**INTRODUCTION**

*Starting paragraph*

Diet quality refers to the caloric value and nutritional composition of food and impacts the behaviour of many species (Ishizaki et al. 2001, Bouvier and Hylander 1982). With dynamic ecosystems, diet quality does not remain consistent across all habitats, especially with human-induced environmental degradation and climate change (White 1978, Tuomainen and Candolin 2011). In low quality diet areas, animals implement numerous mechanisms to ingest key nutrients required for fitness (Chambers et al 1995). Behavioural and physiological mechanisms allow individuals to selectively consume food to meet nutritional and energetic needs. In situations where nutritionally limited diets are inescapable, animals will prioritise consumption of the most important nutrients, usually at the cost of another (Han and Dingemanse 2017). The subsequent nutritional imbalance influences a multitude of factors, including behaviour (Han and Dingemanse 2017).

*Qualifications of Temperament*

The significance of animal temperament (or personality) has been overlooked by many ecologists and evolutionary biologists, despite their ecological implications (Dingemanse and Reale 2005). Only traits that have direct impacts on fitness have been thoroughly explored by scientists, including aggression and anti-predator behaviour (Ellis et al. 2006). Also, some behaviours depend on an accumulation of underlying genes, showing how a number of traits can be correlated (Reale et al. 2007). Some traits prevail in certain environmental conditions, such as boldness in periods of high predation (Reale and Feste-Bianchet 2003). As such, the impact temperament has on fitness should be considered.

Based on the genetic and environmental drivers of behaviour, animal temperament is defined as “individual behavioural differences [that] are consistent over time and across situations” (Reale et al. 2007).

The five traits that make up temperament, according to Reale et al. (2007) are as follows:

1. Shyness-boldness: An animal’s willingness to participate in risk-taking behaviours determines their boldness as well as their reaction to risky, but not new, situations. Boldness has often been studied to observe the response to humans and predators (Boissy et al. 2005).

2. Exploration-avoidance: This trait describes the reaction of an animal to a new situation which is not necessarily risky. Neophobia is a facet of exploration and refers to an individual’s hesitation to interact with an unfamiliar item (Beissinger et al. 1994). Neophobia, however, is only relevant when studying individual variation in exploration (Greenberg and Mettke-Hoffman, 2001), as in this project.

3. Activity: The general activity of an individual in a neutral environment. Activity should be tested in a non-threatening and familiar environment so as not to confound results with exploration and neophobia (Barnett and Cowan 1976, Renner 1990).

4. Aggressiveness: Applicable to a social interaction, aggression is the measure of antagonistic behaviours towards a conspecific.

5. Sociability: Observation of an animal’s willingness to interact with a conspecific in a non-aggressive manner. Avoidance of conspecifics is considered antisocial while actively seeking interactions is considered social.

These qualities form a basic framework for study into animal temperament and, rather than being an exhaustive list, is a simple representation of behaviour (Reale et al. 2007).

Diet quality can have important effects on fitness and vary across species, population and individuals (Sterner and Elser 2002). Low quality prey adversely impact survival and growth of terrestrial arthropods (Raubenheimer 2007, Mayntz and Toft 2006). Behavioural types also relate to fitness-related characteristics such as habitat use and activity patterns (Sih et al. 2012, Wolf and Weissing 2012). In *L. delicata,* high exploratory behaviour has been suggested to be important in colonization success (Chapple et al. 2011). In these studies, we can see how diet quality affects fitness and is therefore essential to understanding the extent of its ecological significance.

*Nutrient dependent behavioural changes*

Diet affects metabolic plasticity with nutrient rich/poor diets trigger different gene expressions and subsequent behaviours (Mason et al 2016, Rocha et al 2016). The two key nutrients that often have the highest impact are protein and carbohydrates. Carbohydrates are the main source of energy, allowing increased levels of activity and quality reproductive efforts (Maklakov et al. 2008). High carbohydrate diets also increase metabolic rate, having further implications on the expression of traits with high energy demands such as activity and exploration (Mathot and Dingemanse 2015).

