Dietary Conservatism: The Influences and Determinants of Alternative Foraging Strategies

Samuel David Preston

A thesis submitted in partial fulfilment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

School of Natural Sciences
(Zoology)

Trinity College Dublin

March 2023

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018)

_____

Sam Preston

Summary

Dietary conservatism – the existence of alternative foraging strategies in response to novel foods – continues to be a little-known and understudied topic of animal behaviour. In every vertebrate population tested so far, some individuals are Adventurous Consumers (ACs) that rapidly accept novel foods as part of their diet, while others are Dietary Conservative (DC) and show prolonged reluctance to incorporate novel foods into their diet. However, there has been little wider recognition of the relevance of this behaviour and many questions about its mechanism and function remain a mystery. In this thesis, I aim to address this oversight to some small degree by bringing together current knowledge of dietary conservatism and exploring some of the fundamental outstanding questions about the behaviour, how it operates and what determines it.

Chapter 1 is a literature review on dietary conservatism. It serves as an introduction to the topic and clarifies terminology and methodology which have become somewhat confused across different studies. I also outline crucial gaps in research on foraging strategies, some of which are the focus of the chapters that follow.

In chapters 2 and 3, I explore how perception of familiarity and novelty differs among individuals with different foraging strategies. Whether or not a food (or, indeed, anything) is familiar or novel is a matter of experience, memory, and cognitive processes like association and generalization. Here is where the fundamental differences between the foraging strategies must lie. In chapter 2, I investigate whether there are degrees of familiarity; does the amount of experience with a familiar food can change animals' preference for it, and does this relationship differ among AC and DC individuals? In chapter 3, I explore how different a food must be from the familiar to be treated as novel by individuals with different foraging strategies, and whether a difference in generalization might explain why AC and DC animals respond differently to novel foods.

These chapters deal with mechanistic causes of different foraging strategies. However, they also address important concerns for experimental design, namely for how long should animals be trained on familiar foods to make them familiar, and how different must a novel food be to elicit different responses from different foraging strategies? In

chapters 4 and 5 I move to explore ecological and evolutionary causes of foraging strategies and foraging strategy expression.

In chapter 4, I investigate how animals' hunger levels affect preference for familiar foods and acceptance of novel ones. Foraging strategy expression can be flexible depending on context and hunger can make animals take greater risks when foraging. It seems reasonable, then, to expect that foraging strategy expression might depend on an individual's hunger level. One intuitive possibility is that, when hungry, animals should be more willing to accept novel foods (behave more AC) to maximize energy gain. This has never been tested and, apart from being useful to know for designing experiments, understanding how animals respond to novelty when experience different levels of hunger has substantial ecological relevance. Hunger varies seasonally and with energy demands (e.g., breeding, growing, moulting), so if foraging strategy expression depends on hunger state patterns of AC and DC behaviour might be expected at a population level as ecological circumstances dictate.

Finally, chapter 5 deals with, in my opinion, the most exciting question in this thesis: what is the advantage of being DC? Explaining the existence of the DC foraging strategy and its coexistence with the AC foraging strategy in all populations is, perhaps, the most important outstanding question in dietary conservatism research. Two hypotheses to explain the benefit of being DC have been mentioned in earlier chapters – the avoidance hypothesis and the efficiency hypothesis. In this chapter I present data from the first explicit test of the latter to determine whether DC individuals are more efficient foragers than AC individuals. Such an advantage might explain not only why the DC foraging strategy evolved but why DC individuals make up a similar proportion of populations across many different studies.

Acknowledgements

First and foremost, I would like to thank my supervisor, Professor Nicola Marples.

I often think about a joke I heard sometime during my education. A rabbit is caught by a fox and begs the fox not to eat him because he's finishing his thesis. "Thesis!" laughs the fox. "On what?"

"My title," explains the rabbit "is 'Rabbits are more fearsome predators than foxes'".

Incredulous, the fox replies, "What kind of thesis is that? You can't hope to defend such a preposterous idea!" (Clearly the fox was also a reviewer.)

"Come to my office and speak to my supervisor," says the rabbit. "Then we'll see if you still think my thesis is stupid."

Curious now, the fox follows the rabbit to his office, which turns out to be a large cave. At the back of the cave is a lioness, surrounded by fox bones.

The moral of the story is, it doesn't matter who you are; it doesn't even matter what your ideas are; when it comes to your PhD it only matters who your supervisor is.

To my knowledge, Nicola hasn't eaten any of my detractors, but her patience, understanding, interest, guidance, knowledge, and belief in my ideas have slowly developed me into a researcher that won't get wiped out by the first fox he meets. She is the reason animal behaviour has become the centre of my research interests (I believe this is true of most undergrads who take her lectures) and, though I prefer to avoid socializing unless absolutely necessary, has always made me feel welcome in the department. No student could ask for a better supervisor. I will be eternally grateful and eternally in your debt.

I would also like to thank to the following people whose significant contributions, sometimes only once but at crucial moments, made this thesis possible:

Alison and Sinead, for their technical expertise and being the people that actually run the department. Without them none of my ideas could have been realized.

Frances, for finding me the funding I eventually won to undertake this degree, and who I suspect actually runs the whole school.

Pepijn, for his *Daphnia*, *Daphnia* expertise, and support throughout, especially during the horrendous move to our new digs.

Dave, for sharing his method for calculating food preference, on which I relied heavily, and also for his suggestions and input (often via Nicola).

Rob, for his expertise, advice, and collaboration to navigate the statistical aspects of most of these experiments. Thank you, also, for sharing some unpublished data for analysis.

Siobhan, for supplying me with a steady stream of demonstrating work – valuable teaching experience, but more importantly a source of income for a postgrad.

My project students, Daniel, John, Whitney, and Camila, without whom many of the experiments couldn't have been conducted.

Andrew, for his initial assistance in getting R program to behave for me with new analyses, and then for connecting me with Dublin Zoo where my final fieldwork was conducted.

Dublin Zoo and the people working there, who were welcoming, eternally helpful, and genuinely interested. Thanks also to the visitors who took the time to not step on my equipment as they walked past – both of you were great.

My mother, for occasional proof reading, unwavering faith, and bedrock of support I'll never be finished needing.

Auntie Paddy, whose financial contribution made moving and living in Dublin during this period possible.

Floriane and Lucy, for looking after my fish and *Daphnia* when I was away.

Mrs Mary Gibbons, who taught me biology and chemistry when I was young and cemented my love of science.

I can't express enough the difference your help made. If you ever need an extra pair of hands, I'll see what I can do for you.

Lastly, I would like to thank Alice. Better half is an understatement. Whatever I needed you gave. There is literally – and I must stress I am using this word in its correct sense – *literally* nothing I have thanked others for that Alice has not also helped with, and more besides. If I began to thank you properly here, the acknowledgements section would be longer than the rest of the thesis. You and you alone never have to address me as "Doctor". (I will be making everyone else do so at least once.) All my love, Sam.

Table of Contents

Declaration....2
Summary....3
Acknowledgements....5
List of Figures....13
List of Boxes....13
List of Tables....14
Chapter 1....17
Novel Foods and the Foraging Strategies that Deal with Them....17
Authors....17
Author Contributions....17
Status....17
Note....17
1.1 Abstract....18
1.1.2 Keywords....18
1.2 Introduction....19
1.3 Defining the DC Foraging Strategy....20
1.4 Maintaining Two Foraging Strategies....23
1.5 The Prevalence of the Dietary Conservatism and the DC Foraging Strategy....26
1.6 Dietary Conservatism: Binary or Continuum?....32
1.7 Testing for Dietary Conservatism: Distinguishing ACs from DCs....33
1.7.1 Latency Tests....34
1.7.2 Evolution Tests....35
1.7.3 Recommendations for Testing for Dietary Conservatism....37
1.8 Ecological and Practical Impacts of Dietary Conservatism....38
Chapter 2....41
How Familiar is Familiar?....41
Authors....41
Author Contributions....41
Status....41
Note....41
2.1 Lay Summary....42
2.2 Abstract....42

2.2.1 Keywords: 42
2.3 Introduction 43
2.4 Methods 46
2.4.1 Fish collection and outdoor housing 46
2.4.2 Water Treatment 46
2.4.5 Experimental food 47
2.4.6 Training 47
2.4.7 Testing 48
2.4.8 Innate Colour Preference 49
2.5 Data Analysis 50
2.5.1 Innate Colour Preference 50
2.5.2 Calculating Preference Scores 50
2.5.3 Determining foraging strategy 51
2.5.4 Relating Preference Scores to Variables 51
2.6 Results 53
2.6.1 Innate Colour Preference 53
2.6.2 Distribution of foraging strategies 53
2.6.3 Preference Scores 53
2.7 Discussion 58
Chapter 3 62
Novel is Novel: the stability of generalization across foraging strategies and experience 62
Authors 62
Author Contributions 62
Status 62
3.1 Abstract 63
3.2 Introduction 64
3.3 Methods 66
3.3.1 Study sites 66
3.3.2 Experimental Food 67
3.3.3 Colour Validation 68
3.3.4 Training 69
3.3.5 Testing 70
3.4 Data Analysis 71
3.4.1 Calculating preference scores 71
3.4.2 Determining Foraging Strategy 71
3.4.3 Modelling 72

3.5 Results 73
3.5.1 Colour Validation 73
3.5.2 Distribution of foraging strategies 76
3.5.3 Preference across the colour gradient 76
3.5.5 Generalization across a novelty gradient 78
3.5.6 Effect of foraging strategy 80
3.5.7 Effect of red baits 81
3.6 Discussion 84
Chapter 4 89
Hunger Games: The effect of hunger on novel food recruitment by animals with different foraging strategies 89
Authors 89
Author Contributions 89
Status 89
4.1 Abstract 90
4.1.1 Keywords: 90
4.2 Introduction 91
4.3 Methods 93
4.3.1 Fish Housing 93
4.3.2 Experimental Food 93
4.3.4 Training and Treatments 93
4.3.5 Testing 94
4.4 Data Analysis 95
4.4.1 Calculating Preference Scores 95
4.4.2 Determining Foraging Strategy 95
4.4.3 Distribution of Foraging Strategies 96
4.4.4 Relating Preference Scores to Treatment 96
4.5 Results 97
4.5.1 Distribution of Foraging Strategies 97
4.5.2 Preference Scores 97
4.6 Discussion 101
Chapter 5 104
Can Efficiency Explain Foraging Strategies? 104
Authors 104
Author Contributions 104
Status 104

5.1 Abstract 105
5.2 Introduction 106
5.3 Methods 109
5.3.1 Overview 109
5.3.2 Study Site 109
5.3.3 Experimental Foods 110
5.3.4 Training 110
5.3.5 Testing 111
5.4 Data analysis 113
5.4.1 Constraining test parameters 113
5.4.2 Determining foraging strategy 114
5.5.2 Statistical Analysis 114
5.5 Results 115
5.5.1 Foraging Strategies 115
5.5.2 Foraging Rate 115
5.5.3 Number of Mistakes 118
5.6 Discussion 121
Chapter 6 124
General Discussion 124
6.1 Cognitive mechanisms underlying foraging strategies 125
6.2 Plasticity of foraging strategy with hunger 127
6.3 Towards standard experimental design 128
6.4 Preference scores 129
6.5 The why of DC 130
6.6 General application of this research 131
7. Bibliography 133
Appendix 1. Supplementary material for Chapter 1 – Novel Foods and the Foraging Strategies that Deal with Them 145
A1.1 Dietary Conservatism in Other Species 145
A1.2 Dietary Conservatism in Vertebrates 145
A1.2.1 Dietary Conservatism in Smooth Newts. 145
A1.2.2. Dietary Conservatism in Common Frogs 149
A1.2.3. Dietary Conservatism in Mice 151
A1.3 Dietary Conservatism in Invertebrates 153
A1.3.1. Dietary Conservatism in African Praying Mantids 153
A1.3.2. Dietary Conservatism in Spiders 155

A1.3.3. Dietary Conservatism in Ladybirds 157
Appendix 2. Supplementary material for Chapter 2 – How Familiar is Familiar?.....161
Appendix 3. Supplementary material for Chapter 5 – Can Efficiency Explain Foraging Strategies? 165

List of Figures

Figure 2.1 Preference scores vs treatment. 54

Figure 2.2. Preference scores vs test day for each treatment: (a) Ex1, (b) Ex4, (c) Ex7, (d) Ex10. 55

Figure 3.1. Colour map of bait gradient corrected for avian visual system (see text). 75

Figure 3.2. Preference scores of all robins for each colour of bait by familiar colour. 78

Figure 3.3. Preference scores for all robins (a) before exposure to red baits and (b) after experience with red baits robins for each colour of bait. 80

Figure 4.1. Preference score vs treatment group for (a) brown familiar fish and (b) green familiar fish. 99

Figure 5.1. Feeding rate of AC and DC birds across each test type. 116

Figure 5.2. Feeding rate of AC and DC birds across trials in (a) test A, only real, familiar baits present (b), test B, real and fake, familiar-coloured baits present (c) test C real and fake baits of both familiar and novel colours present. 118

Figure 5.3. Number of mistakes (attempts to eat or investigate fake baits) by AC and DC birds over successive trials. 119

Figure A1.1. Latencies of individual mice to consume food from the novel odour dish. 152

List of Boxes

Box 1.1. A Note on Terminology 22

Box 1.2. Testing for Dietary Conservatism 32

Box 3.1. Example calculation of preference scores 71

Box 4.1. Preference score calculation example 95

Box A1.1. Preference score calculation example 146

List of Tables

Table 1.1. The four stages of incorporation. 21

Table 1.2. Published and unpublished studies testing vertebrate species for dietary conservatism. 28

Table 2.1. Treatment groups and their experience with brown Daphnia prior to testing. 48

Table 2.2 Distribution of foraging strategies among treatment groups. 53

Table 2.3. Best-fitting model and output relating preference score to foraging strategy (DC.class) and day of testing. 56

Table 3.1. Study sites in this experiment. Note that two sites comprised several disconnected areas. 67

Table 3.2. Stock solution ratios for dying pastry baits. 68

Table 3.3. L*a*b -based colour distances (derived from a combination of luminosity and hue) of each colour bait from our Green bait compared against ideal values of an even gradient. 73

Table 3.4. Number of AC and DC robins in each colour group. 76

Table 3.5. Best-fitting model and output of preference score's relationship with explanatory variables. 77

Table 3.6. Post hoc contrast analysis of explanatory variables from best-fitting model of preference score. 82

Table 4.1. Number of fish grouped by colour, treatment, and foraging strategy. 97

Table 4.2. Best fitting model and output relating preference score and explanatory variables. 100

Table 5.1. Details of robins in each area of the study site. 110

Table 5.2. Fixed effect estimates for best fitting model relating foraging rate to explanatory variables. 116

Table 5.3. Models with the lowest AIC values (within 2 AIC) returned from the global model of foraging rate. 117

Table 5.4. Fixed effect estimates for best fitting model relating number of mistakes to explanatory variables. 120

Table 5.5. Models with the lowest AIC values (within 2 AIC) returned from the global model of number of mistakes made. 120

Table A1.1. Data for individual newts from Experiment 1. 147

Table A1.2. Number of novel and familiar fixations during evolution testing among newts by familiar colour. 149

Table A1.3. Number of spiders in each group (those trained with ebony Drosophila and those trained with yellow) and at each training length. 156

Table A1.4. Preference scores for ladybirds as larvae and adults. 158

Table A1.5. Results of statistical tests on preference score data. 159

Table A2.1. Model of preference score and explanatory variables for fish in treatment Ex1. 161

Table A2.2. Model of preference score and explanatory variables for fish in treatment Ex4. 162

Table A2.3. Model of preference score and explanatory variables for fish in treatment Ex7. 163

Table A2.4. Model of preference score and explanatory variables for fish in treatment Ex10. 164

Table A3.1. Complete best fitting model relating foraging rate to explanatory variables. 165

Table A3.2. Complete best fitting model relating number of mistakes to explanatory variables. 166

Chapter 1

Novel Foods and the Foraging Strategies that Deal with Them

Authors

Sam Preston and Nicola Marples

Author Contributions

I conceived this review and wrote this manuscript. NM provided insight on this manuscript and data from several unpublished theses. I analysed data from unpublished theses.

Special thanks to Rob Thomas who also provided data from unpublished thesis included in this manuscript.

Status

This manuscript has been submitted and is awaiting review. Raw data and R scripts for supplementary material have also been provided for review.

Note

When referred to in other chapters (excluding general discussion), this manuscript is referenced in text as Preston and Marples, in prep a.

1.1 Abstract

Animals show considerable interindividual differences in how they go about the business of living. These differences can lead to surprising behaviours by individuals that appear counterintuitive at best, perhaps none more so than how individuals cope with novelty in their diets. Faced with novel foods, individuals of many species have been shown to display alternative foraging strategies. Some are Adventurous Consumers (AC), rapidly accepting novel foods as part of their regular fare, while others are Dietary Conservative (DC), refusing to incorporate novel foods for long periods despite considerable experience of them.

This review brings together current knowledge of AC and DC foraging strategies, as well as expanding the list of species in which the behaviour has been observed with previously unpublished data. We also propose a framework for standard testing to improve comparability across studies and provide researchers with the tools to include foraging strategy tests in their experiments.

There remains much to learn about the foraging strategies animals employ to deal with novel foods. How different strategies are maintained in a population, the ecological advantages of each, and the mechanisms underlying them are rich areas for future study. Further, the ecological and evolutionary consequences of different foraging strategies remain almost completely unknown. We conclude by highlighting the need to incorporate knowledge of individual foraging strategies into dietary ecology, evolutionary theory, and animal management to improve our theoretical understanding of animal feeding behaviours and our ability to optimize animal welfare effectively.

1.1.2 Keywords:

Behaviour; Dietary Conservatism; Foraging; Novel Foods; Specialization; Vertebrates

1.2 Introduction

Diet is fundamental to animals' ecology. What and how an animal goes about eating is, arguably, the primary way in which it interacts with its environment and is of obvious interest to ecologists, conservationists, and evolutionary biologists alike. While optimal foraging theory (Charnev, 1976) and population- and species-level descriptions of animals' diets (e.g., Raboy and Dietz, 2004; Hill and Dunbar, 2002) have largely defined our thinking about dietary ecology, these approaches do not address individual variation in foraging strategies. Recently, research has highlighted the importance of inter-individual differences in a range of behaviours including foraging behaviours (Wolf and Weissing, 2012; Toscano *et al.* 2016; Marples *et al.* 2018).

Optimal foraging theory predicts that there is one ideal foraging strategy for a given environment and physiological state. However, more recent studies of individual behaviour demonstrate that, while populations do follow the predictions of optimality models (e.g., Kacelnik, 1984; Manatunge and Asaeda, 1998), individuals quite often don't (Reale *et al*. 2007; Skelhorn *et al.* 2016; Marples *et al*. 2018). The reason for this disparity centres round three recent insights into individual behaviour: personality differences, information gathering, and foraging strategies.

An animal's personality can dramatically affect its foraging ecology. For instance, bold three-spined sticklebacks (*Gasterosteus aculeatus*) exploit prey more efficiently than shy individuals (Ward *et al*. 2004). The implications of personality types on foraging behaviour are explored elsewhere (e.g., Toscano *et al*. 2016), and lie outside the remit of this review, but cause clear individual differences in food choice. A second source of individual foraging differences is disparity in knowledge or tolerance to the food type. Animals that appear to make "mistakes" when foraging may instead be gathering information about potential foods (Skelhorn *et al*. 2016) or may have different experience of or tolerances to the foods available (Marples *et al*. 2018).

Finally, and the focus of this review; individuals have been shown to possess different intrinsic foraging strategies relating to their treatment of novel foods (Marples and Brakefield, 1995; Marples *et al*. 1998; Thomas *et al*. 2010). Populations appear to consist of some individuals that display a long-term avoidance of novel foods, displaying a Dietary

Conservative (DC) foraging strategy, while others in the population much more readily accept novel foods and display an Adventurous Consumer (AC) foraging strategy. Appreciation of this divergence in the way individuals deal with novel foods helps to explain some of the unexpected patterns of behaviour seen in foraging animals.

Animals inevitably encounter novel foods during their lives and need to make decisions as to whether to eat them. Individuals will be confronted by new prey types and assemblages whenever they move geographically either through migration or dispersal, and as the environment changes around them. Whether an animal is an AC or a DC individual will therefore determine how it interacts with its environment at a very basic level, underpinning every aspect of their lives, development, and growth.

This review details the current knowledge of these foraging strategies, drawing together data sets to illustrate the prevalence of the behaviour across different taxonomic groups. We present a critical analysis of the methodologies that have been used to date and provide a framework of standard methodologies suitable for future study. The relevance of individual differences in foraging behaviour to wider ecology and conservation is then briefly outlined.

1.3 Defining the DC Foraging Strategy

When an animal encounters novelty it typically displays a short-lived period of aversion to that novelty, termed neophobia (Barnett, 1958). Neophobic responses can be caused by novel environments, objects, or foods (Greenberg, 2003), and variation among individuals in the degree of this response is now a well-studied aspect of animal personality (see Greggor *et al*. 2015 and Crane and Ferrari, 2017 for recent reviews). After neophobia has been overcome, however, animals must decide how they will treat this novel stimulus. For novel foods, animals must decide whether to consume them and, afterwards, use information about the food's profitability to decide whether to eat this food type should it be encountered again.

Incorporating new food types into the diet is a complex process. Research on wild birds (Marples and Kelly, 1999) identified four stages of accepting a novel food (see Table 1.1),

from initial neophobia, through sampling the novel food, before reluctant eating of it and, eventually, full acceptance. The duration of the latter stages depends on whether an individual displays an AC or DC foraging strategy. Individuals displaying an AC foraging strategy quickly recruit novel foods into the diet, while individuals displaying a DC foraging strategy show a substantial delay before doing so.

Table 1.1. The four stages of incorporation. Adapted from Marples and Kelly (1999). An individual's foraging strategy (AC or DC) determines how quickly it passes through stages 2-4.

Stage	Description
Visual Inspection Only	Physical contact with the novel food is avoided. This stage is neophobia.
Sampling	Physical contact with the novel food begins. The food is manipulated, tasted, and occasionally eaten.
Regular Acceptance when Familiar Unavailable	The novel food is consistently eaten but only after familiar foods have been finished.
Full Incorporation/Recruitment	The novel food is accepted as readily as the familiar – no distinction is made between them, and it has been fully recruited into the diet.

