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1 **Tasting novel foods and selecting nutrient content in a highly successful**

2 **ecological invader, the common myna.**

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Abstract

Invasion success is dependent on the ability of a species to discover and exploit novel food resources. Within this context, individuals must be willing to taste novel foods. They must also be capable of evaluating the nutritional content of new foods, and selecting their relative intake in order to fulfil their nutritional needs. Whereas the former capacity is well studied, little is known about the latter capacity. First, using the common myna as a model avian invader species, we quantified the willingness of mynas to taste novel foods relative to familiar ones. Mynas readily tasted high protein (HP) novel foods and consumed them in higher quantities compared to a familiar food. Data showed that at three different levels – mixes, ingredients and macronutrients – intake could not be explained by a random model. In experiment 2, we confirmed that mynas were making their selection based on protein (P) content rather than a selection for novelty per se. When given the choice of three equally unfamiliar foods, mynas again ate disproportionately from the high protein relative to high lipid and high carbohydrate foods. Analysis revealed that mynas consumed amounts of protein that were closer to the ones in their natural diet. Finally, in experiment 3, we measured inter-individual variation in innovation and exploration propensities, and examined associations with inter-individual variation in consumption of specific macronutrients. This analysis revealed that individuals that selected HP pellets were more exploratory and individuals that selected HC pellets were quicker to solve the innovative foraging task. These findings indicate that not only the willingness to taste novel foods, but also the capacity to evaluate their nutritional content, might be central to the myna's substantial ecological success.

1. INTRODUCTION

To establish in a novel environment, invasive birds have to face new ecological challenges such as finding new shelter and recognizing new predators and competitors (Coleman and Mellgren 1994, Griffin et al. 2016). Within this context, there is evidence that the ability to exploit novel food resources is particularly important (Sol et al. 2011, 2012a). Avian species with high numbers of reports of novel feeding behaviours, including consuming novel foods and developing novel foraging techniques, are more likely to become established when introduced to environments outside their native range than species with fewer such reports (Sol et al. 2002, 2005, Wright et al. 2010). Thus, to date, research has established that success in new environments depends heavily upon an invader's ability to consume novel food, develop new foraging behaviours and/or use pre-existing ones in novel contexts (Mayr 1965, Sol and Lefebvre 2000, Sol et al. 2005, 2008, Griffin et al. 2016).

Foods are complex blends of many nutrients each of which has their own effect on the forager (Raubenheimer et al. 2012). Lipid (L) and carbohydrates (C), in particular, play a central role in metabolism and energy storage whereas protein (P) is fundamental to animals' growth and reproduction (McWilliams 2011). Nutritional goals can vary among individuals, for example as a function of age and/or sex (Simpson et al. 2010), but also among geographically isolated populations and among species (Tait et al. 2014). The complexities of nutrient content and their impact on individual health generate a challenge for invaders. Novel foods are likely to differ in their nutritional composition and physiological properties to those foods that invaders have encountered previously (Machovsky-Capuska et al. 2016a). Therefore, not only must invaders be willing to consume novel foods, they must also have the ability to identify the nutritional content of these novel foods and combine their proportional intake to fulfil their nutritional goals (Machovsky-Capuska et al. 2016a).

Exploration and innovativeness in the foraging context have been consistently linked to invasion and colonization of novel habitats in birds (Holway and Suarez 1999, Sol and Lefebvre 2000, Sol et al. 2002, Russell et al. 2010, Wright et al. 2010). Nutritional intake might impact behaviour in several ways. First, it is known that nutritional deficiencies can produce personality biases in later life

61   (Fraňková 1973, Barnes et al. 1976, Almeida et al. 1991, 1993, 1994, 1996). For example, low P intake

62   during ontogeny is linked to increased exploration in adult rats (*Rattus norvegicus*). This link between

63   low P intake and activity might be maintained through life as suggested by research in invertebrates.

64   Crickets (*Anabrus simplex*) deficient in P locomote more than crickets satiated in protein (Simpson et

65   al. 2006). Second, it is possible that the tendency to forage on certain nutrients specifically might

66   enhance certain types of foraging behaviours. For example, urban exploiters, which rely heavily upon

67   human discards rich in L and C, might have an enhanced motor diversity due to their frequent handling

68   of packaged human discards in school playgrounds, supermarket carparks and around fast food outlets.

69   Higher motor diversity is known to facilitate innovative foraging (Griffin et al. 2013a, Diquelou et al.

70   2016). These nutrient-behavioural relationships have yet to be explored in invasive birds.

71   Classified as one of the world's 100 worst invasive species (Lowe et al. 2000), the common 'Indian'

72   myna (*Acridotheres tristis*, recently proposed to be reclassified as *Sturnus tristis* by Christidis and Boles

73   (2008); hereafter mynas) is an excellent model to investigate the physiological and behavioural

74   attributes of ecological invaders (Griffin et al. 2010, 2013a, b, 2014, Sol et al. 2011, 2012b). Introduced

75   to Australia late 19th century (Martin 1996, Pell and Tidemann 1997, Tidemann 2005), mynas are now

76   present along much of the east coast (Grarock et al. 2013). The species is well known for its ability to

77   learn about novel environmental stimuli. Previous research has demonstrated learning of bait avoidance

78   (Feare 2010), the location of human hunters (Dhami and Nagle 2009), novel predators (Griffin 2008),

79   and dangerous places (Griffin et al. 2010, Griffin and Haythorpe 2011). The species is also known to

80   reduce flight distances in areas highly frequented by humans (McGiffin et al. 2013).

81   Based on the traditional approach of quantifying dietary generalism in terms of foods consumed, mynas

82   are documented to be generalist omnivores. Indeed, the species has been reported to consume a broad

83   range of foods including invertebrates, plants, fruits, and human discards (Moeed 1975, 1976, Sengupta

84   1976, Pell and Tidemann 1997, Machovsky-Capuska et al. 2016b). Diet generalism is a well-established

85   predictor of invasion success (Cassey 2002, Blackburn et al. 2009) and is likely to explain some part of

86   the myna's extraordinary worldwide ecological success. On the other hand, mynas are known to taste

87   novel foods (Sol et al. 2012b), but it is not known whether they would choose novel foods when given

88   the choice. It is also not known whether mynas have the capacity to identify the nutritional composition

89   of novel foods and adjust their relative consumption to achieve their nutritional goals. Finally, it is not

90   known whether the tendency to selectively consume certain nutrients is associated with individual

91   variation in exploration and/or innovative foraging behaviour.

