). The GLMM was specified with a binary response denoting whether or not an observed feeding event was ended by an act of intraspecific aggression (1) or whether the bird stopped feeding of its own accord (0). The predictor was a 5-level predictor denoting the contents of the dish at which the observation was made (HP, HC, HL, HP+HC, or HP+HL, with HP as the intercept). Again, a random-factor correcting for the combination in which dishes of different diets were offered was included. Ideally, we would have included individual identity for unique birds as a random factor in this GLMM.

However, given the logistical constraints of marking birds this was not possible. Nevertheless, as discussed above, making reasonable assumptions about sociality it seems likely our sample size is above the median for behavioral field studies on birds ([Taborsky 2010](#)). From GLMMs and LMMs, statistical significance was inferred when an estimated difference had a 95% confidence interval (CI) not spanning 0.

Foraging effort and protein consumption

Multivariate LMMs were also used to assess how foraging effort translates to protein consumption at dishes containing HP foods and mixed foods. Here, the response was grams of HP foods eaten during the day (log transformed + 0.5), and the fixed predictors were a 2-level categorical predictor denoting the contents of the dish (HP diets only or a mix of diets) fitted with an interaction with a numeric predictor for the total amount of time birds were observed eating at the dish during the day (log transformed to linearize and unbound foraging time); this latter predictor was our index of foraging effort. Also fitted was the cumulative number of birds seen eating at the dish that day to correct for instances where consumption was higher simply because more birds were eating from the dish. Finally, we included a random-factor correcting for the combination in which dishes of different diets were offered.

All data were analyzed in the statistical programming environment `R` 3.1.2 (`R Development Core Team` 2014).

The right-angled mixture triangle

RMTs were used to explore the relationships among the proportional content of nutrients in the prey species of myna birds. This model enables a mixture of 3 components (e.g., the macronutrients protein, lipid, and carbohydrates) to be graphed in a 2D plot ([Figure 2](#)). For example, if protein (P), lipid (L), and carbohydrate (C) were present in a food at 15%, 25%, and 35% of total dry weight, respectively, then each would be expressed as: $P = 15 / (15 + 25 + 35)\% = 15 / 75\% = 20.0\%$; $L = 25 / 75\% = 33.3\%$; $C = 35 / 75\% = 46.7\%$, that is, as the percentage contribution of each to the total macronutrient content.

To geometrically define this mixture in an RMT, % P is plotted against % L. Considering that the 3 nutrients in the mixture sum to 100%, plotting % P (first axis) and % L (second axis) will automatically reflect the value of % C in the third axis. This plot enables a visual representation of a 3D mixture and also to compare and model different meta-mixtures from the nutritional composition of diets that are a consequence of mixing multiple foods ([Figure 2](#)).

RMT models provide a simple means of comparing complex data on foods and diets from different sources across species and sites ([Raubenheimer 2011](#)). In the present study, behavioral observations in opportunistic feeding events provided information on the main food items consumed by mynas. Second, [Sengupta \(1976\)](#) provided detailed estimates of the typical masses of individual prey types consumed by mynas. Third, for those prey species previously recorded in the diets of mynas (as mentioned above), we estimated the proximate nutrient composition from [Klasing \(1998\)](#) and for human discard using [US Department of Agriculture, Agricultural Research Service \(2015\)](#). Fourth, the field-based feeding stations enabled us to obtain the macronutrient composition of the dietary component taken from the feeding stations. The use of nutritional geometry allowed us to represent the results of these various components of the data within a single integrative model.