Depending on their foraging strategy, individuals show substantial variation in the rate with which they incorporate novel foods. Initial studies in captive-bred Japanese quail (Marples and Brakefield, 1995) and wild blackbirds and robins (Marples *et al*. 1998) showed that AC individuals could begin eating the novel food consistently after just a few seconds (Marples and Brakefield, 1995) or a single exposure (Marples *et al*. 1998), while DC individuals refused to accept the novel food for extended periods, in some cases failing to do so after 45 minutes of exposure over several trials (Marples and Brakefield, 1995) or only after several months (Marples and Kelly, 1999). In one wild blackbird, refusal to recruit a novel food persisted for over two years (D. J. Kelly, unpubl. data), highlighting how recruitment of novel foods can be so protracted it can take a significant proportion of the animal's life. The foraging strategy an individual displays appears to be genetically

controlled, as selectively breeding Japanese quail successfully produced lines of AC and DC birds in just three generations (Marples and Brakefield, 1995).

Box 1.1. A Note on Terminology

The terminology used to describe dietary conservatism has suffered from confusion in the literature. In particular, dietary conservatism and Dietary Conservative (DC) have often been used interchangeably (e.g., Richards *et al.* 2014; McMahon and Marples, 2017), leading to the use of further terms such as dietary wariness and recruitment latency to describe individuals' foraging strategies. To avoid ambiguity and simplify descriptions, we adopt the following definitions here:

"Incorporation" of a novel food into the diet is a multi-stage process involving sampling, reluctant consumption, and eventually consumption at the same rate as an equally profitable, familiar food (Table 1, stages 2, 3 and 4).

"Dietary conservatism" is the name of the trait in which individuals in a population show alternative foraging strategies in their incorporation of novel food into the diet.

"Dietary Conservative" (DC) is the name of one foraging strategy characterised by a relatively long delay in incorporating novel foods into the diet after neophobia has been overcome. Individuals that adopt this strategy are referred to as DCs or DC individuals.

"Adventurous Consumer" (AC) is another foraging strategy characterised by a relatively short or no delay in incorporating novel foods into the diet after neophobia has been overcome. Individuals that adopt this strategy are referred to as ACs or AC individuals.

Thus, testing for dietary conservatism in a population involves establishing the existence of both AC and DC individuals. The distinction of the terms (importantly between the similarly named dietary conservatism and Dietary Conservative) is further illustrated in Figure B1 for clarity.

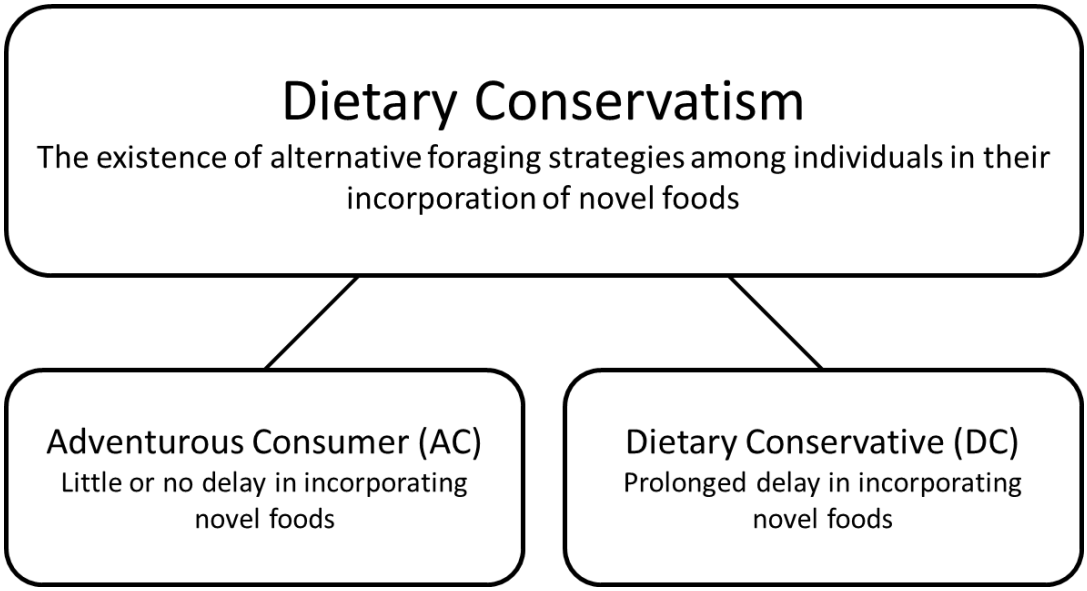

Figure B1. Diagram of dietary conservatism terminology illustrating how dietary conservatism relates to the foraging strategies (AC and DC) displayed by individuals.

1.4 Maintaining Two Foraging Strategies

The existence of the DC foraging strategy is surprising and counterintuitive. That the DC foraging strategy exists alongside the AC foraging strategy is astonishing. Classic optimal foraging view is that all individuals will forage in one ideal way according to their experience and the habitat in which they find themselves. Animals are expected to show an AC foraging strategy by default, consuming any profitable food they encounter and avoid all unprofitable foods (Marples et al 2018), but this is not what is observed in real populations. There are many reasons why individuals might depart from the optimal behaviour for the population (Skelhorn *et al.* 2016; Marples *et al*. 2018), but animals following a DC foraging strategy do so consistently, forgoing foods their AC competitors can exploit simply because that food is novel to them. DC individuals are apparently hampering their competitiveness by limiting their diet unnecessarily. Furthermore, the only difference between a DC individual which rejects a given food, and a DC individual which accepts it, is whether that individual encountered a different morph of the food type first, making the food in question marginally more novel. This apparently trivial asymmetry in experience causes, in DC individuals, a persistent rejection of the "novel" food for protracted periods of time which may encompass a significant proportion of that

individual's lifespan, and long after the avoided food could still be considered remotely novel.

One might expect selection to drive one of these strategies to extinction in any given habitat, but both strategies have been observed in every vertebrate population studied to date (see Table 1.2, below), across a wide range of ecologies. Consequently, the relative advantages of one strategy over the other must either be frequency dependent (where whichever strategy is in the minority has a competitive advantage) or simply negligible.

Why the DC foraging strategy exists and its evolutionary basis remain unexplored questions. Two hypotheses have been proposed to explain its persistence: toxin avoidance and foraging efficiency.

The toxin avoidance hypothesis (Marples *et al.* 2007; Richards *et al*. 2014) suggests that the DC foraging strategy is a way to avoid ingesting harmful foods, similar to neophobia's protective role against toxic prey or in uncertain environments (Greenberg, 2003; Crane and Ferrari, 2017). If the DC foraging strategy and food neophobia share the same ecological function of protection from harmful foods, then DC individuals should have a selective advantage over AC individuals and become more common as the frequency of toxic (or otherwise unprofitable) prey increases (Sherratt, 2003) and as the cost of consuming unprofitable prey increases (Sherratt, 2011).

Marples et al. (2007) found support for a link between the DC foraging strategy and avoiding unpalatable prey. Chicks exposed to a single distasteful food item of a familiar colour reactivated both their neophobia and overall wariness to that colour of food. However, only one study has so far attempted to explicitly test the toxin avoidance hypothesis. Richards et al. (2014) examined the proportion of DC individuals in different species of tropical fish (all poecilids: guppies, mollies, and swordtails) and compared these to the proportion of DC individuals in a temperate fish (three-spined sticklebacks,) with the assumption that toxic prey are more prevalent in the tropics, so the tropical fish species should have higher proportions of DC individuals in any given population. No support was found for the hypothesis. Tropical fish species had smaller proportions of DC individuals than the sticklebacks, suggesting that avoiding harmful prey is not the main advantage of the DC foraging strategy. However, it is worth noting that Richards *et al.*'s

experiment is by no means definitive. It based on the assumption that toxic prey are more prevalent in the tropics, yet we could find no substantial evidence in the literature to support this claim. In addition, the tropical fish tested were store bought (and therefore, presumably, captive bred) and may not have reflected the proportion of DC individuals in the wild. Moreover, their protocol may have underestimated the number of DC individuals (see Testing for Dietary Conservatism, below). Ultimately, evidence for the toxin avoidance hypothesis is lacking, but there is a need for further study before it can be confidently dismissed.

The enhanced efficiency hypothesis, on the other hand, suggests that DC individuals are effectively dietary specialists within a predominantly AC population. Comparing DC and AC individuals is therefore analogous to cross-species comparisons among dietary specialists and generalists, where dietary specialism is advantageous because of greater foraging efficiency (e.g., Michálek *et al.* 2017; García *et al.* 2018), especially when preferred prey are relatively abundant (e.g., Terraube *et al*. 2011). The DC foraging strategy would therefore be a means of specialising on an abundant, familiar prey and DC individuals would be more efficient at exploiting these than AC individuals.

No study to date has explicitly tested the enhanced efficiency hypothesis, however experiments with wild birds found that, when foraging with the threat of a nearby predator, both AC and DC birds selected and consumed only familiar prey, ignoring the novel (McMahon, 2014). As the presence of a predator necessitates speed and efficiency during any foraging, this result suggests that choosing familiar prey over novel offers some efficiency advantage.

The effect of competitors on foraging behaviour also suggests a role of efficiency in the DC foraging strategy. In blue tits, DC foragers behaved even more conservative in the presence of a competitor (McMahon and Marples, 2017). Among domestic chicks, DC birds failed to reduce their conservatism in the presence of a competitor eating cryptic food, while AC foragers became less conservative, presumably enhancing their own foraging efficiency by widening their diet (McMahon *et al*. 2014). These experiments suggest it is more profitable for DC individuals to specialise on familiar food in the presence of competitors.

The mechanism through which specialisation on familiar food increases foraging efficiency is not clear. One possible basis for this advantage is through the formation of a search image, a learned prey pattern which enhances the efficiency in identifying that prey type (Tinbergen, 1960). If DC individuals have a foraging advantage over AC individuals because they use search images, the way in which DC individuals develop, retain, and/or use search images must differ from their AC counterparts. This has yet to be demonstrated. Indeed, exploring the basis of enhanced foraging efficiency by DC individuals would be premature, as it is first necessary to demonstrate that DC individuals are more efficient than AC individuals. One approach to this question would be to compare encounter and consumption rates of AC and DC individuals in environments of varying complexity. Familiar prey can be made easier or more difficult to find, allowing exploration of the effects of both search and handling time on each foraging strategy's efficiency.

1.5 The Prevalence of the Dietary Conservatism and the DC Foraging Strategy

A range of species from diverse taxa have now been tested for the dietary conservatism, i.e., the presence of AC and DC foraging strategies (Table 1.2). Of these, all vertebrate populations tested so far (from >14 species across 4 phyla) have shown evidence of dietary conservatism, with both AC and DC individuals present in each population. In contrast, only one individual among the 42 individual invertebrates tested, from 5 species (3 spider, 1 ladybird and 1 praying mantis species), has suggested the existence of a DC foraging strategy among invertebrates. Its wide prevalence among vertebrate species suggests that dietary conservatism is a fundamental aspect of vertebrate foraging behaviour. Among vertebrates, DC individuals typically make up the minority of populations although, in some populations, higher proportions of DC individuals can be present (see Table 2).

These studies suggest that dietary conservatism is widespread among, but largely restricted to, vertebrates. However, one individual ladybird larva showed a distinct and long-lasting avoidance of novel prey while the remaining 7 larvae and 5 adults did not. This DC larva did not survive pupation, so could not be tested as an adult. The occurrence

of a DC foraging strategy in one invertebrate individual could be an idiosyncrasy of preference, or this finding may betray the presence of the trait at very low frequencies outside the vertebrate clade, but it would be premature to draw firm conclusions. To date, only a handful of invertebrate species have been tested for dietary conservatism, and only with small sample sizes. It is possible that some as yet untested invertebrates may display the behaviour more prominently, or that the behaviour is very rare among invertebrate populations. The experimental methods used may fail to detect dietary conservatism in invertebrates - when testing for dietary conservatism novel foods are typically distinguished from familiar foods by colour, which may not be a relevant cue for foraging invertebrates which rely on, for example, prey movement to elicit attack (Shine *et al.* 2003). Though dietary conservatism has been demonstrated in four vertebrate phyla, work has mostly focused on birds and generalist omnivores. One vertebrate phylum (reptiles) has yet to be examined for dietary conservatism and testing different guilds of foragers may reveal a more complex pattern of foraging strategies than is yet appreciated.

Future studies should aim to expand the number of species tested for dietary conservatism. To develop a better understanding of the distribution and underlying cause of DC and AC foraging strategies in the animal kingdom, studies should attempt to include more species from underrepresented taxa with different ecological roles and life histories, and care should be taken to ensure that novel aspects of food are relevant to the animals being tested.

Table 1.2. Published and unpublished studies testing vertebrate species for dietary conservatism. Unpublished studies are supported by data provided in Appendix 1. The testing methods "Latency" and "Evolution" refer to types of test, outlined in Box 1.2. DC proportion is the proportion of individuals in study that displayed a DC foraging strategy. Where the DC proportion was "Not explicitly defined" the presence of different foraging strategies was inferred from significant variation among recruitment times in the test population. "Colour balanced" refers to whether the novel cues used were balanced to correct for inherent bias.

Species	Population	Sample Size	Dietary Conservatism Observed	DC Proportion	Testing Method(s) used	Colour (or equivalent)-Balanced?	Reference
Birds							
Blackbird (*Turdus merula*)	Wild	19	Yes	Not explicitly defined	Latency	Yes	Marples *et al.* 1998
Robin (*Erithacus rubecula*)	Wild	12	Yes	Not explicitly defined	Latency	Yes	Marples *et al.* 1998
Zebra Finch (*Taeniopygia guttata*)	Captive	9	Yes	Not explicitly defined	Latency	No	Kelly & Marples, 2004
Domestic Chicken (*Gallus gallus domesticus*) – 3 experiments	Captive	120	Yes	Not explicitly defined	Latency	Yes	Marples *et al.* 2007
Robin (*E. rubecula*)	Wild Caught (tested in captivity)	10	Yes	80% (8/10)	Evolution	Yes	Thomas *et al.* 2003

Various bird species	Wild	n/a	Yes	Not explicitly defined	Evolution	Yes	Thomas *et al.* 2004
Great Tits (*Parus major*)	Wild Caught (tested in captivity)	17	Yes	23.5% (4/17)	Evolution	No	Marples & Mappes, 2011
Domestic Chickens (*G. g. domesticus*) – 3 experiments	Captive	108	Yes	38.8% (42/66)	Latency	No	McMahon *et al.* 2014
Blue Tits (*Cyanistes caeruleus*)	Wild Caught (tested in captivity)	11	Yes	81.8% (9/11)	Latency	No	McMahon *et al.* 2016
Fish							
Three-spined Sticklebacks (*Gasterosteus aculeatus*)	Wild Caught (tested in captivity)	52	Yes	32.7% (17/52)	Evolution	Yes	Thomas *et al.* 2010
Three-spined Sticklebacks (*G. aculeatus*) – single fish	Wild Caught (tested in captivity)	60	Yes	25% (15/60)	Evolution	Yes	Richards *et al*. 2011
Guppy (*Poecilia reticulata*) – 3 experiments	Captive (commercial supply)	86	Yes	10.5% (9/86)	Evolution	Yes	Richards *et al*. 2014

Mollie (*Poecilia sphenops*) – 3 experiments	Captive (commercial supply)	80	Yes	5% (4/80)	Evolution	Yes	Richards *et al*. 2014
Platy (*Xiphophorus maculatus*) – 3 experiments	Captive (commercial supply)	83	Yes	14.5% (12/83)	Evolution	Yes	Richards *et al*. 2014
Swordtail (*Xiphophorus helleri*) – 3 experiments	Captive (commercial supply)	95	Yes	7.4% (7/95)	Evolution	Yes	Richards *et al*. 2014
Amphibians							
Common Frog (*Rana temporaria*)	Wild Caught (tested in captivity)	19	Yes	15.8% (3/19)	Evolution	Yes	Unpublished thesis
Smooth Newt (*Lissotritus vulgaris*)	Wild Caught (tested in captivity)	5	Yes	60% (3/5)	Latency	Yes	Unpublished thesis
Smooth Newt (*Lissotritus vulgaris*)	Wild Caught (tested in captivity)	15	Yes	46.7% (7/15)	Evolution	Yes	Unpublished thesis
Mammals							
Mouse (*Mus musculus*)	Captive	40	Yes	15% (6/40)	Latency	Yes	Unpublished thesis

Invertebrates							
African Praying Mantis (*Sphodromantis lineola)*	Captive (commercial supply)	18	No	0%	Latency	Yes	Unpublished thesis
Seven-spot Ladybird - adult (*Coccinella septempunctata*)	Captive (bred in captivity from wild stock)	5	No	0%	Latency	Yes	Unpublished thesis
Seven-spot Ladybird - larvae (*Coccinella septempunctata*)	Captive (bred in captivity from wild stock)	8	Yes	12.5% (1/8)	Latency	Yes	Unpublished thesis
Spiders of 3 species	Wild caught and captive (commercial supply)	11	No	0%	Latency	Yes	Unpublished thesis

1.6 Dietary Conservatism: Binary or Continuum?

Dietary conservatism has largely been treated as a binary phenomenon, with individuals considered to be using either the AC or the DC foraging strategy (e.g., Marples and Brakefield, 1995; Marples and Kelly, 1999; Thomas *et al*. 2003). To some extent, this assumption is a consequence of method – tests that simulate natural selection on a prey population (see "Evolution Tests" in "Testing for Dietary Conservatism", below) have only two possible outcomes: either the novel food is driven to fixation and the individual is classed as DC, or the novel food is driven to extinction and the individual is classed as AC (e.g., Marples and Mappes, 2001; Thomas *et al*. 2003). Other methods (see "Latency Tests", below) have found dietary conservatism expression to be binary when a relatively short, experimental endpoint is used to calculate dietary conservatism expression (e.g., McMahon and Marples, 2017) or continuous when longer end-points are used (Kelly, 2001).

Identifying the genetic component of AC and DC foraging strategies is an essential step in understanding the binary or continuous nature of dietary conservatism. Marples and Brakefield (1995) selectively bred AC and DC lines individuals of Japanese quail and concluded that foraging strategy may be controlled by a single gene. However, foraging strategy expression is heavily influenced by other factors, such as cognition, perceived predator risk (McMahon, 2014), and experience (Marples *et al.* 2007). As such, even if the behaviour is under binary control genetically, it may be more practically useful to treat dietary conservatism as a continuum of foraging strategies. Greggor *et al*. (2015) argue that research regarding neophobia needs to incorporate psychology and ecology for a better understanding of the behaviour, and the same applies to AC and DC foraging strategies. How animals respond to novel foods is a combination of perception, learning, memory, physiological state, and foraging ecology (Turner and Speed, 1999; Marples *et al.* 2018) and developing a complete understanding of dietary conservatism requires incorporating these concepts into future studies.

Box 1.2. Testing for Dietary Conservatism

Testing for dietary conservatism involves demonstrating the existence of alternative foraging strategies. Determining an individual's foraging strategy (AC or DC) involves testing incorporation rates of a novel food. Two distinct methods have been developed to test for dietary conservatism which we term "Latency" and "Evolution" tests.

Latency tests measure the time taken for an individual to consume a novel food e.g., (Marples *et al.* 1998; Marples *et al*. 2007; McMahon and Marples, 2017). Equal amounts of the novel and familiar food type are presented together and the latency to eat the novel food is measured. As individual foragers tend to sample foods they have not yet accepted into their diet (Marples and Kelly, 1999), acceptance of the novel food is determined by individuals eating a certain number of novel food items (usually three) in a predetermined time or number of trials. Individuals that fail to consume the required number of novel food items in that time are then defined as DC and those that do consume the novel food before the time limit are defined as AC.

Evolution tests simulate natural selection among a population of artificial prey made up of novel and familiar food items (e.g., Thomas *et al*. 2003; Marples and Mappes, 2011; Richards *et al*. 2014). Initially, the proportion of novel food items is low (usually 5% of the total, or 1 novel prey in a population of 20) and the majority of the population is made up of familiar prey. Individuals are permitted to forage on this mixture until half of the food is consumed, at which point the trial is stopped. The surviving prey are then counted and the proportion of novel and familiar food items remaining is used to calculate the makeup of the next prey population, scaled back up to the original number. If the foraging individual consistently avoids the novel prey, the proportion of novel prey will increase in consecutive trials. In this way, DC individuals can be identified as those foragers which allow the novel prey to reach fixation in the test population (i.e., the familiar prey goes extinct). Conversely, AC individuals that do not consistently avoid the novel prey are likely to drive the novel prey to extinction in early trials when they are low in numbers. Long-term maintenance of both prey types in the population is very unusual, with one or other prey type typically becoming extinct within 20 trials (Thomas *et al*. 2004).

1.7 Testing for Dietary Conservatism: Distinguishing ACs from DCs

Two distinct approaches have been used to test dietary conservatism and distinguish AC and DC individuals, here termed “Latency” and “Evolution” tests (Box 1.2). In both types of test, individuals are first familiarised with one type of food (the familiar food) by being exposed to it from birth, or offered it without alternative food for at least four days, before being introduced to a novel food. Typically, the novel food differs from the familiar food only in colour (e.g., Marples *et al.* 1998; Thomas *et al.* 2003), but studies have used novel odours (Kelly and Marples, 2004), patterns (Marples and Mappes, 2011), and even natural seeds with

multiple visual, taste, and odour cues (Camín *et al*. 2016). The foraging strategy an individual displays is then determined by the speed with which they accept the novel food.

1.7.1 Latency Tests

Latency tests are direct measures of the time taken to incorporate a novel food. They allow simultaneous measurement of neophobia and observation of the order in which prey are eaten, facilitating comparison among the stages of dietary incorporation (McMahon *et al*. 2016). Another advantage of latency tests (compared to evolution tests, below) is the speed with which individuals can be screened as AC or DC. Individuals that consume as little as three novel food items in up to three exposures can be identified as AC, while those individuals that fail to eat three novel food items across three tests can be identified as DC (e.g., Marples *et al.* 2007; McMahon *et al*. 2016). As DC avoidance is only deactivated over a large number of exposures (Marples *et al*. 2007), the limited exposure individuals have to their novel foods during latency tests is unlikely to affect DC expression in subsequent tests. Thus, latency tests allow for subsequent testing of DC expression by individuals in other contexts (e.g., McMahon *et al*. 2016).

Despite their usefulness, latency tests have weaknesses relating to the endpoint of novel food recruitment. Identifying when incorporation of a novel food has occurred is necessary to understand features such as duration and reactivation of the DC foraging strategy with respect to a given food type. Different latency tests have used different criteria to identify the point of novel prey recruitment. The most common approach for birds has been to define recruitment as consuming the novel food in three successive presentations (e.g., Marples *et al*. 1998; McMahon *et al*. 2016), but sometimes the novel prey is also required to be taken as quickly as the familiar food (Kelly and Marples, 2004). While the latter probably more accurately reflects the endpoint of dietary conservatism, the former is shorter (and requires simpler experimental methodologies) and is less likely to influence subsequent testing.