92   In the present study we combined behavioural observations with captive feeding trials and nutritional

93   geometry to examine food and nutritional selections in common myna birds. In particular, we aimed to:

94   i. determine whether mynas fed with novel foods select their foods, ingredients and nutrients in non-

95   random proportions, ii. establish the macronutrient composition of myna diets using high-protein (HP),

96   high-lipid (HL) and high-carbohydrate (HC) pellets, and iii. determine whether consumptions of

97   particular macronutrient were associated with innovation and exploration propensities.

98   ## 2. METHODS

99   ### SUBJECTS AND HUSBANDRY

100   A total of 21 birds were caught in and around Newcastle (NSW, Australia) using a trap specifically

101   designed for this species (Tidemann 2009). All twenty-one participated in experiment 1, and a subset

102   of ten also participated in experiment 2. Upon capture, birds were transported to the Central Animal

103   House (CAH) at the University of Newcastle, where they were measured (tarsus, beak and wing),

104   weighed and individually marked with coloured plastic leg bands. Birds were then released into outdoor

105   group aviaries (2.0m x 1.0m x 2.0m, H x D x W) and treated for internal parasites during a period of

106   ten days. Mynas were then transferred to a large flight aviary (2.2m $\times$ 1.2m $\times$ 4.4m) where they were

107   held until testing commenced. Food and water was available ad libitum at all times. Birds were fed a

108   commercial brand of puppy dog pellets (SUPERCOAT® Puppy with Real Meat). Dog pellets were

109   used because they are small enough to be swallowed by mynas easily. Dog pellets were the most widely

110   used food to bait myna traps in Australia and were originally selected as suitable bait based on myna

111   food choice tests undertaken in the context of developing a species-specific trap (Tidemann 2009). In

112   addition, free-ranging mynas frequently consume dog pellets from suburban back gardens with dogs

113   (Parsons et al. 2006). Based on these observations and on veterinarian advice, they were an adequate

114   food to feed captive-held mynas and have been used since 2006 in our myna research program.

115   During the food and macronutrient selection experiments, birds were held in individual cages (0.6m x

116   0.6m x 0.6m) located outdoors but protected from weather conditions by a roof. Individual cages were

117   equipped with a perch and a nest box and spatially arranged such that birds were in close visual and

118   acoustic contact. This arrangement facilitates mynas' adjustment to individual housing (Griffin and

119   Boyce 2009, Griffin and Haythorpe 2011, Sol et al. 2012a, Griffin et al. 2013a).

120   All husbandry and experimental procedures were approved by the Animal Care & Ethics Committee of

121   the University of Newcastle (Animal Research Authorities A-2011-127, A-2014-424, and A-2014-425).

122   PROCEDURE

123   **Experiment 1: Novel food selection**

124   To measure the food choices of mynas in the presence of new, unfamiliar foods, 21 mynas were

125   transferred to individual cages and left for two days to acclimatize to their new holding conditions.

126   During this time, each myna had ad libitum access to water, as well as ad libitum access to the now-

127   familiar dog pellet food (see above).

128   Over the course of the following three days, birds were presented with a simultaneous choice of three

129   foods. To create these foods we mixed ingredients that differed in their macronutrient composition: Dog

130   pellets (DP, 14.0% L, 29.0% P and 46.6% C), commercial insectivore powder named Wombaroo® (W,

131   12.0% L, 52.0% P and 18.0% C) and commercial parrot food called Superior Egg & Biscuit Vetafarm®

132   (EB, 8.0% L, 16.0% P and 63.0% C). The foods were created manually under laboratory conditions in

133   order to produce experimental pellets that were visually identical and novel to the captive birds. The

134   ingredients were selected with the primary aim of gradually reducing the proportion of familiar content

135   (dog pellets), and increasing the proportion of novel content. This allowed us to quantify the mynas'

136   willingness to consume increasingly novel foods. The foods varied along a gradient of gustatory

137   familiarity, but also differed in their nutritional composition. The first food (Mix 1), contained solely

the birds' familiar DP and the nutritional composition of the mix (dry mass) was: 15.6% L, 32.4% P and 52.0% C; the second food (Mix 2) contained different proportions of DP and W and the nutritional composition of the mix (dry mass) was: 15.2% L, 44.1% P and 40.6% C; and the third food (Mix 3) contained different proportions of DP, W and EB, and the nutritional composition of the mix (dry mass) was: 13.5% L, 36.7% P and 49.7% C.

In order to measure which of the three foods mynas selected, 7.5 g of each food was placed in one of three small individual dishes (3 cm diameter, 2 cm deep). The three dishes were attached to a wooden board so that they could not be tipped over (see photography in Supplementary material Appendix 1, Figure A1). The 3-dish board was then offered to each bird for three successive 24-h time periods. Each food was presented in each dish once across the three testing days in order to avoid specific location preferences. At the end of each 24-h period, any remaining food was collected and weighed with a high-precision scale. Each cage was equipped with a removable floor tray that collected any dropped food. Any food in the tray was collected and incorporated to the left-over food before weighing. A control board with equivalent amounts of food was placed in an adjacent empty cage in order to measure any change in mass that might have occurred as a consequence of desiccation or humidity. As weight changes of all control foods placed in an empty myna cage were negligible (weight changes across the three days (in percentage of total food consumed): Mix 1: -1.1%; Mix 2: -0.91%; Mix 3: -0.92%), they were not considered further.

Experiment 2: Macronutrient selection

Experiment one manipulated food familiarity, but in doing so, simultaneously manipulated nutrient content. Hence, the purpose of experiment 2 was to manipulate macronutrient content while holding energy content and familiarity constant (i.e. all foods were novel). Ten common mynas were transferred from the group flight aviary to individual cages identical to those used in experiment 1 and allowed two days to acclimatize to their new surroundings. As in experiment 1, birds had access to food (DP) and water ad libitum during acclimatization. Over the course of the following three days, each bird was presented with a simultaneous choice of three semi-synthetic pellet foods that were isoenergetic (2600

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164 kcal/g), but differed in their macronutrient composition (P, L, C) (for details see Machovsky-Capuska

165 et al. (2016b)). During tests, 25 g of each food was placed in one of the three small individual plastic

166 bottles attached to one side of the cage so that they could not be tipped over (see photography in

167 Supplementary material Appendix 1, Figure A1). The three bottles were then offered to each bird for

168 three successive 24-h time periods, as in experiment 1. At the end of each 24 h period, any remaining

169 semi-synthetic food was collected and weighed with a high-precision scale.

170 **Experiment 3: Behavioural analysis**

171 The aim of experiment 3 was to explore potential relationships between individual variation in

172 macronutrient intake and individual variation in exploration propensity and innovativeness.