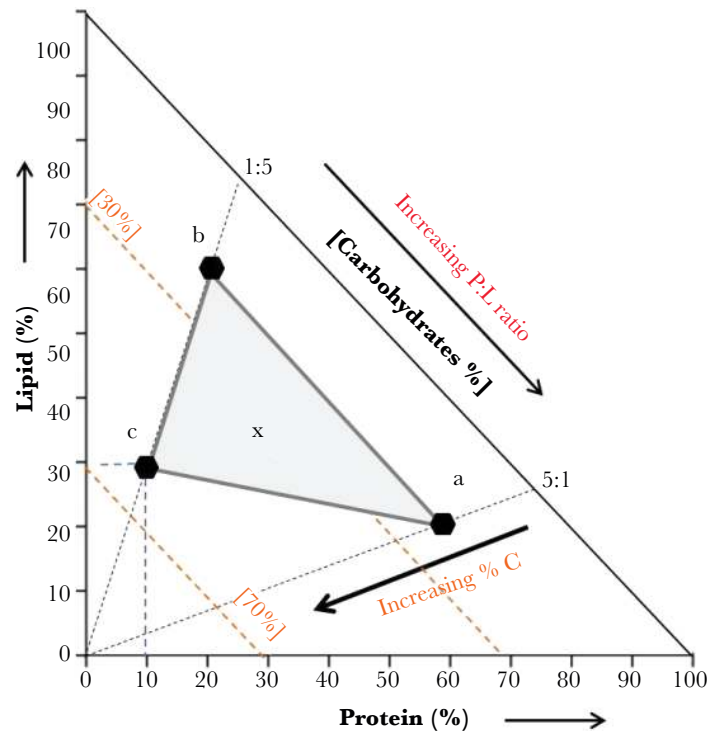


Figure 2

RMTs provide a means to plot 3 components in 2D graphs. In *a* each point represents a mixture of protein (P), lipid (L), and carbohydrate (C). % P and L increase in the normal way along the *x* and *y* axes, respectively, and the P:L balance of a mixture is given by the slope of the radial that connects the point to the origin. % C of a point is determined as the difference between 100% and the value at which a negatively sloped diagonal the point intersects with the 2 axes (Raubenheimer 2011). For example, point *a* contains 60% P, 20% L, and 20% C, with a 5:1 P:L ratio; *b* has the same % C (20%), but a lower P:L (1:5) than *a* and point *c* contains the same P:L ratio but higher % C (60%) than *b*. The plot also illustrates the fact that if an animal mixes its intake from 3 foods (e.g., *a*, *b*, and *c*) then the resulting diet composition is constrained to lie within the triangle connecting these foods (e.g., point *x*) also known as a nutrient niche.

RESULTS

Prey composition of diets

In total, we observed mynas feeding in $n = 256$ separate events, comprising 40.6% on insects, 33.2% on earthworms, 17.6% on human discard, and 8.6% on plants. These frequencies differed significantly from a random distribution ($\chi^2 = 65.0938$, degrees of freedom = 3, $P < 0.0001$).

Consumption of different macronutrients from feeding stations

On average, considerably more HP food was consumed during an experimental day than HC or HL diets, and these differences were statistically significant (LMM $\text{est.}_{\text{HC-HP}}$, CI = -3.372 , -3.901 to -2.844 ; LMM $\text{est.}_{\text{HL-HP}}$, CI = -2.751 , -3.162 to -2.341 ; Figure 3). Although overall consumption from dishes containing HP diets and those containing mixed dishes was similar, significantly more HP pellets were consumed than HC or HL pellets from those mixed dishes (LMM $\text{est.}_{\text{HC and HL-HP}}$, CI = -2.866 , -3.319 to -2.413 ; Figure 3).

Macronutrient composition of prey and diets

The RMT shows that protein-to-lipid ratio (P:L) from the different foods consumed by mynas varied considerably, from 11:1 (earthworms) to 1:1 (bread as a human discard) (Figure 4). In addition, the diet estimated from the experiments showed a higher P:L ratio (6:1) than the diet obtained from natural foods (3:1). The P:L:C ratios were 4.1:1.0:1.0 for pellets selected from the feeders

and 3.3:1.0:1.4 for the natural diets, these representing 55.8% and 47.9% of energy from protein, respectively.