Consumption of three novel food items was shown to be sufficient to indicate a willingness of individual domestic chicks to continue eating the novel food thereafter (Marples *et al*. 2007). However, wild birds, for example, may have a longer period of sampling before recruitment and other taxonomic groups may need to fulfil different criteria to indicate full

recruitment. Other researchers have determined the start of recruitment as pecking at the novel food more than 5 times in a row (Camín *et al*. 2016). This seems unlikely to be comparable to latencies which measure consumption of the novel food. Regular contact with, or manipulation of, a novel food is more representative of the end of neophobia (Marples and Kelly, 1999). Additionally, the novel food may be presented either with (e.g., McMahon *et al.* 2014) or without (e.g., Camín *et al*. 2016) the familiar food during testing. Research has yet to investigate how such differences in food presentation might affect foraging strategy expression.

Another limitation of latency tests of only a few trials is that they can fail to detect the full range of variation in recruitment latencies. Shorter tests have been carried out over one or more trials of 3-10 minutes each (i.e., less than 600 seconds of exposure to the novel foods; e.g., Marples *et al*. 2007; McMahon *et al.* 2014), but populations may have some individuals whose DC would persist for much longer than that. If these individuals were tested until recruitment of the novel food occurred, a broader variation in latencies could be revealed than is normally detected. When designing short latency tests, it is prudent to consider whether sufficient time is given to capture all meaningful variation if statements about variation and duration of dietary conservatism (at least among DC individuals) are to be made.

1.7.2 Evolution Tests

Evolution tests for dietary conservatism simulate natural selection by an individual predator on a population of familiar and novel "prey". See Box 1.2 for a description of this method.

Evolution tests have been applied to birds in the wild (Thomas *et al.* 2004) and in the laboratory (Thomas *et al.* 2003; Marples and Mappes, 2011) and to several species of fish in the laboratory (Thomas *et al.* 2010; Richards *et al*. 2011, Richards *et al.* 2014). Monte-Carlo simulations of evolution tests with birds have shown that the likelihood of a novel morph reaching fixation by chance is very low (probabilities ranging from 0.00002 to 0.0052; Thomas *et al.* 2003; Thomas *et al.* 2004). This indicates that, while driving a novel morph to fixation, individuals must actively and consistently avoid consuming it, including when it forms the majority of the population. As such, evolution tests for dietary conservatism provide a robust method for distinguishing AC and DC individuals. In addition, evolution tests can be easily

applied with little modification to a wide range of species and settings. Any individual that is slow to fully incorporate a novel food into their diet can be identified as using a DC foraging strategy, without the need to consider whether consuming a set number of novel food items represents an equivalent incorporation for different species in different conditions.

One weakness of evolution tests is that occasionally early sampling of novel prey, when they are rare in the population, may send them extinct even by DC individuals which have not incorporated the novel food into their diet. This can lead to the misclassification of DCs as AC individuals. Such problems can be addressed by using more prey in the initial population while presenting the prey types in the same ratio (e.g., introduce the novel prey at a frequency of 5 novel: 95 familiar instead of 1 novel: 19 familiar) or by repeating the test when novel extinction occurs early, requiring confirmation that it was caused by AC foraging and not mere sampling.

Evolution tests require a much greater investment of time and effort to conduct. While AC individuals may consume the novel prey and drive them to extinction in just a single presentation, DC individuals which consume no novel prey but eat half of the total prey in each generation will require a minimum of six successive presentations to drive novel-coloured prey to fixation (assuming an initial proportion of 5% novel prey). Where avoidance of novel prey is strong, but incomplete (i.e., novel prey are occasionally but not consistently eaten), individuals may take significantly longer to send novel prey to either fixation or extinction. In one instance, a stickleback took 22 days to send a novel morph to fixation (Thomas *et al*. 2010). The long duration of evolution tests may also limit the use of individuals for subsequent testing, as exposure to novel foods may allow DC individuals to become accustomed to novelty and reduce their incorporation latencies for other novel food types (Marples *et al*. 2007). Such effects will be uneven across the DC part of the population as DC individuals with weaker preferences for familiar prey, will be exposed to novel prey for longer.

Though evolution tests provide clear criteria for identifying AC and DC individuals, differences in the application of these tests between experiments makes simple comparisons difficult. The initial proportions of novel and familiar foods have varied from 1% novel (Marples and Mappes 2011) to 5% novel when testing birds (Thomas *et al*. 2003, 2004) and fish (e.g., Thomas *et al*. 2010). Similarly, starting numbers have varied from a single novel food item in a total of 20, to three novel prey and fifty-seven familiar prey for groups of three fish (Richards

et al. 2011). The effect of differing initial conditions in evolution protocols has not been explicitly tested. If, for instance, the likelihood of sampling novel prey was influenced by the absolute number of that prey type available, then changing the prey population size might alter the likelihood of identifying an individual as AC or DC.

A practical difficulty with evolution testing is that, ideally, half of the available prey should be consumed in any given round of testing. However, halting a test when exactly half of the prey have been eaten can be challenging, either because of the speed of eating by some subjects, or because of the difficulties associated with observation without causing disturbance, (as in wild studies). If individuals eat more than half of the available prey but avoid novel prey when doing so, they may drive novel prey to fixation in fewer than the minimum six presentations required (cf Thomas *et al*. 2003 and Thomas *et al.* 2010). This lowers the bar for identifying DC individuals compared to trials where individuals may eat only half the food available. If, on the other hand, individuals are permitted to eat all baits in a single trial (cf Richards *et al*. 2014), it is possible for novel prey to be eaten after the familiar prey have been consumed. Such behaviour would suggest a DC foraging strategy, but the individual is likely to be identified as AC because it has driven the novel prey extinct. Thus, it is important for trials to be stopped when half the prey have been eaten. Otherwise, more active eaters are likely to be misidentified and the reliability of the testing method will be reduced.

1.7.3 Recommendations for Testing for Dietary Conservatism

To facilitate comparisons between experiments, we propose the following framework to guide future tests of dietary conservatism.

Studies should either work with a colour-balanced design, or, where that is not possible, aim to use cues for which individuals have no pre-existing bias. This should be ascertained by testing a subset of naïve individuals for a pre-existing preference for any of the colours, odours, patterns, etc. which are to be used. As such a test might influence future foraging decisions, these individuals may need to be excluded from further dietary conservatism testing.

For detailed explorations of the stages of dietary conservatism, as well as screening for the foraging strategies of individuals which will go on to further testing, latency tests are more

useful than evolution tests. For straightforward tests of the prevalence of dietary conservatism among individuals, which are easily adaptable to different taxa, evolution tests are a better choice. In latency tests, novel food should be presented alongside familiar food, simulating a natural scenario where alternative foods are likely to be available.

Full recruitment of a novel food occurs when the forager no longer distinguishes between that novel food and familiar foods. This state can best be identified by comparing rates of consumption of the two food types, though this may not always be practical where foraging cannot be observed directly. In addition, the duration of testing should be sufficiently long to capture all meaningful variation in dietary conservatism expression.

Ideally, for evolution tests, the speed with which individuals can send prey morphs to fixation or extinction should be controlled by strictly halting tests once half of the prey are eaten. Where such control is impossible, it can be simulated by limiting the increase of novel prey morphs in consecutive 'generations' to, at most, double their previous proportion. Effectively, this treats individuals as though they had only eaten half the available food and sets the minimum number of testing generations required to identify a DC individual to six. Where all individuals do not eat precisely half of the available prey, variation in the amount eaten may lead to variation in hunger levels among individuals for subsequent rounds of testing. This, in turn, may alter their behaviour towards novel food. It is possible to control for such variation by offering supplementary feeding to satiation after each test (but not immediately afterwards, to avoid training animals not to eat during tests), coupled with a standard food deprivation period before the test, to ensure similar hunger levels for all individuals.

1.8 Ecological and Practical Impacts of Dietary Conservatism

The DC foraging strategy has potential to determine an individual animal's diet by limiting incorporation of novel foods. At the population level, such choices have the potential to shape evolutionary patterns. Novel prey morphs encountered by DC individuals will enjoy a selective advantage over familiar ones. This advantage is likely to be overlooked if only an "average" response to novel prey across a population (in which most individuals are ACs) is considered. Rather than being consigned to extinction because of an overall selective disadvantage, novel morphs may persist in patches dominated by DC individuals.

This thinking has been applied to the apparent paradox of the evolution of aposematic signals. Marples *et al*. (2005) argue that the disadvantage of being conspicuous may be outweighed by the advantage of being novel, especially if the rare new aposeme arises in the territory of a DC individual. The new aposeme might then reach high enough densities locally to start gaining protection through aposematic signalling. Modelling of such scenarios supports this idea (Lee *et al*. 2010).

Conspicuousness aside, if the presence of DC predators increases the likelihood of recently evolved, novel morphs persisting, dietary conservatism may facilitate the evolution of diversity in general. Small populations of novel prey morphs may be able to escape immediate extinction due to stochastic predation if they occur in territories or areas dominated by DC predators, and eventually grow to a population size where stochastic elimination is unlikely. Further modelling work is needed to investigate how DC predators affect the likelihood of persistence of novel morphs. However, comparing the prey diversity in territories held by DC and AC individuals offers a simple way to test this hypothesis.

Mutation is not the only source of novel prey morphs in the wild. Changes in distribution can bring predators into contact with prey which they have not previously encountered. This is a situation which is becoming increasingly likely as climate change causes shifts in species ranges (Brooker *et al*. 2007; Burrows *et al*. 2014) and the number of introduced, alien species continues to rise globally (Seebens *et al*. 2017). Dietary conservatism may exaggerate the effects of changing ecosystems, for instance by limiting the ability of DC individuals to adapt to new habitats dominated by novel prey or enhancing the survival of aliens that are ignored by DC predators. No research has so far examined how dietary conservatism affects populations expanding their range into new areas, or the survival of invasive species, so there remain significant opportunities to explore this interaction and improve our understanding of how both individuals and populations adapt to new environments.

Dietary conservatism also has relevance beyond the field of dietary ecology if AC and DC individuals differ in respects other than how they treat novel foods, which some research suggests may be the case. Studies on the effects of competition on dietary conservatism expression (McMahon *et al*. 2014; 2017) suggest that foraging strategy is related to how social information is used. Furthermore, social network analysis on great tits suggests AC and DC individuals maintain relationships with conspecifics differently, with DC individuals having

smaller and more close-knit social circles (McMahon, K. unpubl. data). Thus, social interaction seems to be a behaviour unrelated to foraging that co-varies with foraging strategy. There are many research opportunities to determine whether and how animals' foraging strategies are linked to other behaviours.

In addition to its implications for evolutionary patterns and the behaviour of wild animals, understanding alternative foraging strategies with respect to novelty has potential applications in the field of animal management. Weight gain might be improved when changing feed by adopting policies sympathetic to DC individuals (Marples *et al*. 2007). Similar strategies might be employed to promote the welfare of DC individuals in husbandry and *ex-situ* conservation. Reintroduction programs and *in-situ* conservation efforts could also benefit from an understanding of dietary conservatism. An individual's foraging strategy is likely to affect their survival when introduced to a novel habitat, and AC and DC individuals may respond differently to supplemental feeding. An awareness of the ratio of AC to DC individuals may allow a more effective utilisation of resources within the habitat by the reintroduced population.

Finally, it is possible that an understanding of dietary conservatism may allow us insights into fussy eating behaviour in humans. Like the DC foraging strategy in animals, fussy eating in children is heritable (Dubois *et al*. 2013; Fildes *et al.* 2016) and may even be the same trait. It may be worthwhile to explore how humans incorporate novel foods into the diet and the variation that exists.

Overall, there is a need for more dietary conservatism research to leave the laboratory and to explore how animals using AC and DC foraging strategies behave in more natural settings. This would allow results from lab-based studies to have their ecological relevance verified and set the stage for real-world understanding of animals' responses to novel foods and application of that knowledge for practical use. Advances in DNA barcoding technology have made detailed analysis of wild animals' diets possible (Kress *et al*. 2015), offering one potential approach to exploring AC and DC foraging strategies without the need for artificially manipulated novel foods. Meanwhile, advances in remote sensing and micro video-camera technologies offer great potential for real time assessment of foraging decisions in the wild.

Chapter 2

How Familiar is Familiar?

Authors

Sam Preston, Rob Thomas, and Nicola Marples

Author Contributions

I designed this study, collected and analysed the data, and wrote this manuscript. NM provided insight on this manuscript and experimental design. RT assisted with statistical analysis.

Status

This manuscript has been submitted and is awaiting review. Raw data and R scripts for have also been provided for review.

Note

When referred to in other chapters (excluding general discussion), this manuscript is referenced in text as Preston *et al*. in prep b.

2.1 Lay Summary

Familiarity is not all-or-nothing but varies with degree of experience and foraging strategy. Individuals that are slow to accept novel foods prefer familiar food more when they have greater prior experience with it, while fast acceptors rapidly lost their preference for their familiar food irrespective of prior experience.

2.2 Abstract

Animals show an aversion to novel foods and a preference for familiar ones. Familiarity is gained through exposure, but the process by which familiarity develops has largely been neglected in the literature. Is familiarity an all-or-nothing trait? Or is familiarity relative depending on the amount of prior experience? Preference for a familiar food also depends on foraging strategy: some individuals are quick to incorporate novel foods into their diet (Adventurous Consumers, AC), while others refuse to do so and maintain a prolonged preference for familiar foods (Dietary Conservative, DC). To investigate how experience and foraging strategy affected preference for familiar foods, we familiarised three-spined sticklebacks (*Gasterosteus aculeatus*) with dyed *Daphnia magna* for different durations before presenting them with a choice test of novel and familiar-coloured prey. Preference and foraging strategy were determined by the order in which prey were eaten. Our results suggest preference for familiar food increases with the amount of prior experience, but that this depends on the foraging strategy of the individual. For fish with a DC foraging strategy, even a small amount of experience can elicit a transient preference for the familiar food. In contrast, AC fish only maintained a transient preference for familiar food with the greatest prior experience. The findings of this study have implications for both theoretical understanding of ecology and evolution and practical application in conservation and animal husbandry.

2.2.1 Keywords:

Familiarity; Novelty; Dietary Conservatism; Foraging; Behaviour; Sticklebacks; Experience

2.3 Introduction

In a complex and changing world, animals must be able to deal with a potentially infinite range of unfamiliar stimuli. Novelty often elicits an aversive reaction in animals (Barnett, 1958; Greenberg and Mettke-Hofman, 2001), as the risks of interacting with the unfamiliar are unknown (Sherratt, 2011; Brown, 2020). Understanding animals' responses to novelty is crucial to our understanding of their ecology and evolutionary pressures (see Crane and Ferrari 2017 and Crane *et al*. 2020 for reviews). Research that explores the effect of novelty on behaviour has focused on the nature of the novel stimulus (e.g., Domjan 1975; Launchbaugh *et al*. 1997; Mappes and Alatalo, 1997; Thomas *et al.* 2003) and the environmental and social context in which it is presented (e.g., Coleman and Mellgren, 1994; Harris and Knowlton, 2001; Thomas *et al.* 2004; Moretti *et al*. 2015; McMahon and Marples, 2017). However, the process of becoming familiar with a particular stimulus has received comparatively little attention.

How animals determine "familiarity" is a crucial question for improving our understanding of behavioural responses to novelty. Testing responses to novelty requires animals to be habituated to familiar stimuli and circumstances against which their responses can be compared. Protocols and criteria for this familiarisation vary considerably across studies, from Sol *et al*. (2011) familiarising their birds for 2 days to Miller *et al*. (2022) testing them in conditions they were always kept in. In an early experiment on neophobia in different rat (*Rattus norvegicus*) strains, Mitchell (1976) allowed the animals to habituate to their feeding apparatus for 27 days. More recently, Modlinska *et al.* (2015) similarly tested multiple strains of rats, allowing 7 days of familiarisation with their experimental feeding apparatus. In testing birds' responses to novel foods, familiarisation with an experimental food varies from just 3 exposures over 2 days in domestic chicks (*Gallus gallus domesticus*; McMahon *et al*. 2014) to a week of exposures for various wild birds (Thomas *et al.* 2003; Kelly and Marples, 2004; Richards *et al.* 2011), and up to four weeks for wild blue tits (*Cyanistes caeruleus*) in captivity (McMahon and Marples, 2017). The validity of comparing results from these experiments depends on animals having a threshold of experience beyond which stimuli are equally familiar, and this threshold being met by the familiarisation procedure. This caveat also applies to comparisons among individuals within a given experiment, as individuals may receive vastly different amounts of experience with certain stimuli. Do individual birds with

different lengths of experience with a familiar food on a scale of weeks (McMahon and Marples, 2017) or months (Sol *et al*. 2011) treat that food as sufficiently and equally familiar?

Using arbitrary criteria for habituating animals to a set of stimuli against which responses to novelty can be measured also relies on the implicit assumption that "familiarity" is all-or-nothing. This may not be the case. As a trait of stimuli, familiarity may be incremental and based on relative experience. Animals may show greater preference for stimuli with which they have more experience and thus greater certainty concerning its safety or profitability. Suppose an animal is faced with 3 different (but equally profitable) food items – one item it has encountered and eaten once before, the second it has eaten on 5 previous occasions, and the third on 10 or more previous occasions. Which is it most likely to eat first? Should it prefer to eat the third food with which it has had the most experience first, or might it be equally likely to consume either the second or third food types first as it has substantial experience with both? Or will it be equally likely to eat any of the foods available because it has had positive experience with all of them at least once?

When confronted with novel foods, vertebrates have been shown to adopt distinct foraging strategies (Marples and Kelly, 1999). Some individuals adopt a Dietary Conservative (DC) foraging strategy, displaying a prolonged reluctance to incorporate novel foods into the diet. Others adopt an Adventurous Consumer (AC) foraging strategy, rapidly accepting novel foods as part of their regular diet (Marples and Kelly, 1999).

The DC foraging strategy is a counterintuitive behaviour. Dietary Conservative individuals consistently forgo profitable foods simply because they are novel, and despite gaining experience through repeated exposure to the novel food (Marples *et al*. 1998). In any environment, DC individuals might be expected to be outcompeted by AC individuals that exploit a wider range of foods more readily. If the environment is complex or variable, with more opportunity for individuals to encounter novelty, the advantage of the AC foraging strategy should be even greater. Nevertheless, individuals displaying a DC foraging strategy have been observed in every vertebrate population examined to date (Preston and Marples, in prep a). These include some 20 species across four phyla: birds, fish, amphibians, and mammals.

Research on the incorporation of novel foods has shown that animals maintain their preference for familiar food throughout the process of incorporating a new food type into the diet (Marples and Kelly, 1999). That a preference for a familiar food type can be maintained while gaining experience with a novel food type strongly suggests that preference is driven by relative experience to at least some degree. A challenge of studying animals' responses to novelty is the difficulty in acquiring repeated measures of the response from individuals, as after the initial exposure the stimulus is no longer novel (Greggor *et al*. 2015). Variation in the strength of long-lasting preferences for familiar food offers a unique opportunity to study the effects of familiarisation with a single food type.

Using three-spined sticklebacks (*Gasterosteus aculeata*), this study tests the alternative models of acceptance of food as familiar: whether the process of familiarisation has a threshold above which further experience does not change the response to the food, or whether familiarity is relative to the amount of prior experience. The response of the fish is considered in relation to whether they are an AC or a DC forager.

2.4 Methods

2.4.1 Fish collection and outdoor housing

Three-spined sticklebacks were caught using seine and hand nets from Vartry Reservoir, Co. Wicklow, Ireland (53.0505, -6.1896). Following capture, fish were initially held in large outdoor tanks, before being transferred to the laboratory as needed in batches of 24 healthy individuals between 25-40mm in length. In the laboratory, the fish were housed in groups of 6 in white, 10L buckets for 4 days to acclimatize, before being transferred to individual, 1.4L tanks (ZT140T, Aquaneering, San Diego, CA, USA). Tanks were lined with white paper on sides and bottom to provide a uniform background against which to forage.

Fish were initially housed together to reduce stress following transfer to the laboratory environment and to promote eating, as solitary fish were observed to be very reluctant to eat if housed alone immediately after transfer to the laboratory. The water in the buckets and tanks was aerated and fish were provided with sections of semi-transparent tubing (approximately 5cm long and 3cm in diameter) as shelter and hiding spaces.

Of the 24 fish in each batch, 20 were experimental subjects, and 4 randomly selected individuals were designated as “replacements” for use if experimental fish became ill.

2.4.2 Water Treatment

While in buckets, fish were initially kept in a 6L mixture of water collected from their outdoor tanks and dechlorinated water (approximately 50:50) and received a 20-25% water change daily (dechlorinated water). Individual tanks were filled with dechlorinated water and received a >75% water change on alternate days. To limit any stressful effect of water changing on the foraging behaviour of solitary fish, water changes were performed after the experimental trials for individual tanks.

Prior to feeding, particulate waste not captured by filters was removed daily from buckets with a fine-mesh hand net, and from individual tanks with a disposable pipette. On days without a water change, water was treated daily with Seachem Prime (Seachem Prime, Seachem Laboratories, Madison, GA, USA) to detoxify nitrogen waste, and with an

antibacterial and antifungal solution (API Pimafix, Api Fish Care, Chalfont, PA, USA) after the experimental trials.

2.4.5 Experimental food

Fish were fed live and dyed *Daphnia magna* during the experiment. To produce food to which all fish would be naïve, *Daphnia* were dyed using Sugarflair food colouring to create green (Sugarflair Party Green) and brown (Sugarflair Dark Brown) coloured food. The opaque green and brown colours dying produced were distinct from the colour of undyed *Daphnia* likely to be encountered in the wild. Fish were trained with brown as their "familiar" food, and green was used as a novel food.

Daphnia were sieved prior to dying to select individuals with a length of 1-2mm for feeding. These were then rinsed in dechlorinated water, patted dry, and dyed by covering in food colouring for a minimum of 15 minutes, which was found to be sufficient to achieve a strong colour that would persist in water for at least 15 minutes. *Daphnia* were removed from the dye as necessary and rinsed in dechlorinated water before being used. This process killed the *Daphnia*. Rinsing removed excess dye avoiding colour contamination of the tank water. *Daphnia* dyed for longer than 15 minutes (even up to several hours) did not retain noticeably more colour than *Daphnia* dyed for precisely 15 minutes.

2.4.6 Training

Fish were divided into 4 treatments varying in the number of exposures to their familiar food, receiving either 1, 4, 7, or 10 days of exposure, split across the buckets and the individual tanks (see Table 2.1). Prior to exposure to their familiar food fish received live, undyed *Daphnia*. All fish had the same number of days in total in the laboratory before testing, so had equal opportunity to habituate to laboratory conditions.