173 *INNOVATION TEST*

174 One potential approach to quantifying innovative foraging is to measure an individual's propensity to

175 solve novel foraging problems (Webster and Lefebvre 2001, Griffin and Guez 2014). To this end, each

176 myna was presented with either a puzzle box consisting of a closed Petri dish (presented right way up

177 with a flexible handle or upside with a hook attached to the top part) or a small Plexiglas box with a lid

178 and a handle (for examples, see schematic in Supplementary material Appendix 1, Figure A2). Mynas

179 could solve the extractive foraging problem by pulling the small handle or the hook off the top of the

180 puzzle box, or, alternatively, by either levering or pushing the lid upwards. The reward (a dog pellet or

181 a mealworm) was visible but the apparatus needed to be opened to access the food.

182 These tests were conducted early in the morning following an overnight food deprivation period. To

183 avoid a neophobia response to the tasks, the apparatus had been placed in the same location as the

184 mynas' daily feeding dishes with an available food reward the evening before the trial. To begin the

185 trial, the tasks were presented with one visible reward inside but unavailable without solving the task.

186 Trials were video recorded for 30 minutes following the introduction of the apparatus. If the bird failed

187 to make contact with the task within 30-min the first time round, they were given a second opportunity

188 at least 0.5-1.5 h later. To investigate differences between birds in problem-solving propensities, an

189   innovation score was calculated using latency to solve (s) minus latency to first contact the apparatus

190   (s). A score of 1801s was attributed to birds that failed to solve the task.

191   *EXPLORATION TEST*

192   Exploration propensity was assessed in an unfamiliar room under artificial light (Dingemanse et al.

193   2002) (see room's plan in Supplementary material Appendix 1, Figure A3). The room included five

194   artificial trees each bearing five perches. The ground was divided in four areas (87 cm x 116 cm). Birds

195   were moved from the aviary to an individual cage placed on wheels. The individual cages were covered

196   with a large piece of opaque material and rolled into the unfamiliar testing room. The opaque material

197   was gently lifted and the bird left alone to acclimate 10 minutes to this environment before the doors of

198   their cage (1cm x 27cm x 55 cm) was opened remotely by the experimenter. The bird was given a 15-

199   min latency to exit its home-cage, if it had not departed from the cage by the end of that period the

200   experimenter approached the cage to induce exit. Once the bird exited the cage, it was given 10 min to

201   move freely around the room. Birds had the ability to return to the home cage during the test, however

202   the nest box remained closed at all times during testing. White noise was played back through a

203   loudspeaker to mask the sounds of birds in a nearby room.

204   In order to determine exploration tendency, several variables were scored from the videos, including:

205   number of trees, perches and ground areas visited. To assess the bird's activity, we scored the number

206   of movements (flights between trees, hops between perches, walks on the ground and returns to cage).

207   The latency to exit the cage was also scored.

208   STATISTICAL ANALYSES

209   Variables scored for the exploration (i.e. number of movements, number of zones visited, number of

210   floor-zones visited, number of trees visited, number of branches visited, and latency to exit home cage)

211   were compiled in a Principal Component Analysis (PCA), using the correlation matrix and a Varimax

212   rotation, in order to reduce the number of independent variables and obtain an individual exploration

213   score for each bird. Values on the first principal component were used as exploration score for each

214   individual bird.

215   Results were analysed using non-parametric statistics in the form of permutation test (sometimes called

216   a randomization test, a resampling method similar to bootstrapping (Good 2000)). In experiment 1, we

217   tested whether mynas ate randomly from the novel foods provided by comparing the consumption of

218   foods, ingredients and nutrients with a null expectation in which mynas ate equal amounts using

219   approximative general independence tests (repeated measures). In experiment 2, the same approach was

220   used to compare the amounts of each HP, HL and HC foods consumed by the birds. Descriptive statistics

221   for food consumption were also presented as median and range. Significant effects were followed up

222   with post-hoc pairwise comparisons using approximate 2-sample permutation tests stratified by

223   individuals and p-values were corrected using the Bonferroni-Holm correction (noted p').

224   For each semi-synthetic food consumed in experiment 2, correlations between the total amount of

225   macronutrient (P, C, and L) consumed and behavioural data were examined using another form of

226   permutation test, a general Independence test.

227   Following Raubenheimer (2011), we used nutritional geometry (right-angled mixture triangles, RMT)

228   to portray the experimental food choices of mynas faced with unfamiliar foods. For experiment 1, we

229   determined food consumption and diet of mynas at three different scales (foods, ingredients and

230   nutrients) and we compared observed intakes against a null hypothesis that mynas consumed equal

231   amounts. For experiment 2, we obtained the macronutrient composition of diets (expressed as P:L:C

232   dry mass ratios) from the consumption of semi-synthetic foods. We then compared our results with the

233   i) null hypothesis of consumption of equal amounts (dry mass), ii) macronutrient composition (dry

234   mass) of the diet of free-ranging mynas as determined by stomach content analyses (Sengupta 1976,

235   see Machovsky-Capuska et al. 2016b for more details), and iii) macronutrient composition (dry mass)

236   of the diet obtained using an experimental 'cafeteria' design (see Machovsky-Capuska et al. 2016b).

237   All statistical analyses were carried out using R v3.1.1 (R Development Core Team 2014) (for

238   Permutation tests) and JMP® version 10 (SAS Institute Inc., Cary, NC, 1989-2007.10) (for PCA). Two-

239   tailed tests were used throughout and alpha levels were set at 0.05. All visual representations, including

240   RMT models, were created with SPSS Statistics 21 (IBM Corp 2012).

3. RESULTS

Experiment 1

The amount of food consumed by mynas differed significantly across the three food mixes (Mix 1: median = 12.03g, range = 17.53g (4.56-22.09g); Mix 2: median = 17.59g, range = 15.08g (6.36-21.44g); Mix 3: median = 16.20g, range = 16.05g (5.81-21.86g); approximative general independence test: n = 21, χ^2 = 8.75, p = 0.01, Figure 1a). Mix 1 was consumed in significantly smaller quantities than Mix 3 and Mix 2 (approximate 2-sample permutation test with Bonferroni-Holm correction: Mix 1/2: p'= 0.04; Mix 2/3: p' = 0.04), indicating that the food novel in its appearance, but most familiar in terms of its content (DP), was the least consumed food. Mixes 2 and 3, which were equally novel in appearance, but contained increasing proportions of novel contents, were not consumed in significantly different proportions (approximate 2-sample permutation test with Bonferroni-Holm correction: p' = 0.98).

The amount consumed of each of the three ingredients and nutrients also differed significantly after the three days of experimentation (approximative general independence test: ingredients: n = 21, χ^2 = 38.67, p< 0.0001, Figure 1b; nutrients: n = 21, χ^2 = 39.75, p< 0.0001, Figure 1c), showing that mynas fed non-randomly from their foods dishes at the three difference scales analysed.