Aggression over nutrient access at feeding stations

We made 296 observations of birds feeding at the dish containing HP foods, of which 66.4% ended in intraspecific aggression (Figure 5). In the same sampling period, we observed only 6 instances of birds feeding at the dish containing HC foods, none of which ended with intraspecific aggression (Figure 5). At the dish containing HL foods only 2 of 11 observed feeding events ended in aggression, which is a significantly lower proportion than at the dish containing HP foods (GLMM $\text{est.}_{\text{HL-HP}}$, CI = -1.902 , -3.469 to -0.334 ; Figure 5). There was also a smaller proportion of feeding events that ended in aggression at the dish containing HC+HP (3 out of 12) than at the dish containing protein foods alone, but this difference was not statistically significant (GLMM $\text{est.}_{\text{HC+HP-HP}}$, CI = -0.538 , -2.077 to 1.000 ; Figure 5). There was no significant difference between the proportion of feeding events that ended in aggression at the HP+HL dish (protein and lipid foods) versus the HP dish (GLMM $\text{est.}_{\text{HP+HP}}$, CI = -0.053 , -0.646 to 0.540 ; Figure 5).

Foraging effort and protein consumption

Based on 41 observations, the average time spent by birds foraging on HP diets during an experimental session was 607 s (range = 2–2533 s), whereas at the mixed dish the average total time spent by birds foraging during 1 experimental session was

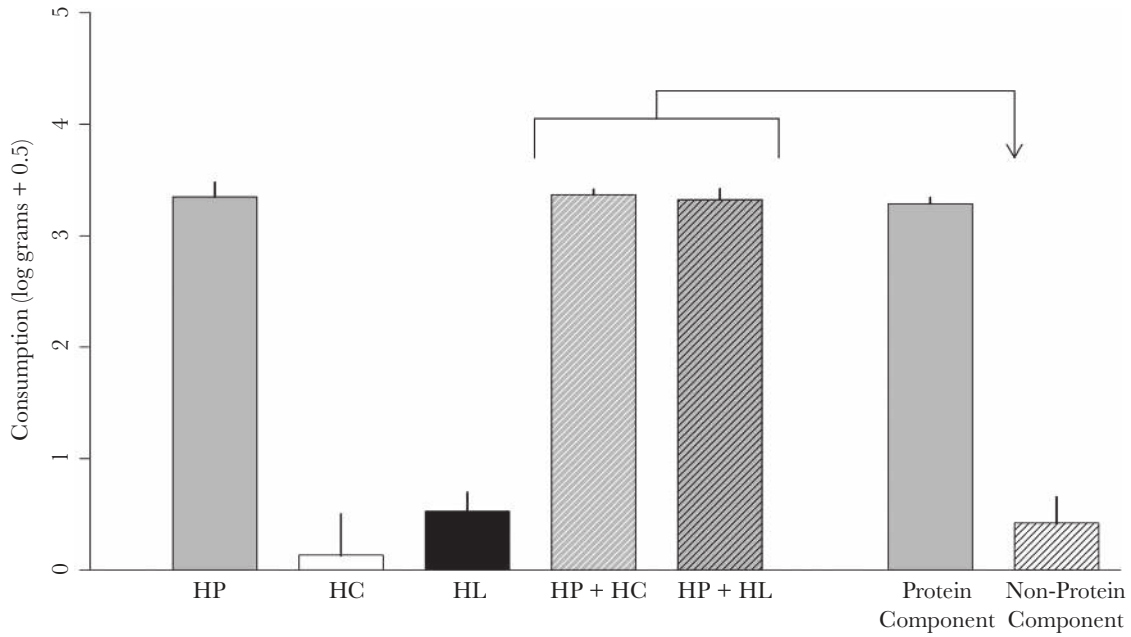


Figure 3

The log mean (+ standard error) grams of food consumed per day from dishes containing foods of differing macronutrient content. Protein (HP) $n = 42$, carbohydrate (HC) $n = 9$, lipid (HL) $n = 17$, mix 1 (HP + HC) $n = 7$, and mix 2 (HP + HL) $n = 7$. Also given is the mean daily consumption or protein and nonprotein (carbohydrates and lipid) components of dishes containing mixed pellets.