Protein is necessary for growth and muscle development (Bowen et al. 1995). A limited protein supply induces higher cannibalistic rates in some invertebrates as they attempt to supplement the shortage (Simpson et al 2006). Yet, in wolf spiders, the opposite was found to be true with females reared on a high quality diet expressing higher rates of sexual cannibalism (Wilder and Rypstra 2008). The inconsistencies across species emphasises the significance of studying them separately rather than assuming they all follow the same pattern. Individuals with access to more protein tend to mate more frequently (Blay and Yuval 1997), outperforming their protein-limited counterparts. Protein deficient invertebrates become bolder since the cost of boldness is not as great as individuals with sufficient protein levels (Dingemanse and Wolfe 2010). When low quality diets are prevalent, the trade-off between carbohydrate and protein benefits becomes obvious (Simpson et al 2006, Maklakov et al. 2008). Optimal target intakes will not be reached and the individual will prioritise the nutrient that will be most beneficial to their fitness (Han and Dingemanse 2017). Diet quality will impact different species in different ways due to the variation in energetic and nutritional demand (Simpson et al. 2004).

Sociability is also influenced by diet quality as it is regulated by neuroendocrine mechanisms (Soares et al 2010). Since poor nutrition has negative effects on neuromuscular development, it is expected that social behaviours will suffer as a result of low quality diets (Akman et al. 2012).

Macronutrient composition and pleiotropic genes generate behavioural correlations where functionally different behaviours are linked (Van Oers et al. 2005). These correlations happen both within- and among-individuals (Han and Dingemanse 2015). Among-individual correlations are influenced by genetics and the environment (Dingemanse and Dochtermann 2013) where the environment alters the expression level of a gene that is linked with a behavioural trait (Filby et al. 2012, Norton et al. 2011). In cases where the gene is pleiotropic, the behavioural changes will be correlated. Thus, environmental effects, such as diet quality, greatly impact behavioural correlations.

Individual animals differ in behaviour consistently within the same population (Bell et al. 2009). Behaviour is considered repeatable when there is variation between individuals and each individual behaves consistently over time (Bell et al. 2009). Behaviours that are linked to energetic needs are predicted to be more plastic as they change in response to the environment (Castellano et al. 2002, Smith and Hunter 2005). That being said, if the behaviour continues to remain consistent after the environmental change, it is still considered repeatable, despite its plasticity. The strength of environmental influences also differs between species, with ectotherms thought to have lower heritability of morphological traits than endotherms (Mousseau and Roff 1987), largely due to their dependence on external factors. Whether repeatability follows the same trend in ectotherms is yet to be completely explored, with preliminary meta-analyses suggesting it does (Bell et al. 2009). However, we know that low quality diets reduce behavioural repeatability in some spider species (Lichtenstein et al. 2016). Males also generally display more repeatability than females, the specific details of which were dependent on the behavioural trait in question (Andrew 1972, Wingfield 1994). However, other studies argue that because of their shared genetic basis, behavioural expressions have little variation (Reddiex et al. 2013). *L. delicata,* specifically, have shown little evidence that suggests sexual differences in behavioural traits and correlations (Michalengeli et al. 2016). Perhaps the differences in nutrient demand induce varied behaviours that impact repeatability in response to environmental changes. While the behaviours remain similar between males and females, the subsequent impacts of diet quality differ.

Diet quality has also been shown to influence behaviour and life history traits (Warner et al. 2007). Nutritional composition in the early stages of an animal’s life relate to behaviours expressed at maturity (Han and Dingemanse 2015), indicating a long term effect. Food available to juveniles determines the proportion of the population that will survive into maturity (White 1978).

The short-term effects have predominately been observed in grazing mammals from an agricultural perspective (Greenwood and Demment 1988, Newman et al. 1994). Their foraging behaviours reinforced selective eating as a means of nutrient compensation. Differences in nutrient availability have been shown to cause changes in courtship behaviours, with reproductive behaviours also being influenced by nutrient balance in food (Maklakov 2008, Bertram et al. 2009). These short-term impacts may have huge implications for subsequent fitness. These impacts need to be explored across all species, rather than a selected few. This project aims to provide insight into the behavioural implications of diet quality in lizards

*Gut Microbe*

Gut microbial communities are susceptible to change dependent on the individual’s diet and have been known to significantly impact a range of behaviours.

*Summarising paragraph*

**METHODS**

**Capture and Husbandry**

Sixty-four female *L. delicata* were collected in the Sydney region in September 2015 by mealworming. For the duration of the experimental period, each female was placed in separate enclosures measuring 35.5 x 27.5 x 15cm with paper substrate, a home hide box and water bowl. Incandescent 100-W bulbs provided a heat chord to allow thermoregulatory opportunities and the average room temperature was 24ºC. Lizards were fed crickets three times weekly and had access to water *ad libitum*.