The macronutrient composition of the diet (dry mass) estimated during the experiment 1 was 37.3 %P (± 0.2 SE): 14.8 %L (± 0.1 SE): 47.9% C (± 0.2 SE).

Experiment 2

The amount consumed of HL, HP and HP foods differed significantly (HL: median = 4.77g, range = 17.21g (0.55-17.75g); HP: median = 30.19g, range = 23.44g (20.08-43.52g); HC: median = 7.00g, range = 24.40g (2.19-26.58g); approximative general independence test: n = 10, χ^2 = 15; p < 0.0001). HP pellets were consumed in significantly greater quantities than HL and HC pellets (approximate 2- sample permutation test with Bonferroni-Holm correction: HP/HL: p' = 0.01; HP/HC: p' = 0.01).

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266   The macronutrient composition of the diet (dry mass) estimated during the experiment 2 was 59.8%P

267   (± 0.8 SE): 22.0 %L (± 0.7 SE): 18.4% C (± 1.0 SE).

268   The RMT model showed the differences of the P:L:C ratios of the diets estimated for experiments 1

269   (P:L:C= 2.5:1.0:3.2) and 2 (P:L:C= 2.7:1.0:0.8) with previously reported experimental feeding trials for

270   mynas (P:L:C= 6.3:1.0:0.1) and also with the reconstructed natural diet (P:L:C= 3.3:1.0:1.4, see

271   Machovsky-Capuska et al. 2016b for more details) (Figure 2).

Experiment 3

273   Correlation analysis revealed the existence of two relationships between behavioural traits and the

274   consumption of specific macronutrients (Figure 3, see full correlation table and scatter plot panel of the

275   correlation analysis in Supplementary material Appendix 2, Table A1 and Figure A2). Individuals that

276   consumed larger total amounts of carbohydrate pellets in a three-way choice between C, L and P were

277   faster to solve a novel extractive foraging task (Figure 3A; approximate general Independence test:

278   n = 10, Z = -1.89, p = 0.04). We found no evidence of a relationship between innovation latencies and

279   intake of the other two macronutrients (P and L) (approximate general Independence test: protein:

280   n = 10, Z = -0.63, p = 0.57; lipid: n = 10, Z = 1.37, p = 0.17).

281   Behavioural variables used to describe levels of exploration were aggregated into a PCA. The KMO

282   test suggested the matrix was appropriate for use in a PCA (KMO = 0.74). The first axis explained

283   58.2% of the variance in the data and was considered to be a good summary of the data (Budaev 2010).

284   Values on this first axis were used as the exploration score of each individual, where high scores

285   indicated high tendency to explore (Table 1). Correlational analyses between each individual's PC1

286   score and its relative intake of each macronutrient during experiment 2 revealed an association between

287   an individual's relative protein intake and exploration tendency. Mynas that consumed more protein

288   had higher exploration PC scores (Figure 3B; approximate general Independence test: n = 10, Z = 1.91,

289   p = 0.04). No relationships were found between an individual's exploration score and either its

290   carbohydrate (approximate general Independence test: n = 10, Z = -0.59, p = 0.58); or its lipid intake

291   (approximate general Independence test: n = 10, Z = -1.31, p = 0.20).

DISCUSSION

In this study, we aimed to analyse mynas' food and nutritional selection through the presentation of novel foods. We intended to identify food selection patterns and also whether the patterns of consumption of some macronutrients were correlated with innovation and exploration propensities. In experiment 1, birds showed significantly less interest for a familiar dog food relative to two other foods, despite the dog food mix being the most familiar in terms of content. Our analyses also showed that mynas fed in non-random proportions at three different scales analysed: foods, ingredients and nutrients. In experiment 2, mynas selectively consumed HP when simultaneously offered a choice between HP, HC, HL pellets. The RMT analyses also revealed that mynas consumed different amounts of protein to their natural diet described by Machovsky-Capuska et al. (2016b). Finally, experiment 3 revealed the existence of two nutrient-behavioural relationships. Mynas that consumed greater amounts of HP pellets had a stronger tendency to explore whereas those mynas that ate more HC pellets were faster to solve a novel foraging problem.

The history of captive myna feeding in the present study, together with their patterns of food choices during testing, strongly suggest that mynas selectively consume novel foods relatively to the nutritional content. In experiment 1, we showed that when mynas were given a choice between foods containing a gradually reduced proportion of familiar content (DP), they chose to consume higher amount of the foods that contained increased proportion of unfamiliar content (EB and W). Birds actually chose novel combination of HP foods.

Experiment 2 confirmed that it was not novelty per se that was driving this selection, but rather protein content, by showing that mynas selected HP relative to HC or HL when all three foods were equally novel. These observations are consistent with the findings of Machovsky-Capuska et al. (2016b). In that study, free-ranging urban mynas selectively chose to consume almost exclusively protein-enriched foods, leading the authors to speculate that free-ranging mynas might be protein deficient. Here, we extend these findings by confirming that mynas are willing to consume novel foods in such proportions that would enable them to reach their nutritional goals.

Animals are likely to detect certain nutrients using olfactory and gustatory cues (Simpson and Raubenheimer 2012). For example, gallinaceous chicks display an unlearned predisposition to peck at foods (Suboski and Bartashunas 1984), which presumably enables them to perceptually locate salt and glucose. In contrast, the identity of plants containing HP, which lacks a perceptual signature, is socially transmitted from mother to chick in gallinaceous chicks (Allen and Clarke 2005). As our mynas were individually held and all foods were visually identical and offered randomly, mynas were not able to mimic each other's food choices. Furthermore, our novel foods had no resemblance to insects (that are also HP foods), meaning that mynas could not have been able to rely on previous experience to select foods more likely to contain protein. Hence, their detection and selection of HP foods were likely to be triggered by different physiological mechanisms including systemic nutrient sensing mechanisms, neural circuits that control feeding behaviour, hormonal feedback from body reserves (Morton and Schwartz 2011) and also post-ingestive regulatory responses that assist in the adjustment of imbalanced nutrients (Simpson and Raubenheimer 2012). A challenge ahead should aim to determine the mechanisms by which mynas sense nutrients and in particular detect HP foods. Also, further experiments will be undertaken to rule out the alternative possibility that mynas only demonstrated taste preferences for particular foods instead of actively regulating their macronutrient intake, although a large body of literature spanning many animal taxa suggest this is unlikely (Simpson and Raubenheimer 2012).