Each bucket received 15 *Daphnia* per fish once per day and were allowed to forage for 10 minutes, after which any uneaten food was removed. During the time that brown dyed food was presented to the fish while they were housed in buckets, all fish partook in feeding, although it was not possible to monitor the exact intake of each fish in the buckets.

Following transfer to individual tanks, fish also received 15 *Daphnia* once daily. To habituate fish to foraging in the presence of an observer during testing, the first 3 *Daphnia* were provided individually with a disposable pipette. Fish displayed a freeze response (tonic immobility) when initially disturbed, which ceased with experience with the training procedure. Fish quickly learnt to attack and follow the pipette during feeding, indicating good habituation to the presence of the experimenter. The remaining *Daphnia* were placed in the centre of the tank and fish were allowed to forage for 10 minutes unobserved. The number of *Daphnia* eaten by each fish was recorded.

Table 2.1. Treatment groups and their experience with brown *Daphnia* prior to testing. Groups were exposed daily to brown Daphnia for different durations with exposures being divided across training in buckets and individual tanks. Fish were fed undyed Daphnia on days where they were not exposed to brown food.

Treatment group	Total days experience with familiar food	Days fed live, uncoloured *Daphnia*	Days in buckets with familiar-coloured *Daphnia*	Days in individual tanks with familiar-coloured *Daphnia*
Ex1	1	9	0	1
Ex4	4	6	1	3
Ex7	7	3	4	3
Ex10	10	0	7	3

2.4.7 Testing

To determine the foraging strategy employed by individual fish, a procedure based on that used by Marples *et al*. (2007) was developed. Fish were provided with 10 brown and 10 green *Daphnia* arranged in a 4 x 5 grid of alternating colours. During *Daphnia* placement, fish were cordoned in one end of the tank using an opaque, plastic card. The introduction of the card often produced another freeze response, which lasted up to several minutes but diminished substantially over subsequent tests (in most cases disappearing entirely). Fish were permitted to forage for 10 minutes after tonic immobility had been overcome or until all food had been consumed. The order in which each coloured food was eaten was recorded and used to

calculate a “preference score” for the familiar food, a technique previously used in wild birds (Marples and Kelly, 1999; Kelly, 2001).

Fish were tested once daily for 5 days. After all tests had been completed for the day, any fish that ate fewer than 20 *Daphnia* were provided additional, undyed *Daphnia* to make up the difference, and allowed to forage for 10 minutes, after which anything still uneaten was removed. This minimised differences in hunger levels due to different numbers of *Daphnia* eaten during testing.

Sixty fish in 3 batches of 20 were tested. Of these, 35 fish completed both training and testing and their data were used in the analysis. Twenty-five fish that failed to complete all training and testing days (e.g., due to refusal to eat during any day of testing) were excluded from analysis.

2.4.8 Innate Colour Preference

To investigate whether there was an inherent or pre-existing bias for either colour of *Daphnia* used in this experiment, 18 naïve fish were placed in individual tanks in the laboratory and allowed to habituate to their new environment for four days, fed on 15 undyed, live *Daphnia* per day. Fish were then presented with 20 dyed *Daphnia* (10 of each colour) and allowed to forage for up to 15 minutes (after tonic immobility had been overcome) until 10 food items had been consumed. Fish that failed to eat at least 8 food items were allowed to forage for a further 15 minutes. After foraging, the remaining *Daphnia* were counted to determine whether naïve fish showed any preference for either colour. Any fish that failed to eat after 30 minutes foraging or failed to overcome tonic immobility after 10 minutes were retested once the following day. After all testing, fish were removed to an outdoor tank for release.

2.5 Data Analysis

2.5.1 Innate Colour Preference

To determine whether naïve fish showed a preference for either brown or green *Daphnia*, the difference in number of each colour eaten was calculated. If fish have an innate bias for either colour this figure should be significantly different from zero. A one sample t-test was applied to data collected during the first 15 minutes testing, excluding 4 fish which failed to eat any *Daphnia* in this time. A second t-test was applied to data from fish that ate at least 10 *Daphnia* during 15 minutes of (receiving no further opportunity to eat) pooled with data from fish allowed a further 15 minutes foraging.

2.5.2 Calculating Preference Scores

Animals are expected to consume preferred food types before non-preferred foods, all else being equal. Based on this assumption, familiar preference scores were calculated using the following formula:

Preference Score = 21 - ∑ (Ordinal position of familiar prey)/Number of familiar prey available.

In this experiment, summing the ordinal positions of familiar-coloured, consumed *Daphnia* (e.g., 1^{st} +2^{nd} + 3^{rd} = 6) and dividing by the total number of familiar-coloured *Daphnia* available (10) yielded a preference score of between 5.5 and 15.5. For instance, if a fish were presented with 10 familiar-coloured 10 novel-coloured *Daphnia* and consumed all 10 familiar prey followed by all 10 novel prey, then its familiar preference score would be calculated by the sum of numbers 1-10 divided by 10 (5.5). These values were subtracted from 21 (sum of maximum and minimum values) so that that highest possible score (15.5) indicated strongest possible preference for the familiar (all familiar- *Daphnia* eaten before any novel-coloured *Daphnia*) and the lowest score (5.5) indicated weakest preference for the familiar (i.e., strongest preference for the novel). Intermediate scores reflected intermediate preferences for the familiar.

Uneaten *Daphnia* were assigned positions as though they had been eaten after all other food, e.g., where only 10 familiar-coloured *Daphnia* were eaten, novel-coloured *Daphnia* were assigned positions 11 to 20. Where both novel- and familiar-coloured *Daphnia* remained

uneaten at the end of the experiment, all remaining *Daphnia* were assigned the mean remaining position as though fish had no preference for these food items. Occasionally, fish would consume both a novel- and familiar-coloured *Daphnia* at the same time, in which case the average of the two positions each would have taken if eaten sequentially was used for both food items.

2.5.3 Determining foraging strategy

Statistical analysis was carried out using R Program version 4.0.2 (R Core Team, 2020). Individuals' preference scores on day 5 of testing was used to determine foraging strategy. Different foraging strategies are known to produce consistent responses to novel foods over long periods (Marples *et al*. 1998). Using the last day of testing should give the best accuracy in identifying individuals' foraging strategies by ensuring their preferences have been maintained over a long period. Furthermore, identifying AC and DC strategies on the fifth day of testing mitigates against any confounding effect of neophobia, which is comparatively short-lived (Marples and Kelly, 1999). By the fifth day, all fish had made contact and/or consumed some novel food, indicating that neophobia had been overcome.

Preference scores on day 5 of testing were scaled -1 to 1 using the "mutate" function in R. Unsupervised K-means cluster analysis was applied to split the fish into 2 groups based on mean, scaled preference scores on day 5 of testing, so that individuals are assigned to the group whose mean is closest to their preference score. Fish in the group with higher preference scores were categorized as being DC (showing stronger preferences for familiar food), while fish in the group with lower preference scores were categorized as AC. As data from the day 5 of testing were necessary to determine foraging strategy, any fish that failed to eat at least 10 *Daphnia* on day five of testing (n=4) were excluded from further analysis.

2.5.4 Relating Preference Scores to Variables

Preference scores were scaled 0-1 with a correction factor to remove scores of 0 and 1 for analysis using the following formula:

Preference Score (Scaled) = (Preference Score – 5.5)/10.5.

Values of 0 and 1 were removed to allow a beta-regression model to be applied to the data, which can only be applied to proportion data between 0 and 1. Scaled preference scores ranged from approximately zero to one, with a maximum of 0.962 indicating strongest preference for the familiar, a minimum of 0.010 indicating weakest preference for familiar (strongest preference for novel), and a mid-value of 0.486 indicating no preference for either food type.

We constructed a global generalized additive model (GAM) with a beta regression family and logit link to model the relationship between scaled preference scores, treatment, foraging strategy, and day of testing for scores on days 1-4 of testing. (Preference score data from day 5 were excluded as these were used to determine foraging strategy, which was also included in the model as an explanatory variable.) Fish identity was included as a random, fixed factor. Interaction terms for foraging strategy and treatment, and foraging strategy and day of testing, were also included in this model. We reduced the global model by stepwise deletion of terms to arrive at the best model based on AICc score.

To determine the within-treatment patterns of preference we constructed separate "within-treatment" GAMs for each treatment group with foraging strategy, day, and the interaction between them as explanatory variables, and fish identity as a random, fixed factor. We used a beta regression family and logit link in each case.

2.6 Results

2.6.1 Innate Colour Preference

No evidence for a pre-existing bias among sticklebacks for *Daphnia* of either colour was found. The difference in number of each colour of food eaten by naïve fish was not significantly different from zero either among fish that ate in the first 15 minutes of foraging (t = -0.40537, df = 13, p-value = 0.6918) or among the pooled sample of fish that ate within 15 minutes and those that received an additional 15 minutes of foraging time (t = 0.31387, df = 16, p-value = 0.7577). We are therefore comfortable that any colour preferences displayed in the experiment are likely to be due to the treatments the fish were exposed to.

2.6.2 Distribution of foraging strategies

Of the 35 fish included in the model, 16 were identified as DC and 18 as AC based on their preference score on day 5 of testing. Distribution of individuals' foraging strategies across treatments is summarized in Table 2.2.

Table 2.2. Distribution of foraging strategies among treatment groups. Treatment groups Ex1, Ex4, Ex7, and Ex10, received 1, 4, 7, or 10 days of exposure to familiar food, respectively.

	Ex1	Ex 4	Ex7	Ex10	Total
AC	3	5	4	5	17
DC	5	4	5	4	18
Total	7	9	9	8	33

2.6.3 Preference Scores

The best-fitting model of preference scores retained three variables: foraging strategy (AC or DC), testing day, and the interaction between foraging strategy and testing day (Table 2.3). Median preference score for DC fish ranged from 0.724 to 0.952, increasing with treatment, suggesting a preference the familiar food that increased with exposure during training. (A preference score of 0.486 shows no preference for either food type – above that value preference is for the familiar, below that it is for the novel.) For AC individuals, median preference scores ranged 0.357 to 0.486, suggesting no preference for either food type or a

slight preference for the novel food. Preference score did not increase across treatments for AC fish, and variation in preference scores was greater among AC individuals than DC individuals for longer familiarization treatments (4 to 10 days familiarization; Figure 2.1).

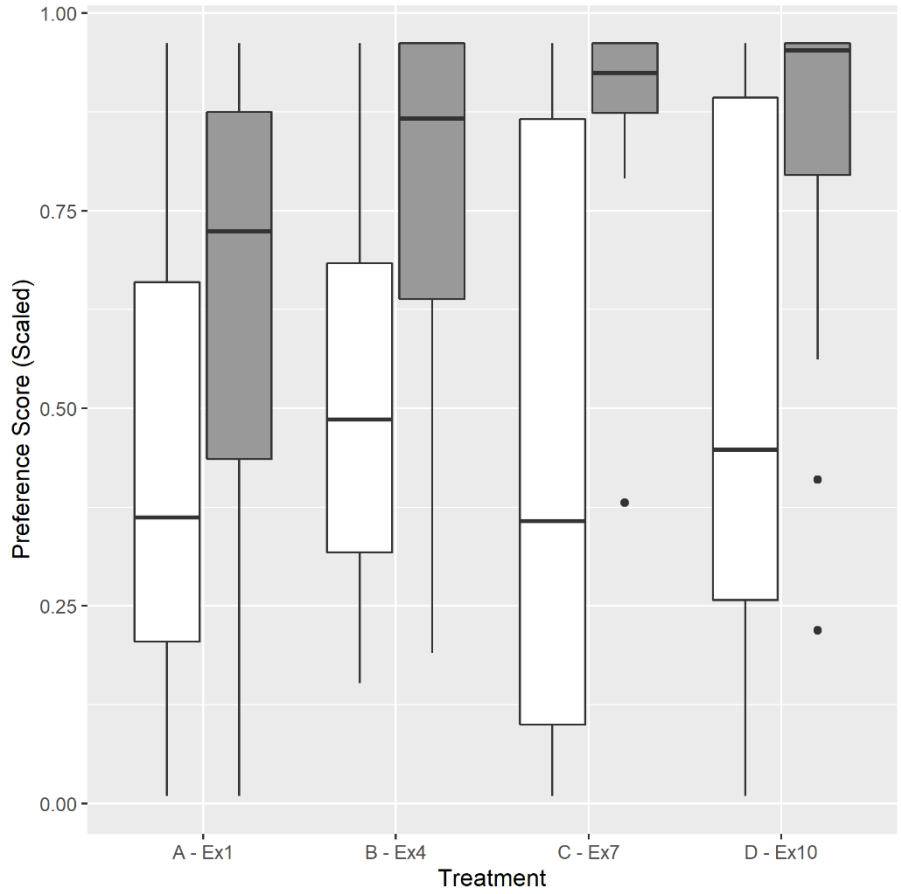

Figure 2.1. Preference scores vs treatment. Preference score scaled 0-1, higher scores indicating greater preference for familiar food. Treatments as in Table 2.1. White boxes denote AC fish, grey boxes denote DC fish.

The interaction between foraging strategy and testing day was significant for all levels (all $p < 0.05$, Table 2.3). Individuals with a DC foraging strategy maintained higher preference for familiar-coloured *Daphnia* than AC individuals over successive days, and this difference grew larger with testing day in most treatments (Figure 2.2).

For all treatments both AC and DC individuals had high preference for the familiar food on the first day of testing. Preference for the familiar food tended to decrease over time and this

decrease was larger for individuals with an AC foraging strategy. This pattern of decline differed across treatments (Figure 2.2).

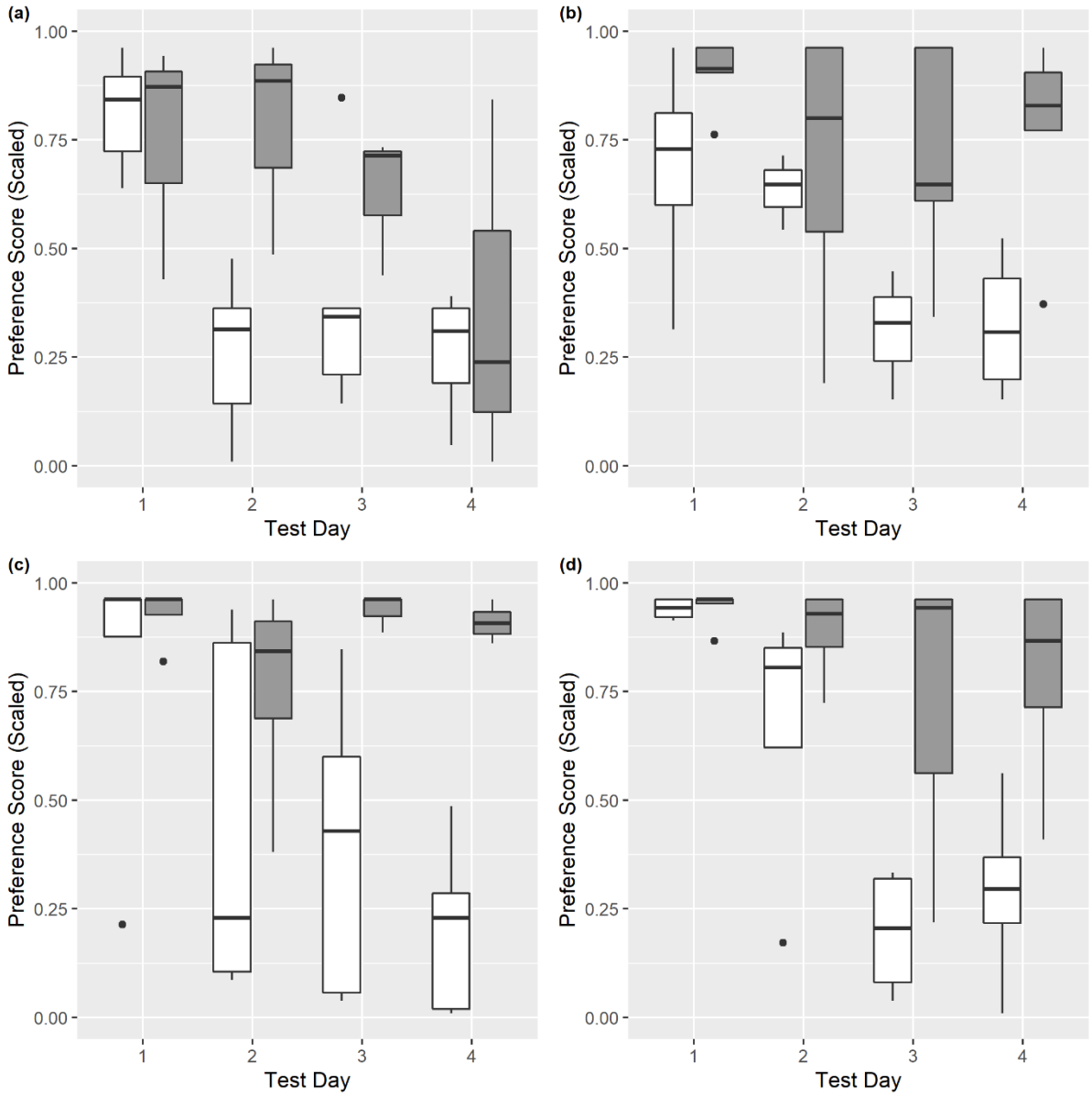

Figure 2.2. Preference scores vs test day for each treatment: (a) Ex1, (b) Ex4, (c) Ex7, (d) Ex10. Preference score scaled 0-1, higher scores indicating greater preference for familiar food. White boxes denote AC fish, grey boxes denote DC fish.

Among AC individuals that received one day of exposure to familiar food prior to testing (Treatment Ex1) preference for the familiar declined by the second day of testing among

those with an AC foraging strategy and remained low on days 3-4 (Figure 2.2a). As experience with the familiar food increased (treatments Ex 4, Ex7 and Ex10), preference score declined more gradually, remaining relatively high on day 2 of testing before declining to low levels on days 3-4 (Figure 2.2b-d), albeit with substantial variation in scores among AC fish that received 7 days exposure to the familiar food (Figure 2.2c). Thus, as the AC fish got more exposure to the familiar food, they preferred it for longer.

Table 2.3. Best-fitting model and output relating preference score to foraging strategy (DC.class) and day of testing.

Family: Beta regression(4.179)
Link function: logit

Formula:
Prefs2 ~ DC.class + factor(day) + DC.class:factor(day) + s(fish, bs = "re")

Parametric coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.3453	0.2685	5.01	5.44e-07 ***
DC.classdc	0.3658	0.3891	0.94	0.347184
factor(day)2	-1.4965	0.3087	-4.847	1.25e-06 ***
factor(day)3	-1.9953	0.3114	-6.408	1.48e-10 ***
factor(day)4	-2.3401	0.3094	-7.563	3.95e-14 ***
DC.classdc:factor(day)2	0.9452	0.4531	2.086	0.036944 *
DC.classdc:factor(day)3	1.3379	0.4532	2.952	0.003156 **
DC.classdc:factor(day)4	1.4847	0.4463	3.327	0.000879 ***

Approximate significance of smooth terms:

	Edf	Ref.df	Chi.sq	p-value
s(fish)	21.56	33	62.05	<2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) = 0.616 Deviance explained = 74.9%
-REML = -54.584 Scale est. = 1 n = 136

In contrast with AC fish, among DC individuals that received one day of exposure to familiar food preference for the familiar remained high on the second day of testing (Figure 2.2a). This preference declined gradually over days 3-4 to reach levels similar to those of the AC fish on

day four. The delayed waning of preference for familiar food is supported by the within-treatment model of preference score, which found a significant interaction between foraging strategy and the second day of testing ($p = 0.014$, Table A2.1), but not the other days.

Among DC fish with more experience with the familiar food, preference for the familiar did not wane for the duration of the experiment but remained high over all four days of testing with only slight declines suggested over time (Figure 2.2b-d). Within-treatment models of preference score found significant interactions between foraging strategy and days 3-4 of testing for treatments Ex7 and Ex10 ($p < 0.01$ in each case, Table A2.3 and A2.4) suggesting that fish with different foraging strategies respond differently to the treatments. DC fish rapidly acquired a sustained preference for the familiar prey after only 4 exposures to it, while any preference that AC fish gained for the familiar prey waned readily even after 10 exposures.

2.7 Discussion

This is the first study to explicitly test how the process of familiarisation with a food type occurs. Our results show that waning of preference for a familiar food depends both on an individual's foraging strategy and prior experience.

Even a limited, positive experience with a food can elicit a significant preference for that food over a novel food. A single exposure to the familiar food was sufficient to cause DC fish to maintain a preference for this food for two days. Greater experience with familiar food caused this preference to be maintained longer, up to four days, suggesting that familiarity is not simply a matter of whether an individual has prior experience with a food type but depends on the amount of experience the individual has had. Familiarity is therefore a quantitative trait of a food for foraging DC fish, at least across this timescale.

Compared to DC fish, preference for the familiar among AC fish increased only slightly with the greatest experience (ten days of exposure). Even with extensive experience, AC fish only maintained their preference for the familiar for a single extra day before readily consuming the novel food.

That preference for the familiar waned significantly in AC fish over consecutive days of testing is not surprising. High preference for familiar food among both AC and DC fish on the first day of testing can be attributed (at least partially) to neophobia, as individuals are initially reluctant to make contact with the novel food (Kelly and Marples, 2004). Once neophobia has been overcome, fish now have a positive experience with the novel food and the process of familiarisation has begun. For AC fish, this brief exposure to novel food was enough to make it as or more appealing than the familiar food the following day (except among those that received the most experience with the familiar food). In contrast, in most treatments DC fish maintained their preference for the familiar despite growing experience with the novel. The implications of this difference in response to experience with the novel food by fish with different foraging strategies are a fertile ground for future study.

The pattern of novel food acceptance among DC fish in treatment Ex1 suggests a possible mechanism for familiarisation based on relative experience. On the fourth day of testing the amount of experience with each food type had become very similar (four days experience with the familiar food and three days with the novel food, i.e., 33% more experience with the

familiar). At this point, DC preference for the familiar decreased to the level shown in the AC fish and the novel food was incorporated into the diet. In all other treatments, fish had substantially more experience (133%-433% more) with the familiar food than with the novel even by day 4 of testing, and incorporation of the novel never occurred among DC fish. This is what might be expected if familiarity is determined by relative experience – incorporation of novel foods only occurs when the difference in experience between novel and familiar food types is sufficiently small.

The graded relationship between familiarity (measured as preference for the familiar) and experience with a given food type found here resembles the well-established response patterns seen in studies of generalization and learning (Ghirlanda and Enquist, 2003). In such studies, the strength of conditioned responses depends on the perceived similarity of the test (unfamiliar) stimulus to the training (familiar) stimulus. Animals' responses are not all-or-nothing but show an exponential decrease in intensity as the test stimulus deviates further from the training stimulus (Pavlov, 1927; Pierrel, 1958; Hanson, 1959). Here, the different levels of experience with novel and familiar foods are analogous to the difference between trained and untrained stimuli. Applying concepts from learning and generalisation research to experience and familiarity as features of stimulus recognition offers a novel way to test animals' perception and understand the dietary choices of individuals with different foraging strategies.