**Diet manipulation**

Diet manipulation was carried out in accordance with previous work in lizards (Warner et al. 2007). To establish diet treatments, crickets were divided into two groups. One group was fed a combination of cat food and an assortment of vegetables (i.e. ‘high quality’ diet) while the other was given only corn (i.e. ‘poor quality’ diet). Crickets were maintained on these diets for two weeks to ensure the crickets in the two treatments were more likely to be of different diet quality. The specific differences in diet quality are unknown but previous studies used a similar method to generate distinct treatments (Warner et al. 2007). Crickets were then fed to random female subjects for 3.5 months, forming two treatment groups. The lizards were kept on the same diet for the duration of the experiment.

**Behavioural assays**

Once the lizards were on the altered diet for 3.5 months, their behavior was assayed daily for 14 days. The three assays of interest were activity, neophobia and sociability. All assays were run consecutively on the same day in a temperature-controlled room set at 28ºC for the duration of the experiment. All assays occurred between 0800-1100hr. We also recorded room temperature during assays to account for any day to day fluctuations in room temperature. To reduce order effects, we randomised neophobia and sociability assays after an initial activity assay. Behaviour was recorded using CCTV cameras (model H.264, CCTV security systems, Melbourne, VIC). Each assay was recorded for at least 20 minutes.

**Activity**

The home hide was replaced with a second, experimental hide, during trials. Assays began as soon as the home hide was removed and. We recorded the total distance the individual moved (cm) as an indicator of activity.

**Neophobia/boldness**

A novel item was fixed on a transparent circular cutout which was placed in the enclosure (Figure #). The items used were either novel objects or food. These items were changed every day to maintain novelty for the duration of the experiment. To quantify neophobia, I recorded the amount of time the lizard spent in the Novel Zone (s) and latency to first interaction with Novel Item (s).

**Social**

Lizards independent of the experiment were placed into transparent containers and introduced into each enclosure (Figure #). The experimental lizard only had visual cues for the social demonstrator. The assays were recorded and measured the total amount of time (s) the individual spent in the social zone.

**Behavioural Scoring**

All behavioural assays were scored blind using the automated tracking software Ethovision XT 11.5. Automated tracking significantly reduces collection time, nullifies inter-observer effects and allows for more precise behavioural measures. Additionally, I was able to control for any carry over effects between assays by over-laying arena settings from different assays within a day.

Arenas were defined in the program for each assay to measure a number of variables in each zone (Fig #). Data from 20 minutes of each video was acquired. In some cases, individuals were not immediately detected during tracking but a full 20 minutes was still acquired, albeit a later segment. Also, some individuals were in the home hide from the beginning of the track and Ethovision was unable to detect and therefore record movement. These missing points were therefore not included in the Total seconds spent inside Arena (TA) for each individual. This information was used to infer total time spent in hide (Th) such that Th = T + (1200 – TA) where T = Original time Ethovision recorded individual in the hide.

**STATISTICAL ANALYSIS**

To fulfil normal distribution assumptions, Total Distance Moved, Time spent in novel zone and time spent in social zone were log transformed. I also only included data for the days where individual lizards were active in all three assays. Using the MCMCglmm R package, I used a Bayesian analysis framework with a Markov Chain Monte Carlo sampling approach to fit all the models. It used 70 000 iterations, a burn-in of 10 000 iterations, and a thinning rate of 100. \*\*Include info about priors where nu = 0.01\*\* The trace plots from each model were observed to confirm that the chains were well mixed.

The final mass of each individual was a fixed effect in the models to account for the impact of body size on behaviour. Models were generated such that there was one each for the high quality diet group, the low quality group and for the entire population with treatment and trait as fixed effects. Repeatability was calculated based on the posterior modes of each model.

**Were there significant correlations and covariances?**

Matrices that show the covariance, variance and correlations between behaviours were generated using the cov2cor function on the posterior modes of each model. Confidence intervals are also presented in the tables and are considered significant when the interval does not include 0. A mantel test was used to assess the significance of any behavioural differences between the matrices for high and low diet treatments.

Separate matrices were created for within and between individual data.

**Did diet impact behaviour?**

T tests visually represented in a box plot

**RESULTS**

**Do behavioural syndromes exist?**

Significant behavioural correlations were observed between most of the behavioural assays in both high and low treatment groups as confidence intervals did not overlap 0 (Table #). The Exploration and Novel latency assays had an extremely high negative correlation between individuals in both treatment groups (high = -0.992, CI = -0.991, -0.541; low = -0.948, CI = -0.998, -0.788). The negative correlation persisted within individuals, indicating a behavioural syndrome despite the within-individual correlation being much weaker (high = -0.193 CI = -0.265, -0.015; low = -0.243 CI = -0.357, -0.086). This means that more exploratory individuals were more likely to approach the novel item quicker. This trend persisted

The repeatability calculations for all traits suggest the existence of behavioural syndromes since the confidence intervals for each assay do not include 0 (Table #). Exploration is the most repeatable in both high (r = 0.386, CI = 0.221, 0.547)and low (r = 0.551, CI = 0.329, 0.693) diet treatments. When considered alongside the strong correlations, the consistent repeatability across both within- and between-individuals indicates behavioural syndromes.