The present study revealed that mynas consuming higher amounts of HP foods were more exploratory when confronted with a novel environment. Moreover, individual's tendency to explore a novel space is a repeatable trait in mynas (Lermite et al. 2017). To reduce the impacts of behaviour unrelated to exploration during this open-field test, specific methodologies were used (i.e. individuals were not food deprived and were free to exit home cage) to limit the expression of stress-related responses and facilitate information acquisition (Dingemanse 2002; Mettke-Hofmann et al. 2002, but see discussions in Carter et al. 2013 and Lermite et al. 2017). Together, these findings suggest that mynas with higher exploratory tendencies are likely to be more sensitive to dietary fluctuations in protein. A relationship between low-protein diet and exploration has been demonstrated in rats (Almeida et al. 1991, 1993,

345   1994, 1996). A lack of dietary-protein has been suggested to cause an increase of "impulsiveness",

346   driving malnourished rats to explore more open-arm novelty (Almeida et al. 1991, 1993, 1994, 1996).

347   This behavioural pattern reflects a decrease in anxiety in the protein-deficient individuals. Protein-

348   malnutrition is suspected by the authors to cause deleterious effects on brain structures underlying

349   inhibitory behaviours in situations promoting anxiety (Almeida et al. 1994). Hence, lower anxiety levels

350   induced by protein malnutrition could act as a mediator for higher levels of exploration in these protein-

351   seeking birds. Moreover, in the context of an urban environment, proteins are expected to be rare and

352   extremely valuable (Eagle and Pelton 1983, Pierotti and Annett 1987, Murphy 1993, Machovsky-

353   Capuska et al. 2016b). Foraging for HP foods in urban environments (e.g. insects) would therefore

354   require longer periods of time spent exploring the environment, which could in turn produce a feedback

355   loop reinforcing the expression of exploratory behaviour in these P deficient individuals.

356   The present study also revealed that mynas consuming higher amounts of HC foods were faster to solve

357   a novel extractive foraging task. Innovative foraging behaviour has been linked to the ability to use a

358   greater variety of motor actions in a foraging context (Griffin et al. 2014). Urban mynas are often seen

359   foraging in school playgrounds, food outlets and supermarket carparks (Sol et al. 2012b).

360   Anthropogenic food sources are often wrapped in different packaging that are likely to substantially

361   challenge and enhance mynas motor diversity skills. Human discards contain high-levels of C relative

362   to P (Pierotti and Annett 1987), and birds adapted to access and consume these food items are likely to

363   develop a taste for HC foods. This might explain the present association between innovative foraging

364   and consumption of C found here.

365   The mechanisms underlying food and nutritional choices and post-ingestive processing in invaders are

366   likely to be a key to understand how these species succeed in novel environments (Machovsky-Capuska

367   et al. 2016b). Here, we demonstrated that common mynas select their foods based on their nutritional

368   composition. The tendency to sample unknown foods to fulfil nutritional requirements is likely to

369   contribute to the invasion success of mynas, but this remains to be tested. Our findings linking

370   macronutrient intake and exploration and foraging innovation highlight the importance of nutrition in

371   the development of exploratory and behaviourally flexible phenotypes in a very successful invader.

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521   404.

522   Supplementary material (Appendix JAV-XXXXX at <www.oikosoffice.lu.se/appendix/jav-xxxxx>).

523   Appendix 1–2.

524

TABLES

Table 1. *Orthogonally (Varimax) rotated component loadings on first axis for the exploration test. Bold*

indicates variables contributing to a component's meaning.

Behavioural variables	PC1
Number of trees visited	**0.87**
Number of branches visited	**0.97**
Total number of zones visited (ground + air)	**0.93**
Number of movements	**0.87**
Latency to first exit the cage	-0.09
Number of floor-zones visited	**0.42**

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SUPPLEMENTARY MATERIALS

531 **Appendix 1**

532 **Fig. A1.** Photos of the experimental setup for experiment 1 and 2. On the left: wooden board with the

533 three small dishes used for experiment 1 (photo courtesy of Charlotte Rousseau). On the right: the three

534 plastic bottles for HP, HL and HC foods used for experiment 2 (photo courtesy of Chloe Peneaux).

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536

537 **Fig. A2.** Schematic of the extractive problem-solving tasks. All containers were glued to an identical

538 sized piece of wood. During the innovation test, each petri dish contained a dog pellet as a reward. The

539 petri dishes (with lids) were presented either: right way up, so the lid could be removed by either

540 levering or pushing the lid upwards, or by grabbing a small piece of tape attached to the edge and

541 pulling; or upside down (i.e. the edges of the top smaller container were contained within the larger

542 bottom container), so the lid had to be removed by grabbing a small hook attached to its surface.
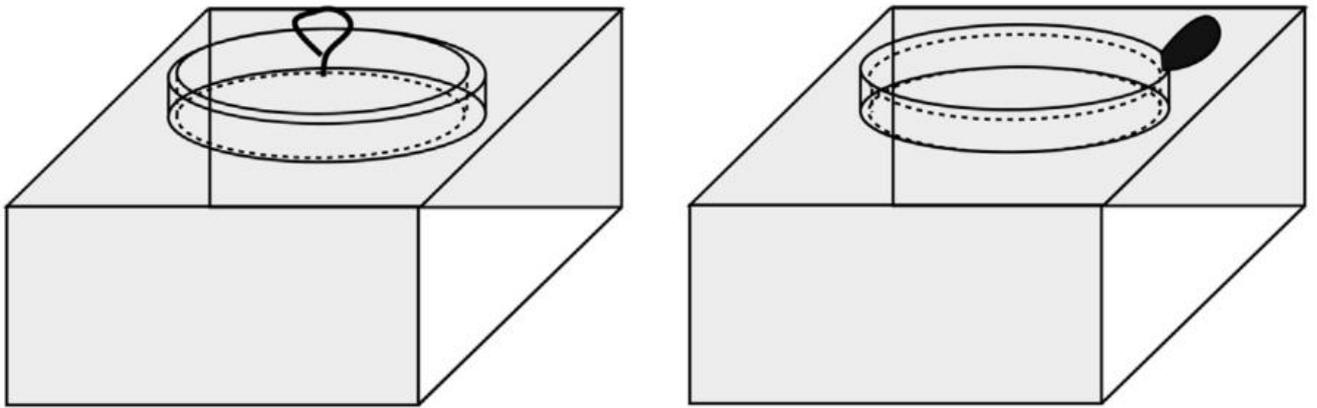
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545   **Fig. A3.** Plan of exploration room (1.7m x 2.80 m x 2.70 m). The room contained five wooden artificial

546   trees of 1.5 m high. Each tree had four lateral branches of 20 cm long. The upper branches were

547   positioned 5 cm below the top (also considerate as a perch) and perpendicular to the lower branches,

548   which were located 30 cm from the top.