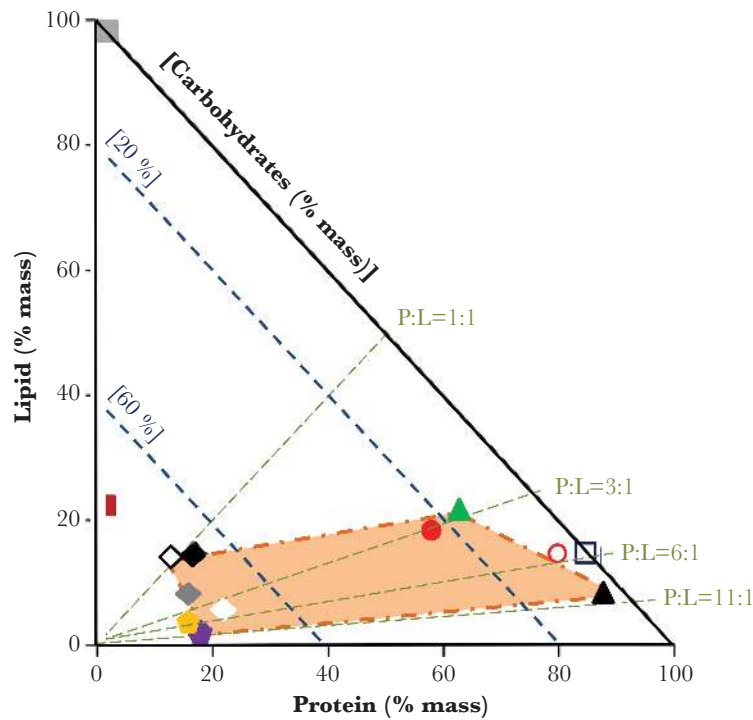


Figure 4

Right-angled mixture triangle showing (as a % of dry weight) the nutrient niche accessible to mynas delineated by short dashed and dotted orange lines as defined by natural foods (triangles and pentagons) and human discard (diamonds) that are part of their natural diet (red solid circle). The squares represent the different semisynthetic food pellets offered in the feeding experiments (hollow blue = HP diet; solid red = HC diet; solid gray = HL diet) and the hollow red circle is the diet estimated from the experiments. Solid black triangle = earthworms; solid green triangle = insects; solid gold pentagon = berries; solid purple pentagon = herbs; solid black diamond = French fries; hollow black diamond = bread; solid gray diamond = crackers and solid white diamond = chocolate-chip cookies.

864 s (based on 14 days, range = 218–2101 s). LMM analyses suggested that at the mixed dish greater foraging effort had very little effect on the amount of protein consumed, estimating a

slight positive but nonsignificant slope (LMM est. τ_{time} , CI = 0.091, -2.404 to 2.586). However, the LMM estimated a significant positive interaction between dish contents and foraging time (LMM

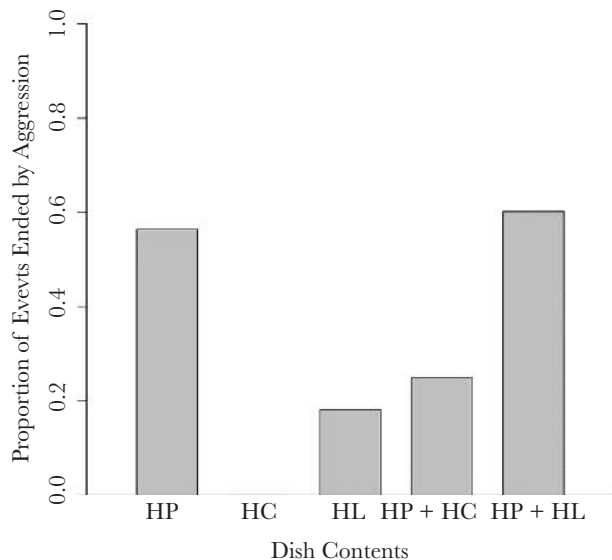


Figure 5

The proportion of feeding events observed to be ended by intraspecific aggression at dishes containing pellets of differing macronutrient content. Protein (HP), carbohydrate (HC), and lipid (HL) diets contained pellets high in those respective macronutrients, mix 1 contained a mix of HP and HC pellets and mix 2 contained a mix of HP and HL pellets; protein $n = 296$, carbohydrate $n = 6$, lipid $n = 11$, mix 1 $n = 12$, and mix 2 $n = 103$.

est. Time:Contents, CI = 0.484, 0.105 to 0.8663). These results suggest that for a given unit of foraging effort, mynas gain more protein at a dish containing HP only foods than from mixed dishes.