* relies on correlations and repeatabilities.

**Does diet impact personality and behavioural syndromes?**

Although there are behavioural syndromes observed, the repeatability differences between high and low diet are not significant (Fig #). The novel duration and social assays in particular are very close together, indicating that individuals fed on a high quality diet do not spend more or less time in the relevant zones than those fed low quality diets. The low diet group has a higher exploration repeatability but is not significant as the confidence intervals overlap.

* Using table from previous question
* Diet treatments do/don’t differ in between
* Diet DID impact repeatability
* Diet impacts between/within/whatever

Figure 2 shows two diet treatments and the variability between them. X = novel, y = exploration

Difference in repeatability for activity 🡪 is it because of between or within individual variation? Explore. If within, across all the individuals, they temd to be much more consistent in how they respond on a day to day basis.

R = IDvar/(IDvar + Rvar)

Rvar = residual variance = within individual variance

**Discussion OCT 5**

1. Answer question, quick synopsis of result -> relate to hypotheses
2. (and onwards) Integrating results with existing research. Core question “does diet impact personality and behavioural syndromes
3. Caveats:
   1. Same behaviours? But in different contexts 🡪 may explain hypercorrelations because of activity? When behaviours are measured closely together, hypercorrelations are common. Just an activity thing – still shows that they’re also correlated
   2. Inability to decompose diet 🡪 What changed? Calories, nutrients, protein:carb, effects on gut microbiota? Not controlled for
4. Conclusion: Broader importance

***Correlations and Covariance***

Between Individuals

HIGH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | **0.279 (0.131, 559)** | **-71.693 (-137.282, -24.375)** | **0.088 (0.003, 0.366)** | **0.181 (0.066, 0.467)** |
| Novel Latency | **-0.922 (-0.991, -0.541)** | **21670.07165 (11245.29, 51247.953)** | - | **-76.332 (-162.785, -27.117)** |
| Novel Duration | **0.303 (0.152, 0.893)** | - | **0.268 (0.100, 0.499)** | **0.269 (0.095, 0.503)** |
| Social | **0.454 (0.239, 0.822)** | -0.804 (-0.994, 0.563) | **0.773 (0.515, 0.992)** | **0.416 (0.198, 0.830)** |

LOW

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Duration | Social |
| Exploration | 0.344 (0.194, 0.798) | -64.956 (-146.449, -25.492) | 0.226 (0.052, 0.525) | 0.301 (0.036, 0.532) |
| Novel Latency | -0.948 (-0.998, -0.788) | 13663.244 (7000.125, 36543.067) | - | -65.371 (-138.861, -21.407) |
| Novel Duration | 0.697 (0.314, 0.920) | - | 0.240 (0.096, 0.629) | 0.260 (0.113, 0.617) |
| Social | 0.581 (0.307, 0.896) | -0.985 (-0.994, -0.575) | 0.950 (0.854, 0.997) | 0.322 (0.114, 0.713) |

*Mantel Test results: Covariance p = 0.299 (Latency), Correlation p = 0.663 (Latency). Covariance p = 0.979 (Duration), Correlation p = 0.351 (Duration)*

All (with treatment as fixed effect)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | 0.337 (0.217, 0.526) | -62.708 (-115.127,  -40.236) | 0.193 (0.066, 0.312) | 0.244 (0.094, 0.393) |
| Novel Latency | -0.791 (-0.974, -0.689) | 18619.103 (11360.42, 34170.011) | -48.099 (-86.029,  -21.021) | -67.210 (-122.972,  -40.481) |
| Novel Duration | 0.693 (0.284, 0.806) | -0.737 (-0.908, -0.426) | 0.229 (0.144, 0.420) | 0.212 (0.133, 0.416) |
| Social | 0.710 (0.365, 0.826 | -0.831 (-0.973, -0.654) | 0.747 (0.664, 0.979) | 0.351 (0.200, 0.597) |