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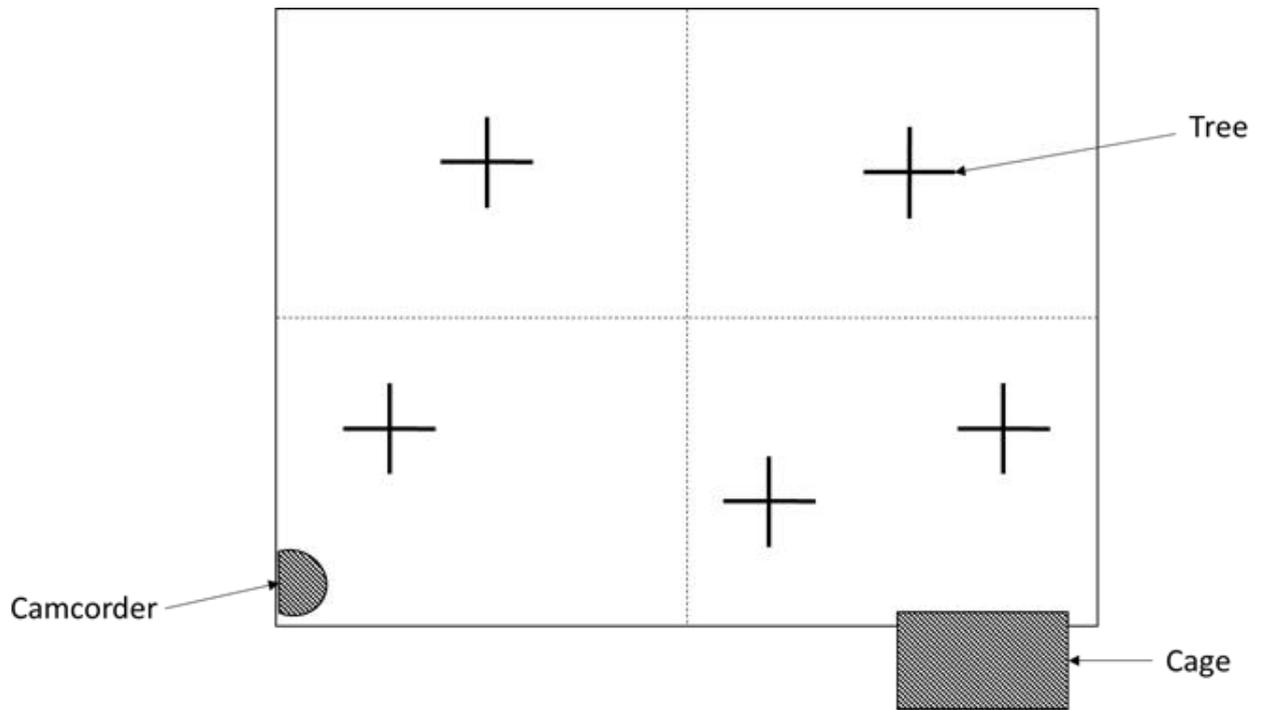
Appendix 2

552   **Table A2.** Correlations between behavioural traits and macronutrient consumptions in Experiment 3.

553   Test values were calculated using Approximate General Independence Tests.

Correlations	Results	
	Z	**p**
Innovation – Protein	-0.63	0.56
Innovation – Lipid	1.37	0.17
Innovation – Carbohydrate*	-1.89	0.04*
Exploration – Protein*	1.91	0.04*
Exploration – Lipid	-1.31	0.20
Exploration – Carbohydrate	-0.59	0.58

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Fig. A2. Scatter plot panel of the correlation analysis between behavioural traits and consumption in