At the other extreme of experience, is also possible that an upper limit exists above which all foods are considered indistinguishably familiar. In this study, preference scores were bounded at the extremes, so the existence of an upper limit to familiarity could not be shown. It would, however, be interesting to investigate whether preference for familiar foods would have waned among DC fish had the experiment been of longer duration.

It is also important to explore how fish respond in more natural conditions. Sticklebacks are a group-living species and previous studies have shown that novel food acceptance is affected by social context (Coleman and Mellgren, 1994). As this experiment tested individual fish in isolation, it cannot reflect the complexities of group living where social learning may change the perception of novel and familiar foods (McMahon *et al*. 2014) and competition may change their relative profitability (Marples *et al*. 2018). Additionally, the presentation of novel food may have sped up its incorporation. In a natural environment, animals presented with

novel foods which they initially reject may move to forage elsewhere in search of their preferred food. In this experiment, hungry fish were unable to roam in search of better feeding grounds and – coupled with the association that all items provided by the experimenter were edible and profitable – were encouraged to sample the novel food until it was accepted. There remains a need to repeat these experiments with larger populations in more natural conditions to better capture the relationship between familiarity and experience.

This study provides valuable insight into the mechanism of novel food acceptance and the perception of familiarity. For studies concerned with responses and habituation to novelty (e.g., Zimmerman *et al*. 2001), a model of familiarity that increases with experience suggests a need for greater rigour in standardizing training of animals to ensure comparability of results.

Beyond refining experimental methods, the question of how familiarity is formed has considerable theoretical implications. Incorporating a model of familiarity based on experience may help better understand dietary ecology, especially as it relates to avoiding profitable prey by DC individuals (Kelly and Marples, 1998). That preferences can be elicited by limited exposure to a particular food suggests a mechanism by which DC animals can develop strong biases for familiar foods. Here, one exposure to the familiar food created a transient preference among DC fish, but given the opportunity to continue searching for their preferred food a DC individual in the wild is likely to build on that early experience as it preferentially selects its more familiar food. If the preferred prey are sufficiently locally available, this would lead to increasing the strength of that preference and specialisation on the familiar. DC individuals might therefore be expected to develop strong preferences for the most common food type, while avoiding relatively uncommon but potentially more profitable foods.

The difference in response to novel prey between fish with the two foraging strategies also has implications for our understanding of the mechanisms underlying these strategies. DC fish appeared to need almost equal exposure to the novel and familiar prey before they accepted the novel prey into their diet. In contrast, AC fish were willing to accept novel prey even when they had much more experience of the familiar prey than of the novel. i.e., their preference for familiar food was very transient compared to that of the DC fish. This suggests that the

difference between the two strategies may be at least partly based on the formation of preference for the familiar prey rather than avoidance of the novel.

Incorporating the effect of experience on familiarity may contribute to better modelling of evolutionary processes. Aposematism - conspicuous signalling by prey of their unprofitability to potential predators – has long been considered paradoxical. A small population of conspicuous mutants is likely to be predated to extinction even as they are educating their predators about their unprofitability (Guilford, 1990). The existence of DC individuals has been posited as facilitating the initial evolution of aposematism, as a rare conspicuous mutant occurring in a DC forager's territory will be rejected in favour of a common, familiar food (Marples *et al*. 2005). In light of our results, we might expect DC individuals to continue their bias against conspicuous prey even as they increase in number, as they will continue to have much greater experience with the common, original prey type, at least until the aposematic prey itself is encountered frequently. By that point, the conspicuous mutation is less at risk of extinction by predation (Marples *et al.* 2005; Mappes *et al.* 2005).

The findings of this study also have practical application in animal management. Better understanding the process of food incorporation in the portion of the population which use the DC foraging strategy will help us tailor the management of animals for release into the wild or under environmental change to encourage them to use the newly available range of foods more effectively. Working with animals' food preferences and their perception of the familiar also has the potential to improve animal welfare, reducing stress and maximizing variety of stimuli for animals both in *ex-situ* conservation and in the livestock industry (Launchbaugh *et al*. 1997).

Understanding the process of familiarisation to foods is of fundamental importance to a wide range of behavioural, ecological and conservation researchers and practitioners. This study has demonstrated that the process of familiarisation is not simple but is a gradual process which differs in mechanism between individuals using different foraging strategies.

Chapter 3

Novel is Novel: the stability of generalization across foraging strategies and experience

Authors

Sam Preston, Whitney Parker, and Nicola Marples

Author Contributions

I designed this study, collected and analysed the data, and wrote this manuscript. NM provided insight on this manuscript. WP assisted with data collection and analysis.

Status

This manuscript is being prepared for publication. Raw data and R Scripts will be added to this manuscript.

3.1 Abstract

Generalizing responses to novel stimuli when they are similar to familiar ones is a universal ability among animals. However, when it comes to diet, individuals show different foraging strategies in response to novel foods: adventurous consumers (ACs) are rapid acceptors of novel foods, while dietary conservative (DC) individuals show prolonged reluctance to incorporate novel foods into their diet. This study explored whether differences in generalization among foraging strategies could explain their responses to novel foods. We presented wild robins (*Erithacus rubecula*) with baits of different hues that formed a gradient from a familiar colour to a novel one. We identified the foraging strategy of individuals and analysed their preference for each colour along the gradient to determine generalization of food acceptance with increasing dissimilarity to the familiar. We then exposed robins to an unpalatable bait of a different novel colour to determine whether a negative experience with novelty would subsequently alter generalization along our novelty gradient.

AC and DC robins showed similar ability to distinguish the different colours and were consistent in determining when foods were sufficiently different to be treated as novel. This suggests no qualitative difference in capacity to generalize between foraging strategies. However, DC individuals had a stronger preference for the most familiar colours and weaker for the most novel than AC individuals, suggesting a difference in response strength due to DC individuals' affinity for familiar foods. Neither foraging strategy showed changes in their generalization pattern following exposure to an unpalatable, novel food. This study offers a novel methodology for testing generalization and dietary choices in wild animals and our results have implications for understanding animals' perception of novelty as well as managing animal diets in the wild and in captivity.

3.1.1 Keywords: Dietary Conservatism; Foraging Strategy; Generalization; Foraging; Novelty; Familiarity

3.2 Introduction

Generalization is the process by which novel stimuli elicit the same response as familiar stimuli because the two have similar characteristics. The ability to generalize is an important tool for animals to cope with novelty as it allows quick and appropriate responses to new situations based on past experience (Ghirlanda and Enquist, 2003; Maes *et al*. 2015). When confronted with novel foods, generalizing can help animals avoid unprofitable prey. A predators' ability to generalize is crucial in the evolution of Mullerian mimicry (Sherratt, 2008; Balogh *et al*. 2010), as negative experience with an aposematic morph results in aversive responses to other morphs or species with similar colours and patterns (Rowe *et al*. 2004; Svádová *et al*. 2009). Generalization can also help animals to exploit profitable prey, broadening their diet to include new foods that share traits with foods they have encountered before (Launchbaugh *et al*. 1997).

As well as generalizing from novel to familiar prey, animals also show distinct foraging strategies that determine how they respond to novel foods (Marples and Brakefield, 1995; Marples *et al*. 1998; Preston and Marples, in prep a). Some individuals show an Adventurous Consumer (AC) foraging strategy, quickly incorporating novel foods into their diet, while others show a Dietary Conservative (DC) foraging strategy and are very slow to recruit novel foods into their diet (Marples *et al*. 1998; Marples and Kelly, 1999). The DC foraging strategy is distinguished from neophobia in part by the prolonged persistence of strong preference for the familiar over novel foods (Marples and Kelly, 1999). Avoidance of a profitable food simply because it was novel when encountered can persist for substantial periods of an individual's life and despite gaining extensive experience with it (Marples *et al*. 1998; Kelly, 2001).

The mechanism underpinning different foraging strategies remains largely unknown. Previous work has shown that foraging strategies are genetically determined (Marples and Brakefield, 1995) but expression can be contextually flexible (McMahon *et al*. 2014; McMahon and Marples, 2017). For instance, DC blue tits (*Cyanistes caeruleus*) were more willing to accept novel foods when they could see what an AC competitor was eating (McMahon and Marples, 2017). However, responding to novelty is also a matter of perception and cognition (Greggor *et al.* 2015), i.e., whether the difference between foods is detectable to the forager and whether they attend to those differences when making a decision whether or not to eat it. In three-spined sticklebacks (*Gasterosteus aculeatus*) AC and DC individuals have been shown

to use prior experience differently in determining preference for familiar over novel food (Preston *et al*. in prep b). DC individuals developed and maintained a preference for familiar food with little experience and maintained their preference for longer with more experience. In contrast, AC individuals only showed a preference for familiar with large amounts of experience, and even then, this preference waned quickly. One possible explanation for this result is a difference in generalization. AC fish may have generalized their response from the familiar food to the novel (which differed only in colour) more effectively than DC individuals, leading to a lack of avoidance of the novel food by the AC fish, and a stable preference for the familiar among DC fish. The fundamental difference between AC and DC foraging strategies may therefore simply be a matter of generalization ability - we might expect individuals that show broad generalization to accept more novel foods and have broader diets (i.e., behave AC) than individuals that discriminate more and generalize less.

In this study we used wild robins (*Erithacus rubecula*) to examine whether AC and DC individuals differed in their generalization from familiar to novel foods. Understanding responses to novelty suffers from a lack of comparability among the novel stimuli used both across and within experiments (Takola *et al*. 2021). For instance, Modlinska and Strvjek (2016) used 5 differed spices to create novel foods in order to test neophobia in brown rats (*Rattus norvegicus*), but whether rats perceived each scent as equally novel and whether we should expect similar levels of response to each food are unknown. To address this, we used image analysis and cone-catch perceptual modelling to create 5 colours of pastry bait that spanned an even gradient from green to yellow in passerine visual perception. We familiarized birds with baits on one end of this gradient, then presented robins with a repeated choice test of all 5 colours. Robins' relative preference for each colour was used to assess generalization and whether AC and DC birds differed in their generalization across the familiar-novel colour gradient. We also tested whether exposure to an unpalatable, novel bait altered the pattern of generalization by birds using either foraging strategy.

3.3 Methods

Wild robins were the study species for this experiment. Robins are known to exhibit different foraging strategies (Marples *et al*. 1998) and are bold, trainable, and commonly fed by people, making them ideal subjects for direct observation while foraging, as used in this study. Once robins were comfortable eating in our set-up, they were familiarised with either green or yellow pastry baits (their "familiar" food). Following familiarisation, robins were presented with a choice test: pastry baits of five colours spanning a gradient from green to yellow (familiar to novel), arranged in a grid. The order in which each colour was taken was recorded to determine preference scores for each colour and to determine individuals' generalization from the familiar end of the gradient to the novel.

3.3.1 Study sites

We conducted our experiments in municipal parks and green areas in urban and suburban areas of Dublin City, Ireland. Four study sites were selected based on accessibility and the presence of territory-holding robins (Table 3.1). Robins were located using playbacks and walkthroughs. Individual birds were visited regularly and offered crushed, skinless peanuts to encourage them to feed in a spot suitable for the experiment. Those that returned and ate regularly were selected for training and testing. In total, 20 robins were selected across all sites (Table 3.1). In two territories, a (presumably breeding) pair of robins returned and ate together regularly. As they could not be separated during the experiment, pairs were treated as a single robin for the purposes of analysis.

Sites were sufficiently far apart that movement of robins among sites was unlikely during the experiment. Sites were visited at the same time daily throughout training and testing either in the morning (between 08:00 and 12:00) or afternoon (between 14:00 and 17:00). The experimental design was colour-balanced across sites and visiting time. Half of the birds receiving green as the familiar colour and yellow as the novel, while half received the opposite combination, and within each colour combination half were visited in the morning and half in the evening.

Table 3.1. Study sites in this experiment. Note that two sites comprised several disconnected areas.

Study Site	**Site details (lat, long coordinates)**	**No. of Robins**	**Familiar Colour**	**Visit Time**
City Centre	3 separate areas in Dublin City: St. Stephen's Green (53.338, -6.259); Merrion Square (53.340, -6.249); Trinity College Dublin (53.344, -6.255)	5	Yellow	Morning
Botanic Gardens	1 area: National Botanic Gardens (53.373, -6.272)	5	Green	Afternoon
Leopardstown Park	2 areas: Leopardstown Park 1 and the adjacent Grange apartment complex 53.280, -6.195; Clonmore park (53.282, -6.202)	4	Green	Morning
Cabinteely	1 area: Cabinteely Park (53.261, -6.156)	6	Yellow	Afternoon

3.3.2 Experimental Food

Crushed, skinless peanuts were used during training to attract robins and habituate them to the experimental set-up. The main experimental food, however, was coloured pastry baits rolled into balls approximately 3mm in diameter. Baits were a mixture of flour, cooking fat, and peanut butter (60:40:10 ratio by weight) with 5ml of food dye solution per 110g. Stock solutions of dye were made from 10% (v/v) Goodall's Green Food Colouring and 60% (v/v) Goodall's Yellow Food Colouring in water. To create green and yellow baits, stock dye solution was added to the pastry mixture. To create a gradient of colours from green to yellow, stock solutions of each colour were combined in ratios that produced an even transition of colours (Table 3.2).

Table 3.2. Stock solution ratios for dying pastry baits. Green Stock was a 10% aqueous solution (v/v) of Goodall's Green Food Colouring. Yellow Stock was a 60% aqueous solution (v/v) of Goodall's Yellow Food Colouring.

Colour	**Green Stock (%)**	**Yellow Stock (%)**
Green	100	0
Yellow-Green 3	75	25
Yellow-Green 2	35	65
Yellow-Green 1	12.4	87.6
Yellow	0	100

3.3.3 Colour Validation

To provide a robust test of generalization, colours on our green-yellow gradient should be equally spaced so that colours two steps apart on the gradient are twice as dissimilar as colours only 1 step apart. We used multispectral image analysis in the open software ImageJ and micaToolbox to measure the L*a*b colour distance in Euclidean space for each of our colours (Troscianko and Stevens, 2015) from our green bait and compared the distribution of our baits against an ideal, evenly distributed gradient. As avian visual systems are different from our own, both in their sensitivity to different wavelengths of visible light and their ability to see in ultraviolet (UV) (Hart *et al*. 2001; Ham and Osario, 2007), we converted our colours using a blue-tit (*Cyanistes caeruleus*) cone-catch model and performed quantitative colour pattern analysis (QCPA) to map our gradient as perceived by avian vision (van den Berg *et al*. 2020). Cone-catch models estimate colour perception based on the different sensitivities of avian photoreceptors (Troscianko and Stevens 2015) and allow perception of colours to be plotted in Euclidean space to determine how colours are likely to be discriminated by animal visual systems. QCPA further adjusts the output to account for the acuity of the receiver's visual system. The blue-tit model parameters were chosen as the closest approximation of a robin visual system, with typical acuity values for a passerine species and with similar visual acuity to that of robins (Donner, 1951).

All image analysis was carried out on a raw image photo of coloured pastry taken against a plain background in good sunlight with a Canon 600D (lens: EFS 18-55mm IS). A blue tit cone catch mapping function in the wavelength range 400-700nm was created using an Xrite Colorchecker Passport 2 to calibrate the reception of the camera system. The open-source

software ImageJ and micaToolBox (Troscianko and Stevens, 2015; van den Berg *et al*. 2020) were used to create a colour map as perceived by avian vision, using the default parameter values in the software where customization was possible.

Avian photoreceptors pigments have peak sensitivities to UV light at wavelengths between 360nm and 370nm. To determine whether UV reflectance might impact birds' perception of our baits as a gradient, we analysed the reflectance of large bait squares (side length approximately 3cm) using a spectrometer. Squares were made as flat as possible to maximize accuracy in reflectance measurement, however the consistency and texture of the pastry made achieving a perfectly flat surface impossible. We measured reflectance of wavelengths between 250nm and 1079nm and compared reflectance in the 360-373nm range.

3.3.4 Training

Training began once a robin was regularly returning to feed. Training was conducted daily, and each session lasted for 10 minutes or until robins had taken some food and flown out of sight, whichever occurred first. Training consisted of three stages, which varied in length among robins. In the first stage, robins were habituated to the presence of the experimenter and trained to arrive on command. Robins were fed crushed, skinless peanuts with the experimenter nearby. On arriving to the feeding spot and whenever an item of food was taken, the experimenter would whistle to provide a stimulus to be associated with the provision of food. This phase was completed when robins were approaching reliably to the whistle. In the second phase, robins were habituated to the experimental background. Crushed peanuts were offered on a white, plastic board (50x50cm) which provided a uniform background for foraging. Once robins showed no hesitation to eat from the board they moved to final stage of training, in which they were familiarised with their experimental food. Depending on site, robins were provided either green or yellow pastry baits (Table 1) on the white background. This became their familiar colour. Baits were initially provided alongside crushed peanuts to encourage birds to sample them. Once the robins started eating the baits, the amount of crushed peanut was reduced over one to three days until only coloured baits were offered, which were then arranged in a grid. This stage was completed when birds were

regularly eating several baits from the board without hesitation and lasted a minimum of 7 days.

3.3.5 Testing

Testing began when training was completed for all robins. Robins were presented with a grid of 20 pastry baits (four of each colour) once daily for five days. Robins were called to the testing board by whistling, but no whistle was given while eating to avoid encouraging birds to eat. Tests lasted for 10 minutes from the moment the robin touched the board. We recorded the order in which baits were eaten (or taken, when one or more baits is removed but not consumed immediately) and used these to calculate preference scores for each colour, based on the assumption that preferred foods will be eaten before less preferred ones. To mitigate the effects of position on bait choice, colours were arranged symmetrically in a five-by-five grid with the middle 5 spaces empty, and the locations of each colour were rotated daily so that each colour spent an equal amount of time in each position on the board.

To determine the effect of an unpalatable, novel food on preference, after robins had been tested for 5 days they were introduced to unpalatable, red baits. Red baits were made by adding 2mL of O'Brien's Christmas Red stock solution (25% v/v aqueous solution) and 2mL of Bitrex (2.5% w/v aqueous solution of Denatonium Benzoate) to 100g of pastry mixture.

Robins were presented with red baits for two days. On the first day, robins were offered many red baits alongside some familiar-coloured baits until they ate at least one and showed a disgust (head shake) response. On the second day, robins were presented with a four-by-four grid of alternating red and familiar baits and allowed to forage for 10 minutes. As several robins were observed to continue eating the red baits even after showing a disgust response on the first day, a drop of Bitrex was added to each red bait on the board before presenting. Following two days of exposure to red baits, robins were presented with the five-colour grid for a further five days, with the order in which baits were eaten recorded as before.

3.4 Data Analysis

All analyses were carried out using R Program version 4.0.2 (R Core Team, 2020).

3.4.1 Calculating preference scores

For each robin, a preference score for each colour was calculated by summing the ordinal position in which baits of that colour were eaten and divided by the number of that colour presented. To facilitate legibility, this number was subtracted from 18.5 so that higher preference scores indicate greater preference, and the minimum value (weakest preference) was 0 and the maximum value (strongest preference) was 16. For any uneaten baits, preference scores were calculated by assigning the average of the sum of the remaining positions and continuing as above. An example calculation is given below.

Box 3.1. Example calculation of preference scores

A robin presented is with 20 baits (four of each colour) and eats five baits in the following order: Green, Green, Green, Yellow-Green 1, Green. The sum of the positions of the green baits (1^{st}, 2^{nd}, 3^{rd}, 5^{th}) would be 1+2+3+5 (= 11) and the preference score for Green would be calculated as

18.5-(1+2+3+5)/4 (= 15.75).

For Yellow-Green, the preference score would be

18.5-(4+13+13+13)/4 (=7.75)

as 3 Yellow-Green baits were not consumed and so are given the average (13) of the positions between 6 and 20. All other colours would receive a preference score of 5:

18.5-(13+13+13+13)/4 (=5)

3.4.2 Determining Foraging Strategy

Data from the fifth day of testing (i.e., before the introduction of unpalatable baits) were used to determine individuals' foraging strategies). These data were excluded from other analyses. The fifth day of testing was chosen to allow reliable identification of individuals' foraging

strategies, by ensuring preferences have been maintained over a long period. Additionally, identifying AC and DC strategies on the fifth day of testing mitigates against any confounding effect of neophobia, which is comparatively short-lived (Marples and Kelly, 1999).

Preference scores for the most novel food on day 5 of testing were scaled -1 to 1 using the "mutate" function in R. Unsupervised, K-means cluster analysis was applied to split birds into 2 groups by assigning individuals to clusters with mean preference scores on day 5 closest to their own. Robins in the group with higher preference scores (above 13.5) were categorized as DC (showing stronger preferences for familiar food), while those in the group with lower preference scores were categorized as AC. A Fisher's exact test was used to determine whether familiar colour affected foraging strategy distribution.

3.4.3 Modelling

As we were interested in examining the effect of relative novelty on preference, we transformed bait colours to a novelty scale from 1-5 where 1 represented the most familiar colour and 5 the most novel colour irrespective of which colour (Green or Yellow) was familiar. We constructed a global generalized linear model (GLM) with a gaussian distribution. Preference score was the response variable and we used the following explanatory variables: novelty scale, foraging strategy, experience with red baits (before/after), familiar colour, and site, and whether birds foraged alone or as pairs. We also included two-way interaction terms between foraging strategy and novelty scale, foraging strategy and familiar colour, familiar colour and novelty scale, and between experience with red baits and each of foraging strategy, novelty, and familiar colour. Interaction terms were chosen to investigate relationships likely to be biologically relevant. Individuals with different foraging strategies might be expected to respond differently to the degree of novelty of a food, and which colour was familiar or novel may impact AC and DC individuals differently if an inherent bias exists in association one colour with profitable food. The interaction between familiar colour and novelty scale was included to investigate bias in generalization along the novelty gradient. Interactions between red baits and other variables were included to determine whether experience with an unpalatable, novel food affected individuals differently depending on foraging strategy, changed the pattern of generalization along the novelty scale, or interacted

with an inherent bias in colour preference. Day was not included as an explanatory variable due to a covarying relationship with exposure to red baits. All explanatory variables were treated as factors with discrete levels.

We used the step function from the "stats" R package to derive the best-fitting model. *Post hoc* contrasts were applied to the best fitting model using the "emmeans" R package to determine where preferences differed along our novelty scale, between foraging strategies, and before and after experience with unpalatable, red baits.