DISCUSSION

Nutrition is fundamental to almost all aspects of an animal's existence and is thus central in the adaptation of an animal to its niche (Raubenheimer 2010). Invasive species are characterized by their ability to overcome ecological challenges and adapt to novel environments, where foods are often unfamiliar (Sol et al. 2012). Urban environments are artificially modified ecosystems in which quality foods are patchily distributed (Dickman and Doncaster 1987), potentially presenting considerable challenges to foraging birds. Under these circumstances, access to a profitable food source can involve competition and agonistic behaviors (Weir and Grant 2004; Sol et al. 2012). Here, we provide behavioral and experimental evidence that myna birds do not forage primarily for energy intake, but preferentially select protein over carbohydrates and lipid. These results are consistent with previous findings in starlings (*Sturnus vulgaris*, Crabb 1978; Twedt 1985). Furthermore, mynas show agonistic behaviors in order to gain access to protein-containing diets and will even selectively consume protein from mixed dishes despite the increased costs in terms of foraging effort.

Preference for protein over nonprotein energy

Animals are known to feed selectively from those prey available according to nutrient composition to maximize fitness (Jensen et al. 2012; Simpson and Raubenheimer 2012). Our observational data show that mynas relied on insects, worms, and human discards in their diets, which is consistent with previous findings (Moeed 1975, 1976; Sengupta 1976; Pell and Tidemann 1997; Newey 2007). Insects and worms are highly nutritious and are believed to be the major source of protein in many avian diets (Klasing 1998), in contrast with human discards, which generally have low concentrations of protein relative to lipids and carbohydrates (Pierotti and Annett 1987). Dietary protein levels are known to play an important role in annual cycles of breeding and moult in birds, although little is known about the role of protein intake regulation in shaping the life histories of wild birds (Martin 1968; Twedt 1985; Klasing 1998; Halpin et al. 2014). Given the apparent preference of mynas for protein-rich natural foods, the nutritional and fitness benefits of a diet that combines HP foods (i.e., worms and insects) with HL and HC human discards warrants further investigation. Based on our findings, 3 nonexclusive hypotheses may be suggested 1) mynas spread their feeding over a variety of foods to ensure an adequate intake of micronutrients; 2) mynas eat human discard to balance their protein gain with adequate lipids and carbohydrates, which are naturally low in insects and earthworms (i.e., as nutritionally complementary foods); 3) mynas consume discards to gain any available protein in the food, even in small quantities, at the cost of overconsuming lipid and carbohydrates to meet their protein requirements (this latter process is believed to be a nutritional strategy adopted by a number of species; Simpson and Raubenheimer 2012; Raubenheimer et al. 2015). If so, then discards are supplementary to natural foods in the diet as per our third hypothesis.

Regarding the first hypothesis, an important aspect of the design of our feeding trials is that the foods were standardized for micronutrient content (see Table 1 for more details). We can thus be confident that these foods were not being eaten to supplement a micronutrient deficiency incurred in the normal foraging environment (in which case we would expect the 3 foods to be equally targeted), and also that preference for the HP pellets related to their macronutrient content. Our results therefore suggest that the mynas in the study specifically targeted HP foods, even when HL and HC foods were freely provided. With regard nutritional complementarity, it has been suggested that mynas consume high amounts of berries and herbs during spring and summer while feeding their offspring (Moeed 1976; Sengupta 1976). Berries are seasonal fruits that have the highest concentrations of carbohydrates among the natural components of the diet and thus may play a fundamental role as nutritionally complementary foods (Figure 4). Our nutritional model (RMT) shows that human discards have similar proportions of carbohydrates to berries, and it is plausible that during the nonberry season, mynas consume more human discard and other foods as a source of carbohydrate (Figure 4). Further studies are needed to test the possible seasonality of myna diets in urban and rural environments.