564   macronutrients in Experiment 3.
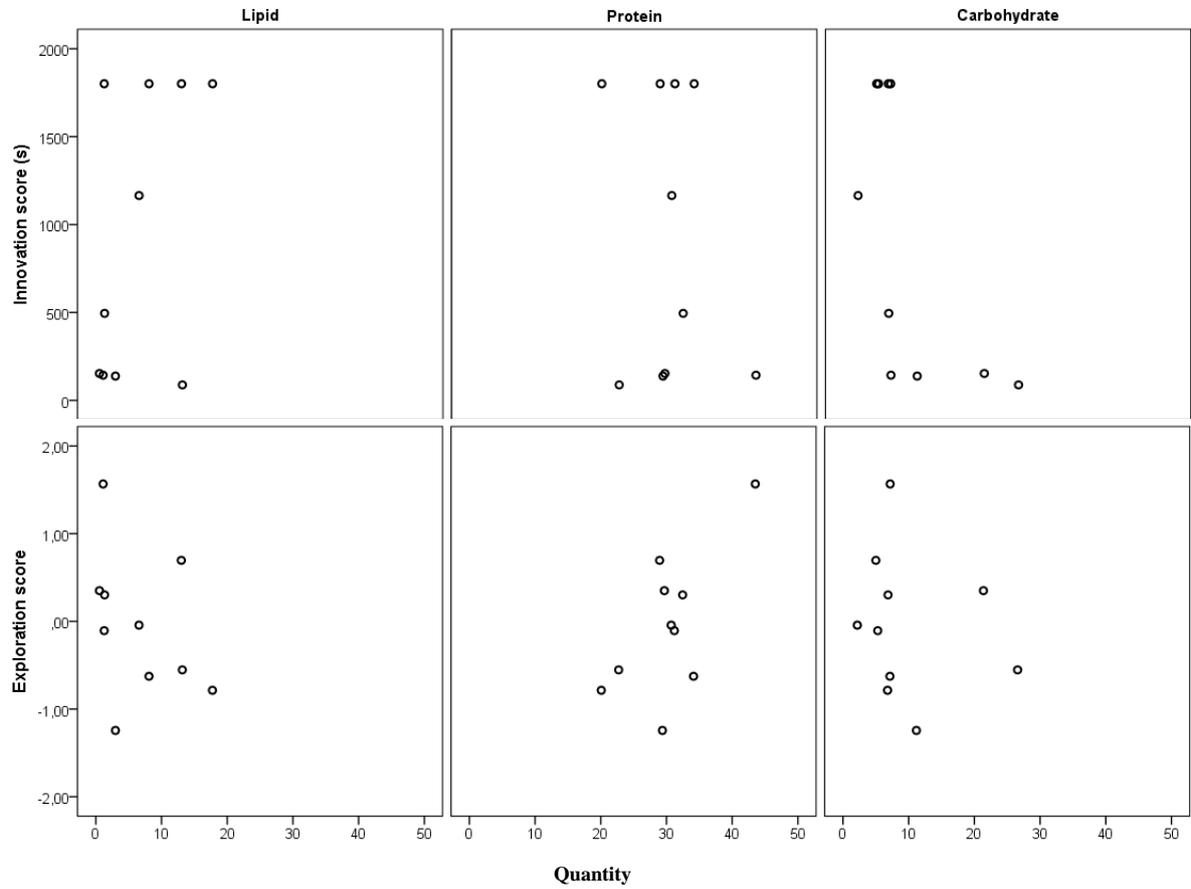

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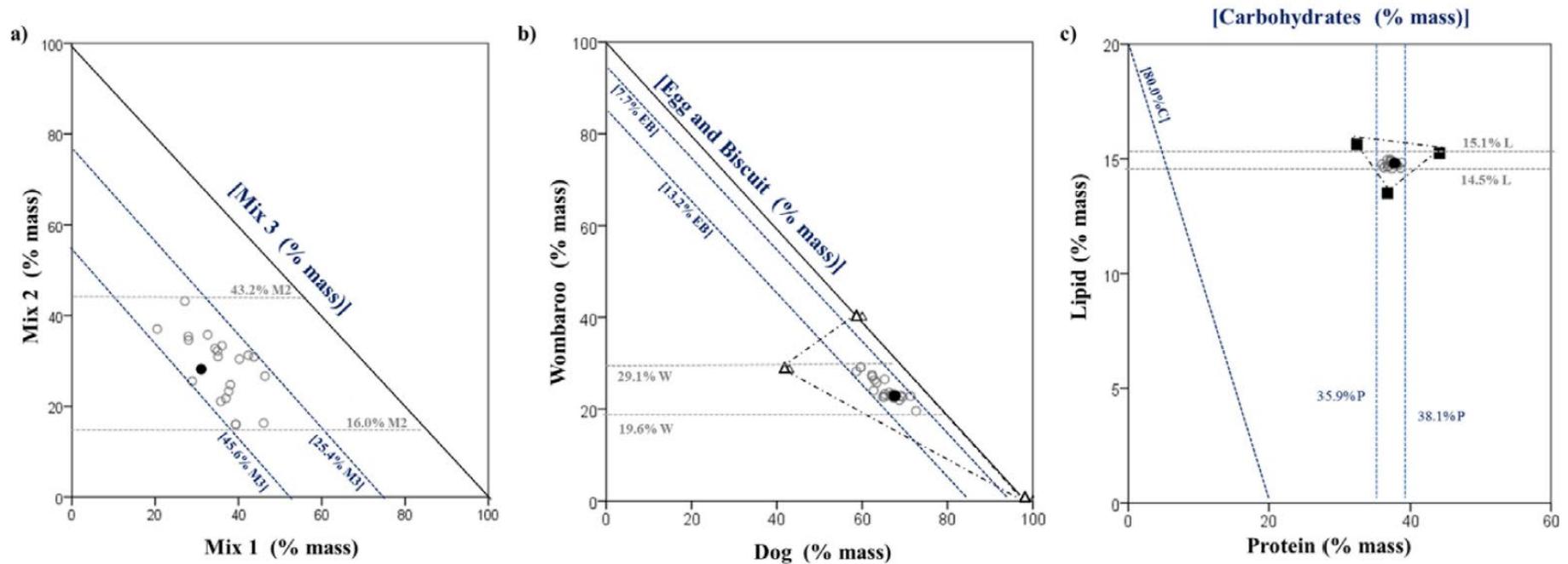
Fig 1. Right-angled mixture triangle showing (as a % of dry weight) the foraging choices of captive mynas during the three days at different scales. **a)** Myna diet (black hollow circles) clusters around the range of Mix 3 intakes, **b)** The model shows the region of ingredients' space (dotted line) that was accessible to the birds (black hollow circles) given the three mixes (black hollow triangles) they were provided during the experiment and **c)** Nutritional niche accessible to mynas delineated by dotted lines as defined by the three different mixtures (black filled squares) offered to the birds. The three plots compare the consumption of foods with a null hypothesis that mynas consume equal amounts (black filled circles).
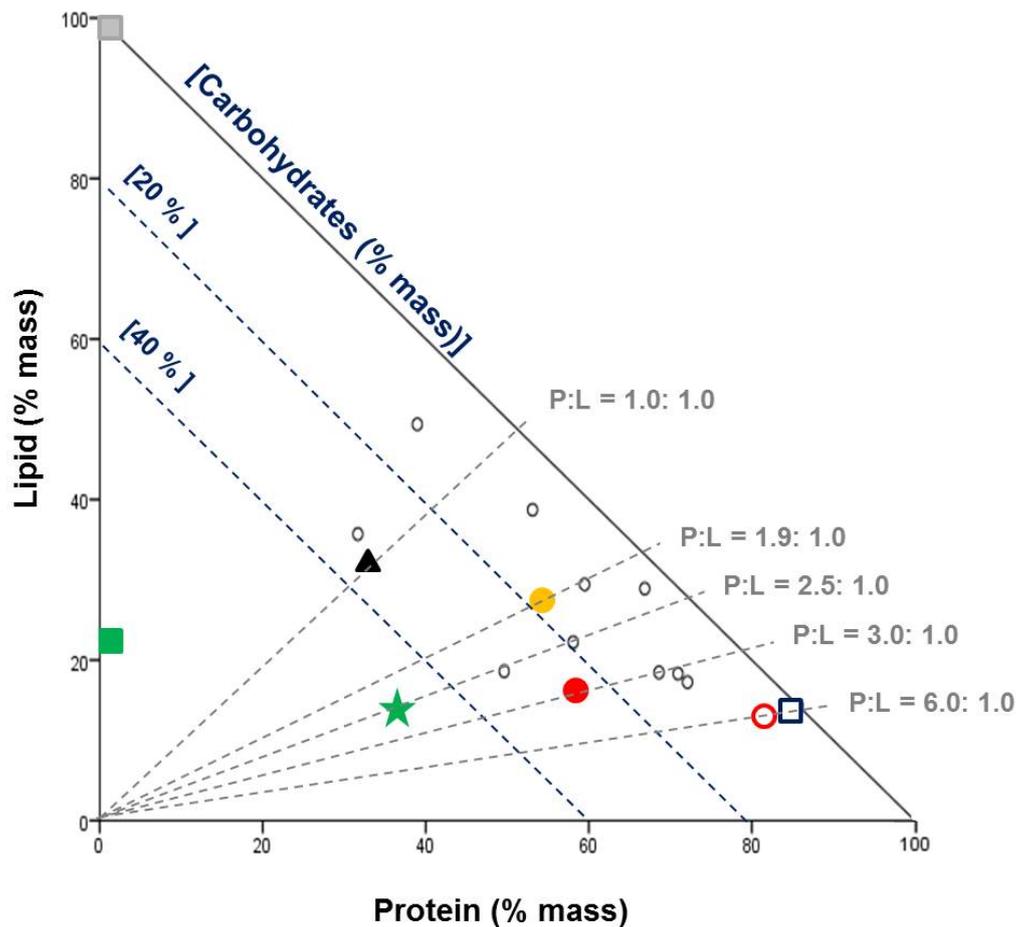
Fig 2. Right-angled mixture triangle showing (as a % of dry mass) the macronutrient preferences in common mynas. Empty circles represent macronutrient intakes of individual birds and the squares