3.5 Results

3.5.1 Colour Validation

Our bait colours were a reasonable approximation of an even gradient of hues from Green to Yellow (Table 3.3). Our intermediate colours were slightly closer to Green than an ideal, evenly distributed gradient of colours, however the percentage difference from the ideal position on the gradient was less than 13% in each case. Deviation from the ideal distribution was restricted to the Green end of the spectrum, with colours closer to Yellow more closely matching their ideal distance along the Green-Yellow gradient. This resulted in greater colour separation among colours with more yellow (Yellow-Green 2, Yellow-Green 3, and Yellow) than between colours with most green (Green and Yellow-Green 1).

Table 3.3. L*a*b -based colour distances (derived from a combination of luminosity and hue) of each colour bait from our Green bait compared against ideal values of an even gradient. Distances between each colour and the one above are also shown. For evenly distributed colours along an ideal gradient, distances from each colour should be 3.505 (14.02 divided by 4).

Colour	**Distance from Green**	**Ideal Distance from Green**	**% Difference from Ideal**	**Distance from Previous Colour**
Green (G)	0	0	0	0
Yellow-Green 3 (YG3)	3.06	3.51	-12.8	3.06
Yellow-Green 2 (YG2)	6.62	7.02	-5.7	3.56

Yellow-Green 1 (YG1)	10.36	10.53	-1.6	3.74
Yellow (Y)	14.02	14.02	0	3.66

Figure 3.1 shows a perceptual map of our bait colours based on the blue-tit cone-catch model. Colours fall along an approximately straight line so they should be perceived as a one-dimensional gradient from Green to Yellow. In most cases colours are a similar distance apart (i.e., an even gradient), but with slightly greater separation at the green end of the gradient. In particular, the distance between Yellow-Green 2 and Yellow-Green 3 is substantially greater than any other pair of adjacent colours. This is the reverse of the pattern seen when considering only L*a*b values. Overall, perceptual difference between some colours might be expected to make generalization from one to the other more difficult, depending on which end of the gradient is familiar.

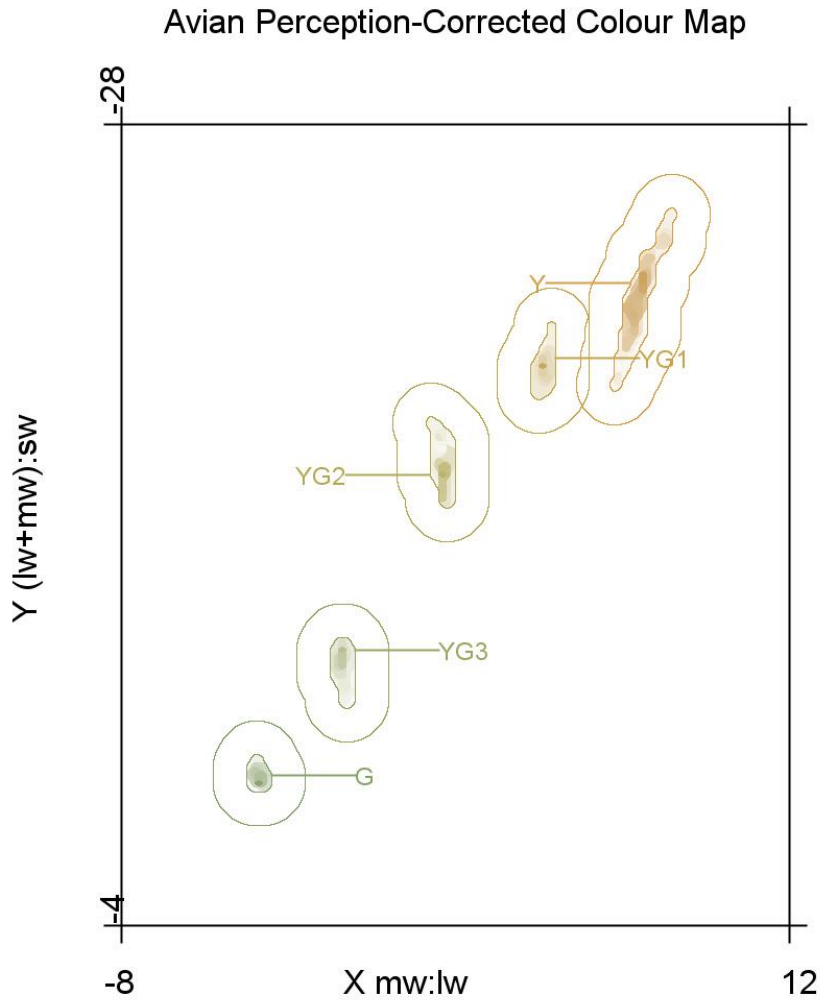

Figure 3.1. Colour map of bait gradient corrected for avian visual system (see text). Axes represent variation in colour space along a green-red dimension (x-axis) or a blue-yellow dimension (y-axis). Colours are labelled as in table 3.3.

Spectrographic analysis revealed fractional UV reflectance of around 0.25 for all baits in the range of peak UV sensitivity (360-373nm). The greatest difference in total reflectance at any wavelength in this range was only 0.04 (0.22-0.26) which was within the error range expected given the uneven surface of the bait squares. In addition, the amount of UV reflectance did not correlate with either the amount of either colour of dye or the total amount of dye in each bait. These results suggest UV reflectance of baits was not a dimension relevant to our colour gradient, which was limited to the yellow-green wavelengths of visible light.

3.5.2 Distribution of foraging strategies

Of the twenty robins in this experiment, 13 were identified as AC and 7 as DC (Table 3.4). Distribution of AC and DC individuals across colour groups was not significantly different from random (Fisher's exact test, p-value = 0.6424), indicating that the colour with which birds were familiar did not alter their foraging strategy.

Table 3.4. Number of AC and DC robins in each colour group.

	Foraging Strategy	
Familiar Colour	AC	DC
Green	5	4
Yellow	8	3
Total	13	7

3.5.3 Preference across the colour gradient

The best-fitting model derived from our global model retained foraging strategy, novel scale, experience with red, and the interactions between foraging strategy and novel scale and between novel scale and red experience as explanatory variables (Table 3.5). The results of our *post hoc* contrast analysis are presented in (Table 3.6).

Table 3.5. Best fitting model and output of preference score's relationship with explanatory variables. Foraging strategy is coded as "AC_DC" and exposure to red baits is coded as "Postred". "Colour" refers to colour of baits along the novelty gradient.

Model:				
Pref.Score ~ factor(AC_DC) + factor(Colour) + factor(Postred) + factor(AC_DC):factor(Colour) + factor(Colour):factor(Postred)				
Deviance Residuals:				
Min	1Q	Median	3Q	Max
-6.9798	-1.2692	-0.1287	1.2515	8.4508
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	11.1136	0.2465	45.081	< 2e-16 ***
factor(AC_DC)DC	1.1348	0.3080	3.685	0.000243 ***
factor(Colour)n1	-0.6549	0.3486	-1.879	0.060644 .
factor(Colour)n2	-4.7838	0.3486	-13.721	< 2e-16 ***
factor(Colour)n3	-6.2015	0.3486	-17.788	< 2e-16 ***
factor(Colour)n4	-6.4279	0.3486	-18.437	< 2e-16 ***
factor(Postred)y	-2.2686	0.2964	-7.654	5.15e-14 ***
factor(AC_DC)DC:factor(Colour)n1	-0.1924	0.4355	-0.442	0.658795
factor(AC_DC)DC:factor(Colour)n2	-0.9672	0.4355	-2.221	0.026612 *
factor(AC_DC)DC:factor(Colour)n3	-2.2038	0.4355	-5.060	5.10e-07 ***
factor(AC_DC)DC:factor(Colour)n4	-2.3106	0.4355	-5.306	1.42e-07 ***
factor(Colour)n1:factor(Postred)y	0.2823	0.4192	0.673	0.500863
factor(Colour)n2:factor(Postred)y	3.2697	0.4192	7.800	1.75e-14 ***
factor(Colour)n3:factor(Postred)y	3.7331	0.4192	8.906	< 2e-16 ***
factor(Colour)n4:factor(Postred)y	4.0579	0.4192	9.681	< 2e-16 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

3.5.5 Generalization across a novelty gradient

Familiar colour was not retained by the best fitting model. Whether birds had green or yellow as their familiar colour did not affect preference for baits (Figure 3.2) Additionally, irrespective of which direction along our gradient (Green to Yellow or Yellow to Green) birds generalized, the same pattern of preference was observed.

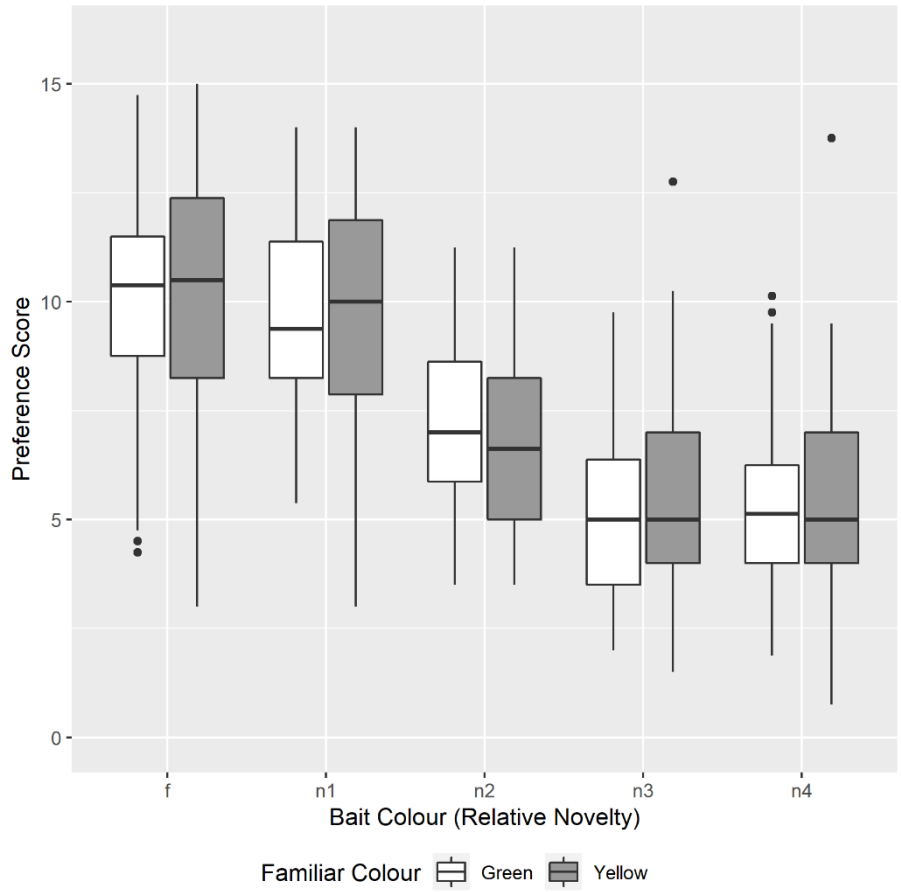

Figure 3.2. Preference scores of all robins for each colour of bait by familiar colour. Bait colours are arranged from least to most novel where f is the familiar colour, n1 is the colour one step away from familiar on the gradient, n2 is two steps away, n3 is three steps away, and n4 is the most novel colour, four colour steps removed from the familiar.

Robins showed greatest preference for familiar baits, and preference decreased as novelty increased (Figure 3.3). This response curve is S-shaped and pronounced in the first half of the experiment (before red baits, Figure 3.3a), becoming flattened but still present in the second

half (Figure 3.3b). *Post hoc* contrast analysis confirmed the statistical significance of this pattern (Figure 3.3 and Table 3.6). Before exposure to red baits, birds showed no difference in preference for either of the most familiar baits (f and n1), suggesting they generalized easily across small deviations from the familiar. As novelty increased, birds showed disproportionately lower preference for each bait. Both AC and DC birds showed significant decreases in preference scores between baits n1 and n2 and between baits n2 and n3 (adjusted p values ranging from <0.0001 to 0.0012). These decreases suggest birds viewed baits n2 and n3 as qualitatively different and were no longer effectively associating them with their preferred, familiar food. At the higher end of the novelty scale, preference was universally low: a further increase in novelty (from n3 to n4) produced no additional decrease in preference. Following exposure to red baits, the pattern of significance remained largely the same, except that AC birds no longer showed a significant decrease in preference from bait n2 to n3 (adjusted p value = 0.0633).

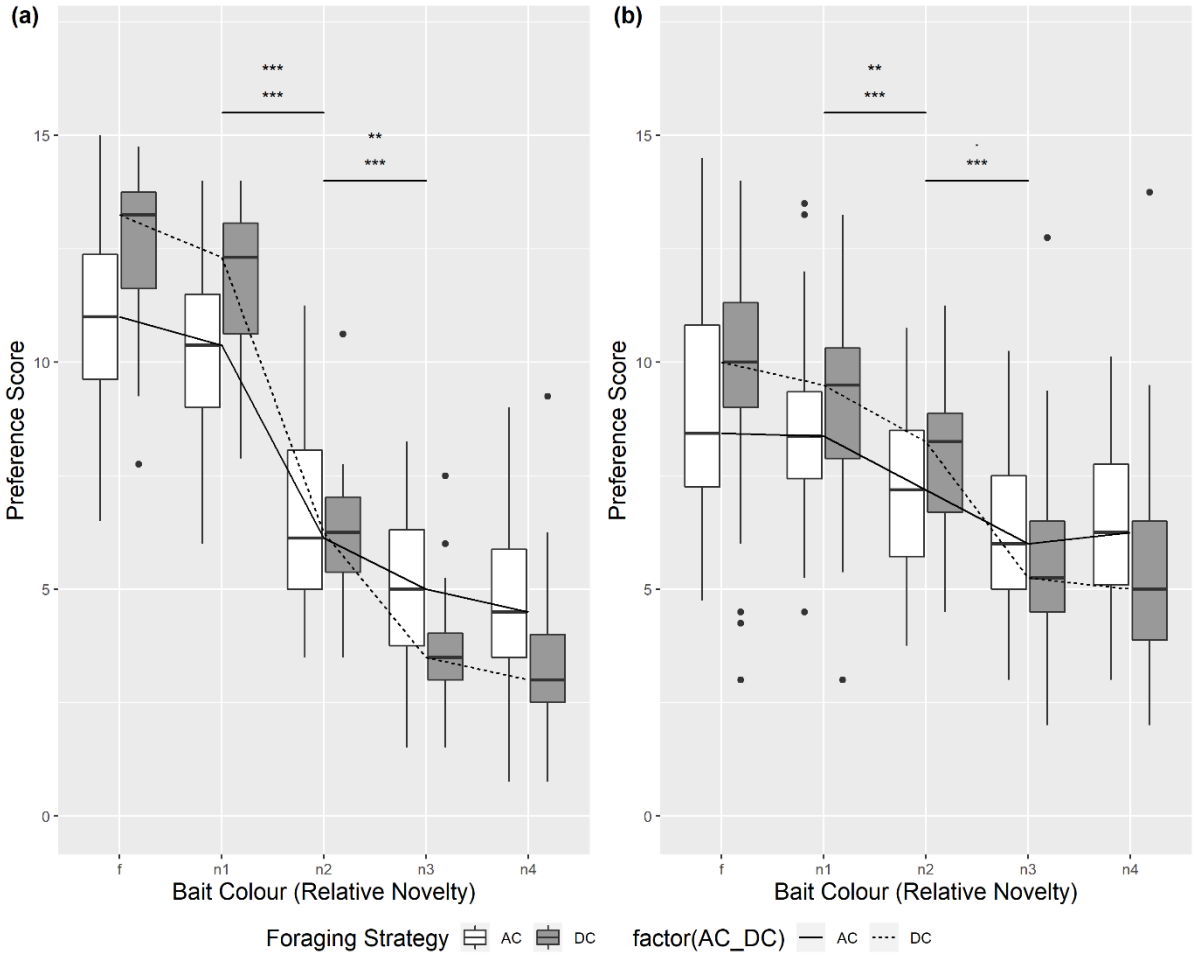

Figure 3.3. Preference scores for all robins (a) before exposure to red baits and (b) after experience with red baits robins for each colour of bait. Bait colours are arranged from least to most novel where f is the familiar colour, n1 is the colour one step away from familiar on the gradient, n2 is two steps away, n3 is three steps away, and n4 is the most novel colour, four colour steps removed from the familiar. Lines show trends in medians for AC (solid line) and DC (dashed line) birds. Bars show decreases in preference score between n1 and n2 baits and between n2 and n3 baits (*post-hoc* tests), with significance levels for AC individuals displayed on top of significance levels for DC individuals. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

3.5.6 Effect of foraging strategy

AC and DC birds different in their strength of preference for familiar and novel baits. DC individuals showed higher preference for the most familiar baits (f and n1) and lower preference for the most novel (n3 and n4) than AC individuals (Figure 3.3). This relationship

is indicated by significant interactions between foraging strategy and novel scale (Table 3.5) and *post hoc* contrast analysis supported this conclusion (Table 3.6). DC birds had significantly higher preference for familiar baits (bait 1) both before (adjusted p = 0.0059) and after (adjusted p = 0.0059) exposure to red baits. Higher preference for slightly novel baits (bait 2) among DC birds was marginally non-significant before red bait exposure (adjusted p = 0.0516), but significant after (adjusted p = 0.0499). Lower preferences among DC birds compared to AC birds for baits 4 and 5 were significant both before (adjust p values = 0.0132 and 0.0035, respectively) and after (adjusted p values 0.0129 and 0.0037, respectively) exposure to red baits. There was no significant difference in preference for baits of intermediate novelty (bait 3) among AC and DC birds (adjusted p = 1.0000 in all cases).

Though ACs and DCs differed in the magnitude of preference for each bait, both showed the same S-shaped pattern of generalization.

3.5.7 Effect of red baits

Contrary to our expectations, preference for more novel foods did not decrease following two exposures to unpalatable, red baits. For both AC and DC individuals, mean preference scores for the most familiar baits, decreased by 19.7% (f) and 18.4% (n1) after encountering red baits (adjusted p values < 0.001) while mean preference scores for the more novel baits increased by 15.7% (n2), 32.3% (n3), and 41.9% (n4) (adjusted p values < 0.050 for n2 and < 0.0001 for n3 and n4). As individuals are expected to gradually increase their acceptance of novel foods with repeated exposures (Marples and Kelly, 1999; Preston *et al*. in prep b), this result is consistent with the effect of time on acceptance of novel foods (preference decreasing for familiar foods is simply a corollary because of how preference scores are calculated). Additionally, the pattern of generalization was qualitatively similar both before and after exposure to red baits. As such, it is difficult to assign an effect of experience with unpalatable, novel baits *per* se on preference and generalization.

Table 3.6. ***Post hoc*** contrast analysis of explanatory variables from best-fitting model of preference score. For legibility the table is divided into three sections: (a) shows contrasts between different foraging strategies (AC_DC); (b) shows contrasts among different bait colours (Colour); (c) shows contrasts between the first and second half of the experiment, i.e., before (n) and after (y) red baits (Postred).

Colour (a)	Postred	AC_DC	contrast	estimate	SE	df	t.ratio	p.value
f	n	.	DC - AC	1.13482	0.308	875	3.685	0.0059
n1	n	.	DC - AC	0.94244	0.308	875	3.060	0.0516
n2	n	.	DC - AC	0.16758	0.308	875	0.544	1.0000
n3	n	.	DC - AC	-1.06901	0.308	875	-3.471	0.0132
n4	n	.	DC - AC	-1.17582	0.308	875	-3.818	0.0035
f	y	.	DC - AC	1.13482	0.308	875	3.685	0.0059
n1	y	.	DC - AC	0.94244	0.308	875	3.060	0.0499
n2	y	.	DC - AC	0.16758	0.308	875	0.544	1.0000
n3	y	.	DC - AC	-1.06901	0.308	875	-3.471	0.0129
n4	y	.	DC - AC	-1.17582	0.308	875	-3.818	0.0037
(b)								
.	n	AC	Colour n1 - f	-0.65492	0.349	875	-1.879	0.6736
.	n	AC	Colour n2 - n1	-4.12885	0.349	875	-11.843	<.0001
.	n	AC	Colour n3 - n2	-1.41773	0.349	875	4.066	0.0012
.	n	AC	Colour n4 - n3	-0.22638	0.349	875	-0.649	1.0000
.	n	DC	Colour n1 - f	-0.84729	0.420	875	-2.015	0.5651
.	n	DC	Colour n2 - n1	-4.90371	0.420	875	-11.663	<.0001
.	n	DC	Colour n3 - n2	-2.65432	0.420	875	6.313	<.0001
.	n	DC	Colour n4 - n3	-0.33320	0.420	875	0.792	0.9997
.	y	AC	Colour n1 - f	-0.37264	0.319	875	-1.169	0.9865
.	y	AC	Colour n2 - n1	-1.14146	0.319	875	-3.580	0.0088

.	y	AC	Colour n3 - n2	-0.95423	0.319	875	-2.992	0.0633
.	y	AC	Colour n4 - n3	0.09837	0.319	875	0.308	1.0000
.	y	DC	Colour n1 - f	-0.56502	0.397	875	-1.425	0.9344
.	y	DC	Colour n2 - n1	-1.91632	0.397	875	-4.833	<.0001
.	y	DC	Colour n3 - n2	-2.19083	0.397	875	-5.525	<.0001
.	y	DC	Colour n4 - n3	-0.00844	0.397	875	-0.021	1.0000
(c)								
f	.	AC	y - n	-2.26859	0.296	875	-7.654	<.0001
f	.	DC	y - n	-2.26859	0.296	875	-7.654	<.0001
n1	.	AC	y - n	-1.98632	0.296	875	-6.702	<.0001
n1	.	DC	y - n	-1.98632	0.296	875	-6.702	<.0001
n2	.	AC	y - n	1.00106	0.296	875	3.377	0.0182
n2	.	DC	y - n	1.00106	0.296	875	3.377	0.0181
n3	.	AC	y - n	1.46455	0.296	875	4.941	<.0001
n3	.	DC	y - n	1.46455	0.296	875	4.941	<.0001
n4	.	AC	y - n	1.78930	0.296	875	6.037	<.0001
n4	.	DC	y - n	1.78930	0.296	875	6.037	<.0001

3.6 Discussion

This study is the first to test how generalization and foraging strategy interact. Depending on their foraging strategy, individual animals respond to novel foods in very different ways (Marples *et al*. 1998; Kelly and Marples, 1999). Generalization is a means of coping with novelty by responding the same to novel stimuli as familiar ones when those novel stimuli resemble those familiar ones (Ghirlanda and Enquist, 2003). Differences in generalization ability among AC and DC individuals offer a plausible mechanism for their different treatment of novel foods. In this study, robins with different foraging strategies showed quantitative differences in their preference for novel and familiar foods characteristics of their foraging strategy but did not show qualitative differences in their generalization along a novelty gradient. Experience with another, unpalatable novel bait did not alter these patterns, which remained stable over the duration of the study.