The feeding trials showed that mynas had a clear preference for HP diets over HC and HL diets. These results suggest that in their urban foraging environments mynas are more commonly deficient in protein, with greater access to nutritionally imbalanced food sources (human discards) high in carbohydrates and fats (Figure 4). A decrease of naturally available protein may be related to the increase in the number of man-made structures in an urban environment, which has been suggested to negatively impact

the distribution of natural foods available to urban birds (O'Leary and Jones 2006). It has been suggested that wild Australian birds are likely to be more limited by protein than nonprotein energy. Australia is known for its nutrient poor soils that encourage plants and trees to produce excess of carbohydrates in their leaves (Orians and Milewski 2007). Thus, insects consuming leaves of Australian plants also consume high levels of sugar, forcing birds, like mynas, to imbibe more sugar than they need in order to meet their protein requirements (Woinarski et al. 1989). The prioritization by mynas for HP pellets, along with their almost complete indifference toward HL and HC pellets, shows that foods with a HP concentration are preferred given free-choice. However, the amino acid intake targets of mynas are unclear and warrant further study. For example, if mynas regulate their intake of digestible lysine, methionine and threonine more closely than alternative amino acids (as is the case in, e.g., farmed avian species, Cowieson 2005) then the amino acid composition of food items may exert as strong a leveraging effect on food choice and intake than does the protein concentration per se. Thus, systematic assessment of the amino acid composition of common HP food items within the habitats of myna birds and within geographical areas vulnerable to expansion of their range may give valuable insight for management of this species in future.

Foraging effort and aggression over protein diets

Macronutrient imbalance is well known to elicit aggression in some insects (Grover et al. 2007). It has been suggested that carbohydrates are the major source of aggression in Australian birds (Low 2014). Conversely, our behavioral analyses, which provided macronutrients ad libitum, showed that the majority of aggressive encounters occurred when myna birds were feeding on protein-containing diets (either solely HP foods or mixes containing protein). By way of contrast, aggression over foods other than the HP one was a relatively rare event, and given that we were not able to differentiate individual birds it is even possible that these rare events are attributable to just few individuals. Data from computational approaches clearly demonstrate that contest competition over access to limiting nutrients is thought to lead to clear and stable dominance hierarchies (Lihoreau et al. 2014; Senior et al. 2015). In fact, mynas are known to display dominance hierarchies in their population structure and foraging flocks with sex and age been predictors of dominance (Moed 1975; Feare and Craig 1998; Old et al. 2014). They have also been suggested to show agonistic behavior to defend and control nests and those food sources that are extremely valuable to them (Lowe et al. 2011; Old et al. 2014). An exciting avenue of research in the nutritional ecology of mynas will be to explore the potential role that the availability of environmental protein plays in development of myna dominance hierarchies.

Avian predators are known to trade-off foraging effort and the physiological costs of consuming toxins against the benefits of gaining essential nutrients (Halpin et al. 2013, 2014). Our analyses suggest that mynas are willing to pay a cost in terms of foraging time in order to selectively consume HP diets out of a mix (HP+HC or HP+HL). This finding further demonstrates the value of this macronutrient to urban mynas. One possible mediator of the cost-benefit trade-off of selectively consuming protein out of the mixed dish may be the intensity of intraspecific competition for more "high-value" protein-rich food sources. Specifically, it is possible that as the intensity of competition at a high-value protein food source increases, an individual will become more likely to pay the time-costs associated with obtaining protein from other sources. Indeed

theoretical work has demonstrated that the intensity of contest competition can drive organisms to alter their nutritional strategy (Senior et al. 2015). Mynas may well make a good model organism for future experimental work on how the economics of foraging for protein in a complex environment alter as conspecific competition intensifies.

In demonstrating protein preferences in the diet of mynas, our study provides novel insights into the nutritional ecology of this successful invader. A challenge ahead is to integrate field data of the sort reported here with experimental studies of macronutrition in the laboratory, to understand the role of nutrition in the decisions made by mynas and other invasive species foraging in complex environments. This knowledge will also have direct practical relevance for the study of invasive species in general. First, understanding the nutritional drivers of invasion will provide novel insights in to how invasive species succeed in novel environments. Second, understanding physiological responses to nutritional flexibility and nutritional stress is likely to enhance our knowledge of ecological interactions and dispersal dynamics in relation to the quality of food sources. Third, a broader understanding of the nutritional ecology of invasive species could enhance monitoring programs, providing a new dimension to management techniques. Overall, we believe that mynas hold considerable potential as a model system for studying the key nutritional traits that underpin the ecological flexibility of highly successful invasive species.

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