ALL (with Treatment:trait as fixed effect)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | 0.326 (0.217, 0.526) | -66.980 (-115.127,  -40.236) | 0.181 (0.066, 0.312) | 0.209 (0.009, 0.393) |
| Novel Latency | -0.937 (-0.976, -0.682) | 15684.951 (11360.42, 34170.01) | -52.983 (-86.029, -21.211) | -69.318 (-122.972,  -40.481) |
| Novel Duration | 0.648 (0.313, 0.791) | -0.866 (-0.902, -0.472) | 0.238 (0.144, 0.420) | 0.253 (0.133, 0.416) |
| Social | 0.614 (0.391, 0.824) | -0.926 (-0.973, -0.659) | 0.865 (0.696, 0.986) | 0.358(0.200, 0.597) |

Within Individual

HIGH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | 0.464 (0.409, 0.593) | -32.282 (-46.081,  -2.412) | 0.107 (0.014, 0.193) | 0.076  (0.013, 0.178) |
| Novel Latency | -0.193 (-0.265, -0.015) | 56207.643 (47426.42, 69409.688) | - | -25.812  (-49.382, 10.927) |
| Novel Duration | 0.171 (0.046, 0.314) | - | 0.822 (0.668, 0.999) | 0.087 (-0.036, 0.188) |
| Social | 0.129 (0.024, 0.278) | -0.122 (-0.236, 0.051) | 0.113 (-0.047, 0.234) | 0.742 (0.614, 0.904) |

LOW

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | (0.327, 0.491) | 35.768(-56.750,  -11.855) | 0.071 (0.015, 0.184) | 0.184 (0.110, 0.269) |
| Novel Latency | -0.243 (-0.357, -0.086) | (46345.27, 67697.385) | - | -41.664 (-64.935, -15.380) |
| Novel Duration | 0.131 (0.032, 0.298) | - | 0.740 (0.649, 0.979) | 0.085 (0.020, 0.206) |
| Social | 0.403 (0.257, 0.517) | -0.239 (-0.349,  -0.092) | 0.133 (0.038, 0.294) | 0.539 (0.440, 0.681) |

*Mantel Test results: Covariance p = 0.703 (Latency), Correlation p = 0.347 (Latency). Covariance p = 0.317 (Duration), Correlation p = 0.981 (Duration)*

ALL (With treatment as fixed effect)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | 0.438 (0.393, 0.512) | -33.683 (-42.696,  -12.277) | 0.095 (0.042, 0.163) | 0.135 (0.077, 0.186) |
| Novel Latency | -0.216 (-0.260, -0.081) | 55728.755 (50774.69, 66792.334) | -77.296 (-104.403, -61.855) | -33.584 (-49.845, -12.588) |
| Novel Duration | 0.157 (0.069, 0.259) | -0.360 (-0.458, -0.294) | 0.829 (0.712, 0.938) | 0.065 (0.010, 0.157) |
| Social | 0.251 (0.146, 0.328) | -0.175 (-0.241, -0.058) | 0.088 (0.020, 0.210) | 0.661 (0.565, 0.751) |

ALL (With Treatment:Trait as fixed effect)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel Latency | Novel Duration | Social |
| Exploration | 0.441 (0.394, 0.517) | -28.497 (-45.637, -14.347) | 0.080 (0.039, 0.162) | 0.123 (0.073, 0.184) |
| Novel Latency | -0.184 (-0.273, -0.086) | 54493.992 (49253.53, 65799.137) | -78.198 (-107.227, -63.313) | -29.205 (-57.010, -9.817) |
| Novel Duration | 0.136 (0.061, 0.254) | -0.376 (-0.461, -0.300) | 0.796 (0.708, 0.933) | 0.087 (0.015, 0.160) |
| Social | 0.232 (0.146, 0.323) | -0.158 (-0.255, -0.050) | 0.124 (0.020, 0.210) | 0.626 (0.572, 0.764) |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Exploration | Novel (Latency) | Novel Duration | Social |
| High | 0.386 (0.221, 0.547) | 0.288 (0.165, 0.485) | 0.246 (0.090, 0.386) | 0.349 (0.178, 0.524) |
| Low | 0.551 (0.329, 0.693) | 0.208 (0.096, 0.396) | 0.264 (0.128, 0.465) | 0.362 (0.183, 0.601) |

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**Boxplots**



*High mean = 5.65, Low mean = 5.82, df = 62,*

*p = 0.28.*



High mean = 2.52 Low mean = 2.31 p = 0.50

grouped by ID Nov Duration

High mean = 760.23 Low mean = 777.89 p = 0.76

Df = 62 Grouped by ID Nov Lat

*High mean = 3.76 Low mean = 3.08 p = 0.098*

*Df = 62 Social*