In classic studies of generalization along a gradient, animals are trained to respond to a (rewarded) stimulus and then tested for their response to unrewarded stimuli of decreasing similarity (e.g., Hanson, 1959; Terrace, 1964; Blough, 1972). Stimulus variation is usually bidirectional along the stimulus gradient (e.g., both increasing and decreasing wavelength), rather than unidirectional (e.g., only increasing wavelength). Plotting response against stimulus produces a Gaussian curve as animals generalize their response when the test stimulus is similar to the trained one, but that generalization drops off as the test stimulus becomes more dissimilar in either direction along the gradient (Ghirlanda and Enquist, 2003).

In this study, the response curve of both AC and DC robins showed a typical pattern of generalization. Small deviations from the familiar produced a generalized response as the familiar and first novel were equally preferred. Larger deviations (second novel) resulted in weaker generalization and lower preference. The lowest preference among birds was for the two most novel baits (third and fourth novel), suggesting little, if any, response generalization from the familiar. This produced S-shaped response curves equivalent to one side of a Gaussian curve as our stimulus gradient varied only in one direction (from a familiar colour to a novel one). Preference scores were unaffected by which colour was familiar, suggesting that birds effectively treated our colour palette as an even gradient despite small deviations from a uniform distribution of colours.

While both foraging strategies showed a typical generalization curve, the shape of this curve was different for AC and DC individuals. DC robins had higher preferences for baits near the familiar end of the gradient and lower preferences for those at the novel end, resulting in a steeper, more extreme curve than AC birds. However, this difference was not due to differences in birds' treatment of novel colours relative to one another. Both AC and DC birds generalized from the familiar to the first novel bait but treated the second novel bait as different from the familiar, and the third and fourth novel baits as different from the second but the same as one another. Individuals of both foraging strategies recognized and agreed as to which baits were sufficiently novel to elicit a difference in generalization and at what point generalization effectively ceased but differed in how they used that information. Despite assessing relative novelty in the same way as AC birds, DC robins cared more about whether it was novel and retained higher preference for the most familiar colours and lower preference for the most novel.

If differences in generalization ability were responsible for the differences between the foraging strategies, we would expect DC birds to show poorer differentiation in preference among novel baits than AC birds. For instance, DC birds might have treated the second, third, and fourth novel baits as equally novel while AC robins distinguished between the second and third, and perhaps also the third and fourth novel baits. That both foraging strategies showed qualitatively the same differentiation among the different colours suggests that AC and DC individuals' responses to novel foods is not based on differences in their ability to generalize. Rather, foraging animals have a stable view of how different a novel prey item must be to be considered novel which is independent of their attitude to eating familiar and novel prey.

Different generalization ability offered an intuitive mechanism for the differences between foraging strategies. However, generalization is a fundamental feature of animal behaviour that functions consistently across stimulus type (Ghirlanda and Enquist, 2003). In humans, individual differences in generalization have been related to differences in perception ability (Zaman *et al.* 2021; Zaman *et al.* 2022). However, to our knowledge, no studies have noted differences in organisms' capacity to generalize. Our finding that generalization was robust across foraging strategies with similar perceptual ability is therefore consistent with previous work. To explain the proximate difference between AC and DC individuals' responses to novel foods, future studies should explore other mechanisms such as differences in learning or

memory (do DC individuals acquire and retain information about familiar foods more quickly or better, leading to maintained preferences?), or differences in the use or retention of search images (mental images that facilitate finding prey) when foraging (Gibb, 1962; Dawkins, 1971).

While other mechanistic explanations for the differences between foraging strategies seem appealing, it would nevertheless be interesting to explore generalization by different foraging strategies in more detail. This present study used a palette of 5 colours to create a gradient. Expanding the design with a novelty gradient composed of more colours might uncover subtle differences in generalization among AC and DC individuals. If a colour were added to the gradient between, for instance, the familiar bait and the first novel, would birds of both foraging strategies show the same preference for and generalize effectively across three colours (familiar, new bait, and first novel)? Or would preference start to wane earlier along the novelty gradient, in particular for DC birds that now distinguish between the familiar and the first novel because an intermediate closer to the familiar exists? Marples (1993) showed that birds can learn to discriminate between prey of slightly different size when their attention is focused on that cue. Perhaps with more opportunity to discriminate between small differences in colour (to which our robins are already paying attention) AC and DC birds may reveal differences in where generalization begins to wane.

In addition to the above, this experiment deals only with a single species: European robins. It remains to be seen whether AC and DC individuals of other species show the same response to a novelty gradient. Moreover, as robins are dietary generalists (Morelli *et al*. 2019), even DC individuals might be expected to rely heavily on generalization ability when foraging. More specialist species may show greater difference in generalization among foraging strategies, especially if differences in generalization ability is merely part of the mechanism between the AC/DC divide and not the whole story.

Beyond foraging strategies, this study also represents the first quantitative measure of the threshold of generalization by colour in a wild bird species. Colour is a vital cue for predators, particularly when dealing with aposematic prey and their mimics (Svádová *et al*. 2009; Rönkä *et al*. 2018). However, testing generalization across different prey cues must often rely perceptions of pattern based on pattern (Brodie III and Jane, 1995; Rowe *et al.* 2004) or crude manipulations of colour (Svádová *et al*. 2009). Combining perceptual models with image

analysis allows unambiguous measurement of differences in hue as perceived by predators. Data here are the first step in developing a quantitative framework to predict how robins respond to prey that vary in colour and expanding this methodology to other species offers an opportunity to broaden understanding of the thresholds of generalization that determine wild animals' foraging behaviour.

Marples *et al*. (2007) showed that chicks familiarised with multiple novel foods were more willing to accept a subsequent novel food, effectively generalizing their positive experience with novelty to new food types. However, a negative experience with an unpalatable food can quickly reverse acceptance of that food type. Here, we tested whether a negative experience with a novel food would alter generalization by making birds more reluctant to consume novel baits more dissimilar to the familiar. Following exposure to unpalatable red baits, robins in this study showed no reversal of their acceptance of baits at the novel end of our gradient. Indeed, preference for novel baits increased in the latter half of the experiment, suggesting that birds were gradually accepting novel foods as would be expected with increased sampling (Marples and Kelly, 1999). The apparent lack of a response to red baits may have been due to an insufficiently off-putting stimulus, leading to no association of the novel bait with unpalatability. Previous studies have used a single exposure of Bitrex to effectively elicit an aversive response in birds (Marples *et al*. 2007; Skelhorn, 2011), but robins in this study were observed to continue consuming red baits even after showing head shake responses characteristic of disgust (Johnston *et al*. 1998; Skelhorn, 2011)! An alternative explanation is that robins did associate red baits with unpalatability but did not generalize their aversion to other novel baits. Perhaps multiple colours of unpalatable, novel baits would be more successful in associating novelty with distastefulness. It is also possible, however, that individuals' prior experience with those colours in the gradient made them sufficiently familiar (Preston *et al*. in prep b) that introducing a new novel bait could have no effect on how that familiarity is perceived and understood.

Unusually for generalization studies, all stimuli were equally rewarded during the testing phase of this experiment. This approach provides insight into how patterns of generalization change with positive experience. It is interesting to note that in the latter half of the experiment (after exposure to the red baits) acceptance of novel foods increased, but birds continued to show a distinctive (albeit flattened) generalization curve across the novelty

gradient. Despite (presumably) learning that the novel-coloured baits were profitable, birds appeared to continue relying on generalizing from experience in their foraging choices. Interestingly, the response curves AC and DC birds remained distinct over time, suggesting their reliance on generalization waned either at a similar rate, or that AC birds' positive experience with the novel foods outweighed their use of generalization more rapidly. Work on fish showed that experience with the familiar was more important to DC individuals than AC ones, resulting in different rates of waning preference depending on foraging strategy (Preston *et al*. in prep b). Future studies might benefit from an explicit test of the flattening of generalization curves over time in AC and DC individuals to determine whether a difference exists and what factors (e.g., positive experience with novel or memory of experience with the familiar) drive it.

This study adds to the limited number of generalization experiments conducted on wild animals in a natural setting. The dietary choices animals make in laboratory experiments are likely to be different from those made in the wild, as free-ranging animals can choose to leave the experiment to seek preferred food elsewhere (Sherratt, 2008). Understanding how animals classify novelty and generalize from the familiar in their diet has considerable practical implications for how we feed animals in the wild and in captivity. This study provides valuable information on how a single passerine species perceives and treats novel foods and offers a useful methodology for exploring these processes further in both birds and other animals.

Chapter 4

Hunger Games: The effect of hunger on novel food recruitment by animals with different foraging strategies

Authors

Sam Preston and Nicola Marples

Author Contributions

I designed this study, collected and analysed the data, and wrote this manuscript. NM provided insight on this manuscript.

Special thanks to Alice Butler for assisting with data collection and animal care.

Status

This manuscript is being prepared for publication. Raw data and R Scripts will be added to this manuscript.

4.1 Abstract

Hunger is a major motivator of animal foraging behaviour. However, hungry animals are caught in a dilemma between not having the resilience to cope with unwise food choices and needing to eat something urgently. This makes novel foods with unknown profitability a particular risk for hungry animals, but we know little about how hunger changes the expression of foraging behaviour in relation to novel foods. We investigate how hunger state affects acceptance of novel foods in the context of individual foraging strategies (Dietary Conservatism) among three-spined sticklebacks (*Gasterosteus aculeatus*). We found that fish which were hungry were less willing to eat a novel food than fish which were not hungry, and that this was true irrespective of foraging strategy. These results support a model of calculated risk-taking where individuals explore unknown foods when they are less physiologically stressed, and costs of unprofitable foods are relatively low. Our results add to our understanding of how animals' internal state impacts decision-making when faced with incomplete information about their environment and suggest new methods for mitigating the impacts of novelty in animal management.

4.1.1 Keywords:

Behaviour; Dietary Conservatism, Familiarity; Foraging; Hunger; Novelty; Sticklebacks;

4.2 Introduction

Hunger state has a profound effect on animals' behaviour. Hunger can make animals take greater risks when foraging in the presence of predators (Gotceitas and Gordon, 1991; Godin and Crossman, 1994; Bridges, 2002), changes individuals' foraging tactics (Ledvai *et al.* 2004), changes group structure as hungry individuals associate with poorer competitors (Reebs and Saulnier, 1997), and can even change the role individuals play in socially cooperative species (Parthasarathy *et al*. 2022). How hunger state affects animals' responses to novel foods, however, remains largely unstudied.

Predators vary consumption of prey based on known risks and rewards that allow an assessment of the prey's profitability (Marples *et al*. 2018). Work on birds has shown that individuals vary their intake of chemically defended prey based on their current toxin load (Skelhorn and Rowe, 2007), the nutritional value of defended prey (Halpin *et al*. 2014) and the relative availability of undefended prey (Carle and Rowe, 2014). However, when the toxin load of defended prey is variable, the profitability of that prey becomes uncertain and the risk/reward trade-off of consuming that prey is unknown, so predators consume fewer novel prey (Barnett *et al*. 2014). Novel foods, naturally, have unknown profitability associated with them, so animals are expected to show caution in consuming them based on this uncertainty (Sherratt, 2011). For hungry animals, novel foods are a special challenge, as they must balance the conflicting forces of unknown risk and need to eat. Should a hungry individual consume a novel food it encounters because its nutritional requirements are urgent, or should novel foods be avoided because the consequence of consuming unprofitable prey when physiologically stressed may be death?

Many animals display a short-lived avoidance to novelty, termed neophobia (Barnett, 1958). Because hunger can increase risk-taking behaviour (Gotceitas and Gordon, 1991; Godin and Crossman, 1994; Bridges, 2002), it seems reasonable to assume that hunger should reduce neophobia towards novel foods (food neophobia). In fact, hunger has been suggested as the mechanism by which parasitic burden decreases neophobia in predators, by increasing the energy demands of the host and making them less aversive towards novel foods (Freire *et al*. 2022). However, few animal studies have directly addressed the effect of hunger on responses to novel foods. Two such studies found a positive relationship between novel food acceptance and hunger state: Heinrich (1995) found that ravens increased neophilia

(particularly for food items) with hunger; Harris *et al*. (2000) found that hungry rats were more flexible in forming associations between novel odour and food, suggesting a mechanism for reducing neophobia. Overall, there is a considerable dearth of knowledge on how animals' current, physiological state affects responses to novel food. In contrast, a larger body of research exists on hunger and food neophobia in humans, but with conflicting findings. In humans, hunger has been suggested to increase (Pliner *et al*. 1995), decrease (Perone *et al*. 2021), or have no effect on food neophobia (White *et al*. 2023).

In addition to neophobia, vertebrate species have been shown to exhibit dietary conservatism (Preston and Marples, in prep a): individuals in a population adopt different foraging strategies to deal with novel foods. Some individuals display a Dietary Conservative (DC) foraging strategy, characterised by prolonged reluctance to incorporate novel foods into the diet, while others adopt an Adventurous Consumer (AC) foraging strategy, rapidly accepting novel foods as part of their regular diet (Marples and Kelly, 1999). While an individual's foraging strategy is at least partially genetically controlled (Marples and Brakefield 1995), expression of both AC and DC foraging strategies is plastic, modified both by experience (Marples *et al*. 2007) and the context of predators (McMahon, 2014) and competitors (McMahon *et al*. 2014; McMahon and Marples, 2017). How hunger affects different foraging strategies has not been previously studied, and it is reasonable to expect that AC and DC strategies might respond differently to the problem of balancing nutritional need and unknown risks.

We investigated the effect of hunger on foraging strategy expression in fish using wild-caught, three-spined sticklebacks (*Gasterosteus aculatus*) as a model (hereafter, sticklebacks). Dietary conservatism has been previously shown in sticklebacks (Thomas *et al.* 2010; Richards *et al*. 2011; Preston *et al.* in prep b) and sticklebacks are a well-established model for understanding foraging behaviour (e.g., Milinski and Heller, 1978; Godin and Crossman, 1994; Quesenberry *et al*. 2007), making them an ideal species for this study. Our results add to our understanding of animal foraging strategies and have practical implications for controlling the impact of individual foraging strategies on interactions with novel foods.

4.3 Methods

4.3.1 Fish Housing

Fish were initially housed in large, outdoor tanks and transferred to the laboratory in batches of 24 as needed. Healthy individuals between 25-40mm in length were selected and kept in groups of 6 in 10L, white buckets for four days before being transferred to individual 1.4L or 2.8L tanks (ZT140T, Aquaneering, San Diego, CA, USA). Tanks were covered with white paper on sides and bottom to provide a uniform background for foraging. Tanks were housed under natural light conditions supplemented with an artificial light:dark regimen (12:12), to ensure that all batches received a minimum of 12 hours light condition. Temperature during testing was 15-25^{o}C.

Water in the buckets and tanks was aerated and fish were provided with sections of semi-transparent tubing (approximately 5cm long and 3cm in diameter) as shelter and hiding spaces. Water treatment (nitrogen and particulate waste removal) followed the procedure in Preston *et al*. (in prep b).

Of the 24 fish in each batch, 20 were experimental subjects and 4 were randomly designated as replacements should any experimental fish became ill.

4.3.2 Experimental Food

Fish were fed live and dyed *Daphnia magna*, 1-2mm in length during this experiment. Sugarflair food colouring paste (Sugarflair Colours, Essex, UK) was used to dye *Daphnia* either green (Sugarflair Party Green) or brown (Sugarflair Dark Brown). The dying followed the protocol described in Preston *et al.* (in prep b). Sticklebacks from this population have previously been shown to have no innate preference for either green- or brown-dyed *Daphnia* (Preston *et al*. in prep b).

4.3.4 Training and Treatments

Each batch of 24 fish was assigned either green or brown *Daphnia* as their familiar food (termed “green-familiar” and “brown-familiar” fish, respectively, alternating across batches). The alternative colour was used as the novel food in each case. Fish were trained on their

familiar food for four days while housed in groups by providing 15 appropriately coloured *Daphnia* per fish per day. Fish typically ate 50-100% of *Daphnia* provided, with the proportion eating increasing each day. All fish partook in feeding, although it was not possible to monitor the exact intake of each fish in the buckets.

After four days of training, fish were transferred to individual tanks and assigned to one of three treatments (Table 1). Fish received either 10, 15, or 20 familiar-coloured *Daphnia* per day for three days, establishing different hunger states and building their experience with their familiar colour of *Daphnia*. During this stage, fish were also habituated to the presence of an observer by feeding the first three *Daphnia* daily with a disposable pipette. By the third day, all fish followed and attacked the pipette in anticipation of food and ate quickly in the presence of the experimenter.

4.3.5 Testing

A latency test for dietary conservatism (Marples *et al.* 1998; Preston and Marples, in prep a) was used to determine preference for the familiar. This test is described in full elsewhere (Preston and Marples, in prep a). Briefly, fish were presented with 10 familiar-coloured and 10 novel-coloured *Daphnia* and allowed to forage for 10 minutes. The order in which prey of each colour were eaten was recorded and used to calculate a score indicating the preference for the familiar-coloured *Daphnia* (hereafter "preference score"), based on the assumption that preferred foods will be consumed before non-preferred foods. Higher preference scores indicate a greater preference for the familiar, and the highest score possible is achieved if all familiar *Daphnia* are eaten before any novel *Daphnia*.

Fish were given three tests, one per day. To standardize satiation, any fish that failed to eat all 20 *Daphnia* on test days were offered live, undyed *Daphnia* equal to the amount uneaten during the test. Following each test day, fish received an additional day of their treatment regimen (10, 15, or 20 familiar *Daphnia*) to re-establish hunger levels.

4.4 Data Analysis

All statistical analysis was carried out using R Program version 4.0.2 (R Core Team, 2020).

4.4.1 Calculating Preference Scores

Preference scores were calculated as described in Preston *et al*. (in prep b). The ordinal positions of each familiar food item eaten was summed and divided by the number of familiar prey available. The result is then subtracted from 21 so that high preference for the familiar is represented by a larger preference score. An example calculation is given below.

Box 4.1. Preference score calculation example

Suppose an individual presented with 10 familiar-coloured prey (F) and 10 novel-coloured prey (N) eats all 20 in the following order: F, F, F, F, F, F, F, F, N, N, F, F, N, N, N, N, N, N, N, N. The preference score is calculated by summing the ordinal positions of the familiar prey (1+2+3+4+5+6+7+8+11+12 = 59) and dividing by the number of familiar prey available (59 / 10 = 5.9). This score is subtracted from 21 (the sum of the maximum and minimum values possible) to yield a familiar preference score of 15.1.

4.4.2 Determining Foraging Strategy

Following Preston *et al*. (in prep b), individuals' preference scores on the last day (day 3) of testing were used to determine foraging strategy. By the third day, all fish had made contact with and/or consumed some novel food, indicating that neophobia had been overcome and that preference scores should reflect foraging strategies.

Preference scores were scaled -1 to 1 using the "mutate" function in R and unsupervised K-means cluster analysis was applied to split the fish into 2 groups. Fish in the group with higher preference scores were categorized as being DC (showing stronger preferences for familiar food), while fish in the group with lower preference scores were categorized as AC.

As data from the third day of testing were used to determine foraging strategy, any fish that failed to eat at least 10 *Daphnia* on day three of testing (n=2) were excluded from the analysis.

4.4.3 Distribution of Foraging Strategies

Individual foraging strategies are thought to be genetically determined (Marples and Brakefield, 1995) and consistent within individuals over long periods (Marples and Kelly, 1999). However, it is possible that the foraging strategy an animal displays is also impacted (perhaps even primarily) by the animal's unique internal state (Marples *et al*. 2018). To determine whether hunger level affected expression of foraging strategy, Fisher's exact tests were used to determine whether the distribution of foraging strategies across treatments and the distribution of foraging strategies within colour groups differed significantly from random.

4.4.4 Relating Preference Scores to Treatment

Preference scores were scaled 0-1 with a correction factor to remove scores of 0 and 1 for analysis using the following formula:

Preference Score (Scaled) = (Preference Score – 5.5)/10.5.

Scaled preference scores ranged from a maximum of 0.962 indicating strongest preference for the familiar, to a minimum of 0.010 indicating weakest preference for familiar (strongest preference for novel), and a mid-value of 0.486 indicating no preference for either food type.

We constructed a global generalized additive model (GAM) with a beta regression family and logit link to model the relationship between scaled preference scores, treatment, foraging strategy, familiar food colour, and day of testing for scores on days 1 and 2 of testing. Fish identity was included as a random, fixed factor. Interaction terms for foraging strategy and treatment and foraging strategy and day of testing were also included in this model. We reduced the global model by stepwise deletion of terms to arrive at the best model based on AICc score.

4.5 Results

4.5.1 Distribution of Foraging Strategies

Fifty-one fish provided data for this experiment (Table 4.1). Of these, 17 were brown-familiar fish and 34 were green-familiar. A heatwave during the experiment caused a sudden die off, resulting in uneven sample sizes for brown familiar fish across treatments. Following the heatwave, a final batch of green-familiar fish was tested, but no further experimental testing was possible. Thirty fish were identified as DC compared to only 21 AC; however, these were not distributed equally across colour groups. Among brown familiar fish, the majority of individuals were AC (10/17), while most green familiar fish were identified as DC (23/34).

No significant relationship was found between foraging strategy and treatment group (Fisher's exact test, $p = 0.5534$), nor when considering each colour group separately. (Fisher's Exact tests: brown familiar, $p = 0.665$; green familiar: $p = 0.482$).

Table 4.1. Number of fish grouped by colour, treatment, and foraging strategy.

	Familiar Colour/ Foraging Strategy			
	Brown		Green	
Treatment	AC	DC	AC	DC
Hunger – 10 *Daphnia*/Day	5	3	2	10
Moderate – 15 *Daphnia*/Day	1	2	4	7
Satiated – 20 *Daphnia*/Day	4	2	5	6

4.5.2 Preference Scores

The best fitting model of preference scores retained foraging strategy, familiar colour, treatment, and test day, as well as the interactions between familiar colour and treatment and between familiar colour and test day as explanatory variables (Table 4.2). DC fish had higher preference scores than AC fish ($p = 0.006$). Preference for familiar food was greatest among hungry fish (Treatment A) and decreased with increasing satiation (Treatments B and C, negative estimates for variables in Table 4.2; see also Figure 4.1). Correspondingly,

acceptance of novel food increased with increasing satiation. However, the pattern of novel food acceptance was influenced by whether the familiar food was brown or green. Positive estimates for the interaction terms between familiar colour and Treatments B and C show that green-familiar fish retained higher preference for the familiar than brown-familiar fish when satiated (p values = 0.0034 and 0.0015 for interactions with Treatments B and C, respectively). Preference for familiar food was close to maximum in all treatments among green-familiar, DC fish, and decreased somewhat among the most satiated, AC fish (Figure 4.1a), while in brown-familiar fish both AC and DC fish approached a no-preference value (0.486) in the most satiated treatment group (Figure 4.1b). The significant interaction between familiar colour and test day ($p < 0.001$) also shows that preference for familiar food waned less among green-familiar fish than among brown-familiar fish over the two days of testing.