represent macronutrient compositions of the three semisynthetic food pellets offered during the captive feeding trials (hollow blue square = HP diet; solid green square = HC diet; solid grey square = HL diet). The green star represents the macronutrient composition of the diet estimated in experiment 1; the gold circle represents the macronutrient composition of the diet estimated in experiment 2; the solid red circle represents the natural diet estimated from Sengupta (1976, for more details see Machovsky-Capuska et al. 2016b) and the hollow red circle represents the diet estimated from free-ranging mynas obtained from Machovsky-Capuska et al. (2016b). The black triangle represents the null hypothesis for a balance dietary nutrient intake consuming equal amounts of the three macronutrients.
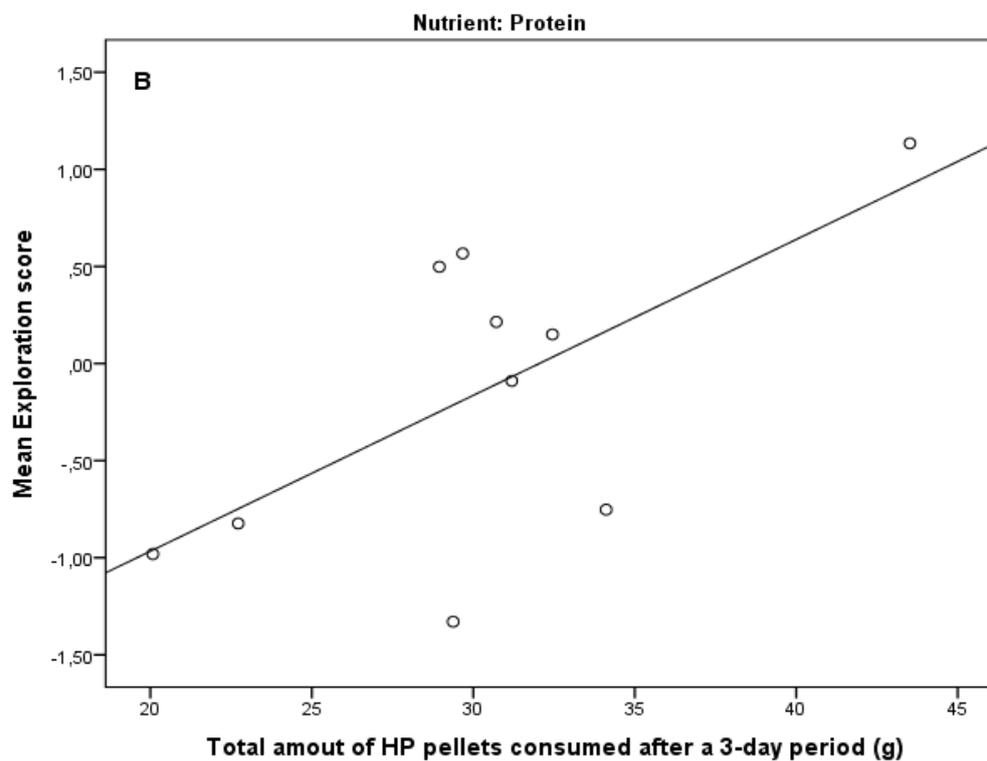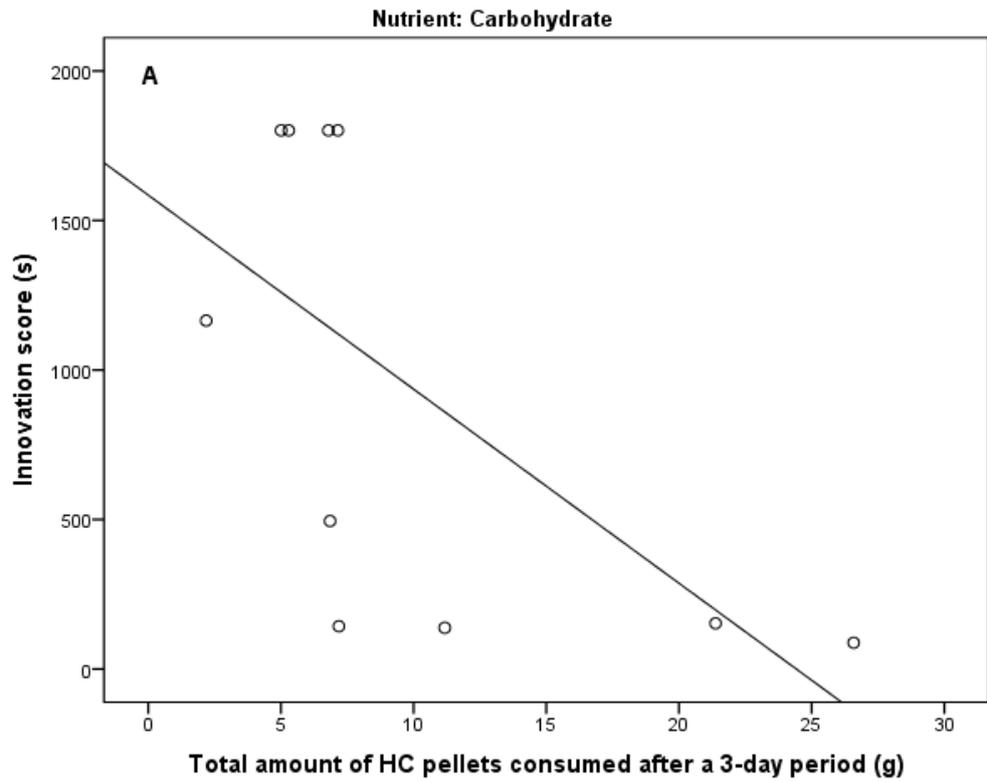
Fig 3. Correlations between: A. mean (±SE) Innovation score and total amount of HC pellets after a 3-day period (g); B. mean (±SE) Exploration score and total amount of HP pellets consumed after a 3-day period (g). Innovation score = solving latency – first contact latency.