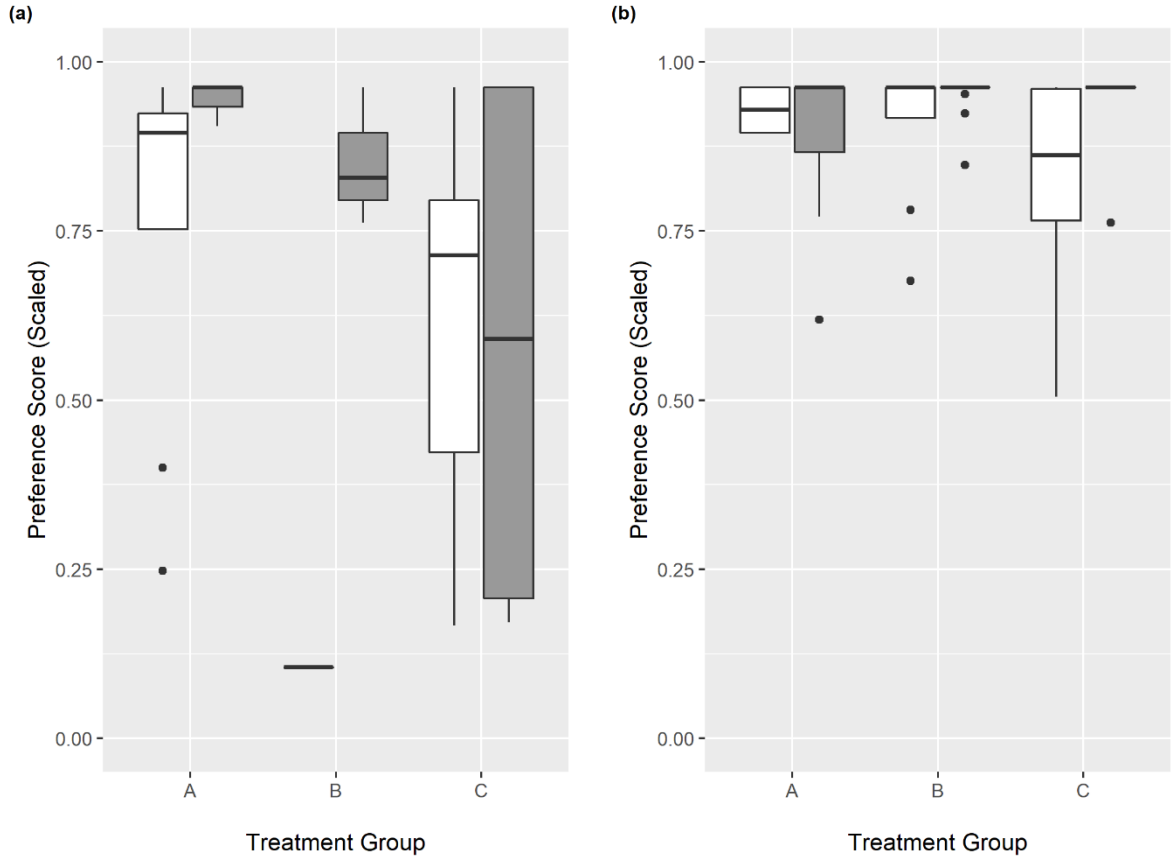

Figure 4.1. Preference score vs treatment group for (a) brown familiar fish and (b) green familiar fish. White boxes denote AC fish. Grey boxes denote DC fish. Treatment groups are arranged most (A) to least (C) hungry, (details in Table 1). Higher preference scores indicate greater preference for familiar food.

Table 4.2. Best fitting model and output relating preference score and explanatory variables.

Family: Beta regression(12.628)

Link function: logit

Formula:

pref.score ~ factor(treatment) + colour + acdc + factor(screening) +

colour:factor(screening) + colour:factor(treatment) + s(fish, bs = "re")

Parametric coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.6929	0.2987	9.016	< 2e-16 ***
factor(treatment)B	-1.3186	0.4912	-2.684	0.007269 **
factor(treatment)C	-1.3215	0.3479	-3.799	0.000146 ***
colourg	-0.8920	0.3782	-2.359	0.018344 *
acdcdc	0.6553	0.2075	3.157	0.001592 **
factor(screening)2	-2.0395	0.2468	-8.262	< 2e-16 ***
colourg:factor(screening)2	1.6555	0.3101	5.339	9.37e-08 ***
factor(treatment)B:colourg	1.6975	0.5804	2.925	0.003447 **
factor(treatment)C:colourg	1.4529	0.4566	3.182	0.001462 **

Approximate significance of smooth terms:

	Edf	Ref.df	Chi.sq	p-value
s(fish)	17.67	44	33.11	0.000479 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.701 Deviance explained = 75.5%

-REML = -109.32 Scale est. = 1 n = 99

4.6 Discussion

Hunger is an important motivator of animal behaviour with profound effects on foraging behaviour. This present study is the first to examine how hunger affects incorporation of novel foods into the diet by individuals with different foraging strategies. Conflicting with previous studies suggesting that hunger reduces food neophobia (Heinrich, 1995; Harris *et al*. 2000) we found that hunger made sticklebacks more conservative in their foraging decisions, i.e., reduced their willingness to eat novel food. For brown-familiar fish, this effect was independent of foraging strategy: both AC and DC fish were more willing to accept novel food when satiated and maintained their preference for familiar when hungry. Among green-familiar fish, AC individuals followed this same pattern, but DC individuals did not. Green-familiar, DC fish maintained a strong preference for familiar food both when hungry and satiated. Thus, our results suggest hunger reduces willingness to incorporate novel foods into the diet largely independent of foraging strategy, however a strong effect of food colour was also evident.

Hunger is expected to modulate individuals' responses to novel food as it increases risk-taking behaviour (Gotceitas and Gordon, 1991; Godin and Crossman, 1994; Bridges, 2002). However, hungry animals are physiologically stressed, so the risk of consuming unfamiliar prey which may be chemically defended or otherwise unprofitable (and thus a waste of time and energy; Marples *et al*. 2018) increases with hunger. Our results therefore align with an individualistic model of animal foraging behaviour, where animals use information to make informed decisions regarding profitability and risks based on experience and current physiological status (Marples *et al*. 2018). In this model, incorporating novel foods into the diet is a calculated risk made by individuals that can afford the consequences, rather than a desperate gambit by those with an urgent need for food.

It is worth noting that this study did not encompass the extremes of hunger that an animal might experience. Severe hunger status may override aversions to novel foods as the risk of starvation increases beyond the risk of the unknown food. It would be interesting to extend the range of hunger states used in this study to see if a response curve of novel food acceptance high at the extremes of hunger and satiation and low at intermediate hunger levels might be produced. Non-linear responses to novel food depending on degree of hunger may also provide an explanation as to some of the conflict between the results presented

here and the expectation that hunger reduces food neophobia (an expectation with limited experimental data to support it). Alternatively, as neophobia and novel food incorporation are different processes (Marples and Kelly, 1999), apparent conflict among results may be caused by hunger affecting each process differently. Hunger may cause animals to take more risks in sampling novel foods but reduce acceptance of those foods as a regular part of the diet.

Both AC and DC foraging strategies coexist in many, if not all, vertebrate populations (Preston and Marples, in prep a). Despite this, the benefits of a DC foraging strategy remain unknown. How do DC individuals, which refuse to add profitable foods to their diet simply because they are novel, compete with AC individuals that readily exploit new, profitable foods?

Two hypotheses have been proposed for the evolutionary advantage of the DC foraging strategy: 1) the DC foraging strategy may represent a specialism by individuals in handling particular prey, thereby increasing efficiency of prey intake (Preston and Marples, in prep a); 2) by avoiding unknown foods, DC individuals are less likely to consume toxic or otherwise unprofitable prey (Richards *et al*. 2014; Preston and Marples, in prep a). These hypotheses suggest different outcomes of hunger on foraging behaviour when confronted with novel foods. If the DC foraging strategy is a matter of efficiency, then hungry DC individuals might be expected to broaden their diet and accept available novel foods because of their immediate need. Alternatively, if the DC foraging strategy is protective against unknown risks, then both AC and DC individuals might be expected to avoid novel foods that might be harmful when in a physiologically vulnerable state. Our results offer support for the latter, that the DC foraging strategy reduces the likelihood of consuming unprofitable prey.

Stimulus characteristics play a crucial role in determining preferences (Heinrich, 1995), especially colour (Marples *et al*. 1998; Marples *et al*. 2007; Skelhorn *et al*. 2008; Spence and Smith, 2008; see also Schuler and Roper, 1992 for a review of innate colour bias in predators). Nevertheless, our finding that colour had a pronounced effect on novel food acceptance was surprising. Previous studies that tested naïve sticklebacks from the same population found no evidence of a preference between green- and brown-dyed *Daphnia* (Preston *et al*. in prep b; S Preston, unpubl. thesis). Despite this, green-familiar, DC fish showed little or no increase in novel food acceptance with increasing satiation. We speculate this is due to a bias for green food, which fish trained on coloured *Daphnia* appeared to prefer whether it was novel or

familiar. Why only fish familiar with dyed *Daphnia* and not naïve fish show this bias is unknown, but may be caused by some aspect of training or housing, or perhaps a predisposition to form an association between green and food, once dyed *Daphnia* of any colour have been encountered. This bias may cloud the relationship between foraging strategy and hunger in accepting novel foods if brown-familiar, DC fish were substantially more likely to accept the novel food because it was green, rather than due to increasing satiation. Thus, while our results offer support for a protective role of the DC foraging strategy, they are by no means conclusive. Future studies should aim to use alternative colour combinations for which biases do not appear to test whether a relationship exists between foraging strategy and hunger.

This study expands our understanding of animal's foraging decisions. That responses to novel foods depend on hunger level has important implications not only for standardising research methodologies to facilitate comparability, but also improving the relevance of results by testing animals under physiological states likely to be encountered in nature. These results also have practical application in animal management. If satiated individuals are more accepting of novel foods, this offers a simple method of introducing animals to new diets without subjecting them to periods of hunger. Indeed, it suggests that it is counter-productive to do so. The impact of hunger on animals' feeding behaviour remains a rich area for future, empirical research and incorporating individuals' foraging strategies offers an effective way to understand the complexities of dietary decision-making.

Chapter 5

Can Efficiency Explain Foraging Strategies?

Authors

Sam Preston, Camila Quintero-Berns, and Nicola Marples

Author Contributions

I designed this study, collected and analysed the data, and wrote this manuscript. NM provided insight on this manuscript. CQB assisted with data collection.

Special thanks to Rob Thomas for providing insight on analysis methods.

Status

This manuscript is being prepared for publication. Raw data and R Scripts will be added to this manuscript.

5.1 Abstract

Individual animals show different foraging strategies in response to novel food. Some are adventurous consumers (ACs), rapidly incorporating novel foods into their diet and utilizing them when they are available. Others are dietary conservative (DC) and show prolonged reluctance to incorporate or utilize novel foods they encounter. Why the DC foraging strategy evolved and how it coexists with the AC foraging strategy are the most fundamental, unanswered questions concerning this behaviour. There are no obvious advantages to DC individuals choosing to restrict their available food resources simply because they are novel, and AC individuals might be expected to outcompete them in many (if not all) environments. Nevertheless, the two foraging strategies have been found in every vertebrate population so far examined for them.

Here we investigate the hypothesis that, by ignoring novel and specialising on familiar prey, DC individuals are more efficient foragers of familiar prey types than AC individuals. I presented individual wild robins (*Erithacus rubecula*) with a range of foraging scenarios and compared their foraging efficiency in different tests with their foraging strategy. My results did not find conclusive support for the efficiency hypothesis but suggest that substantial, practical challenges exist to testing wild birds' foraging strategy. I conclude by discussing the implications of these results for the validity of the efficiency hypothesis and offer insights for future studies

Keywords: Animal Behaviour; Dietary Conservatism; Foraging Efficiency; Foraging Strategy; Robin

5.2 Introduction

Novel foods pose a challenge for foraging animals. Optimal foraging theory predicts that animals should maximize their energy intake rate (Krebs *et al*. 1983; Pyke, 1984), so it might be expected that new foods should be exploited as they become available. However, novel foods carry unknown risks and have unknown profitability (Sherratt, 2011), complicating the decision of whether or not they should be consumed (Marples *et al*. 2018). Individual animals of many species show distinct foraging strategies in response to novel foods (Preston and Marples, in prep a). Some are adventurous consumers (ACs), rapidly incorporating novel foods into their diet. Others are dietary conservative (DC) and show prolonged reluctance to exploit novel foods even when they are as profitable as familiar foods (Marples *et al.* 1998; Marples and Kelly, 1999). While these foraging strategies are flexible in their expression according to various contexts (McMahon 2014; McMahon and Marples, 2017), they appear to be genetically determined behaviours (Marples and Brakefield, 1995) and consistent over an individual animal's life (Marples and Kelly, 1998; Kelly, 2001).

The advantages of being AC are straightforward and easy to understand. After a brief period of sampling (Marples and Kelly, 1999), profitable, novel foods are then regularly consumed when encountered, thus maximizing resource utilization and energy intake rate when foraging. The DC foraging strategy is much less comprehensible. DC individuals voluntarily restrict the food resources they exploit to familiar prey and forgo incorporating many other, profitable prey types simply because they are novel. Indeed, DC individuals can show strong preference for familiar prey with which they have only marginally more experience than the novel foods they ignore (Preston *et al*. in prep b).

Why the DC foraging strategy exists at all remains a mystery. There are no obvious advantages to an animal limiting food resources available to it based solely on prior experience. How DC individuals can coexist with AC individuals that would be expected to outcompete their picky rivals is even less understood.

Two hypotheses have been proposed to explain the evolutionary benefit of being DC. The first is that DC individuals are better able to avoid unprofitable foods. This idea has received mixed support. Experiments examining the prevalence of the DC foraging strategy among tropical fish (which were expected to have evolved in environments with more toxically defended

prey than temperate fish) found instead similar proportions of DC individuals as among populations of a temperate fish species, a finding which did not support the avoidance hypothesis (Richards *et al*. 2014). However, experiments on fish with different hunger levels found that individuals tended to prefer familiar food when physiologically stressed, suggesting that avoiding unknown and potentially unprofitable prey can be advantageous when the costs of consuming unprofitable prey are high (chapter 4).

The avoidance hypothesis, however, does not explain the relatively consistent proportions of AC and DC individuals seen in many populations. In most studies, DC individuals make up a small proportion of the population, usually around 25-30% (Preston and Marples, in prep a). If the DC foraging strategy was most advantageous when unprofitable prey were common, we would expect to see higher proportions of DC individuals in these environments and lower proportions (or possibly none) in environments where the risks associated with consuming novel prey are low and they are outcompeted by AC individuals.

The second hypothesis proposes that the DC foraging strategy confers an advantage in foraging efficiency on familiar prey types. Because of their large and often exclusive preference for familiar foods, DC individuals are effectively dietary specialists on the familiar within a wider population of AC generalists. Dietary specialist and generalist species can coexist even when niches overlap because their relative fitness fluctuates with the composition of the available foods. When the specialist's food is abundant, they have a fitness advantage, while the generalist is at an advantage when the available prey is more diverse (Hanski *et al.* 1991). However, unlike traditional specialists, DC individuals are not limited to a specific prey type but whatever prey they perceive as familiar. Consequently, the proportion of DC individuals in a population need not be tied to the relative abundance of one prey type but to the overall diversity of prey, leading to a more stable ratio of AC and DC individuals across many different habitats and environments.

No study has previously attempted explicitly to test the efficiency hypothesis. One experiment on wild birds found that both AC and DC individuals preferentially selected familiar food in the presence of a mock predator (McMahon, 2014). The inference from this result was that when birds perceive a risk that promotes rapid food acquisition, focusing on familiar items confers an advantage in handling time and efficiency. This suggests a potential mechanism by which DC individuals may glean a competitive edge by specialising on familiar

prey. Other advantages a DC foraging strategy might have include improving searching efficiency if, for instance, AC and DC individuals utilize search images (Gibb, 1962; Dawkins, 1971) in different ways. While individuals with different foraging strategies appear to be equally able to distinguish different prey types using visual cues (although there is some unpublished evidence of differences in taste perception), DC individuals use this information differently and retain a strong preference for the familiar (chapter 3). This may be due to DC individuals relying more on a specific search image to guide them in searching for suitable prey. Experiments on birds suggest that a search-based efficiency advantage may be possible, as many birds preferred their familiar prey over novel prey even when foraging against a background where the familiar prey were more cryptic than the novel (Thomas *et al*. 2004). By focusing on familiar prey, DCs may also need to invest less into brainpower to learn about and recall a wide range of prey types.

Whatever the mechanism, improving foraging efficiency is a plausible explanation for the existence of the DC foraging strategy and its coexistence in populations with the AC strategy. Whether and how different foraging strategies exhibit different efficiencies in foraging has considerable implications for the evolution of foraging strategies, our understanding of foraging ecology, and the cognitive mechanisms that underpin predators' decision-making.

This study is the first test of the efficiency hypothesis to explain the DC foraging strategy. We presented wild robins (*Erithacus rubecula*) with a variety of foraging scenarios and measured their rate of food intake and the number of times an unprofitable, non-food item was examined. These data were then interpreted in the context of individuals' foraging strategies. We discuss implications for the efficiency hypothesis in light of these results.

5.3 Methods

5.3.1 Overview

Wild robins were the study species for this experiment. Robins are known to exhibit different foraging strategies (Marples *et al*. 1998) and are bold, trainable, and commonly fed by people, making them ideal subjects for this study. Once robins were comfortable eating in our set-up, they were familiarised with either green or yellow pastry baits (their "familiar" food). Following familiarisation, robins were presented with a series of three foraging tests and the rate of food capture and number of mistakes made recorded to determine foraging efficiency. A final choice test was then used to determine the foraging strategies of individual birds.

5.3.2 Study Site

We conducted this study in Dublin Zoo, Dublin, Ireland (latitude and longitude: 53.357, -6.305). Dublin Zoo contains a high density of robins habituated to people, making it very suitable for testing large numbers of birds and close observation for recognition of individuals. Robins were located using playbacks and walkthroughs. Individual birds were visited regularly and offered crushed, skinless peanuts to encourage them to feed in a spot suitable for the experiment. Those that returned and ate regularly were selected for training and testing using coloured pastry baits. In total, 15 robins were selected for training across the zoo (Table 1). This selection method resulted in an uneven distribution of subjects across the two areas of our study site, as robins in area 1 were less willing to return consistently to feeding sites.

To colour-balance our experiment we divided our study site into two areas and trained robins on different colour baits in each. Robins in Area 1 (the old zoo, southern half of Dublin Zoo) were familiarized with green pastry baits. Birds in Area 2 (African Plains, northern half of Dublin Zoo) were familiarized with yellow pastry baits. This arrangement aimed to reduce the possibility that robins familiar with one colour would move into an area where the opposite colour was being used as the familiar. Within each area robins, and the locations where we fed and tested them, were chosen to maximize the distance between birds and prevent non-target individuals from feeding. Physical characteristics of individual birds were also used to confirm identity. All robins in Area 1 were visited and fed before birds in Area 2. Robins'

foraging strategies were determined after the experiment, making is impossible to arrange an equal distribution of AC and DC birds in each area.

Table 5.1. Details of robins in each area of the study site. Area 1 comprised the Old Zoo. Area 2 comprised the African Plains section of Dublin Zoo. AC and DC refers to number of individuals of each foraging strategy.

Area	Familiar Colour	# Robins	AC	DC
1	Green	6	4	2
2	Yellow	9	3	6
		Total	7	8

5.3.3 Experimental Foods

Crushed, skinless peanuts and live mealworms were used during training to attract robins and habituate them to the experimental set-up. The main experimental food, however, was coloured pastry baits rolled into balls approximately 3mm in diameter. Baits were a mixture of flour, cooking fat, and peanut butter (60:40:10 ratio by weight) with 5ml of food dye solution per 110g. Food dye solutions made from 10% (v/v) Goodall's Green Food Colouring and 60% (v/v) Goodall's Yellow Food Colouring in water.

5.3.4 Training

Training began once a robin was regularly returning to feed. By excluding robins that did not consistently feed on crushed peanuts, we may have excluded the most DC individuals.

Training was conducted daily between 08:00 and 11:00. Each session lasted for 10 minutes or until robins had taken some food and flown out of sight. Training consisted of three stages, which varied in length among robins. In the first stage, robins were habituated to the presence of the experimenter and trained to arrive on command. Robins were fed crushed, skinless peanuts with the experimenter nearby. On arriving to the feeding spot and whenever an item of food was taken, the experimenter would whistle to provide a stimulus to be associated with the provision of food. This phase was completed when robins were approaching reliably to the whistle. In the second phase, robins were habituated to the experimental background. Crushed peanuts were offered on a white, plastic board (50x50cm) which provided a uniform

background against which to forage. Once robins showed no hesitation to eat from the board they moved to final stage of training, in which they were familiarised with their experimental food. Depending on site, robins were provided with either green or yellow pastry baits (Table 1) on the white background. This became their familiar colour. Baits were hand-rolled balls approximately 3mm in diameter, and initially provided alongside crushed peanuts to encourage birds to sample them. Once the robins started eating the baits, the amount of crushed peanut was reduced over one to three days until only coloured baits were offered, which were then arranged in a square grid of 20-36 baits. This stage was completed when birds were regularly eating several baits from the board without hesitation. All birds were trained on their familiar baits for a minimum of 7 days on the experimental board prior to testing.

5.3.5 Testing

Tests were carried out once daily at the same time as training had been conducted. In all tests, baits were presented solely on the white board. Robins were presented with a grid of 36 baits spaced 5cm apart, resting in small holes to prevent them from rolling off the board. Birds were allowed to forage until approximately half of the pastry baits were eaten or for three minutes (beginning when robins made first contact with the board), whichever was shorter. Robins that failed to eat half of the baits within that time had invariably left the board and did not return during that day's trial. Depending on the test, the grid presented consisted of a combination of novel and familiar baits, and real (pastry) baits and "fake" baits. Fake baits represented inedible (and unprofitable) prey, with an attendant handling cost for foragers. To create the fake baits, a two-part epoxy putty (milliput® silver-grey) was rolled into approximately 2mm balls in the same manner as the pastry baits, which were then painted using acrylic paints to resemble either green or yellow real baits.

Three tests (A-C) were conducted to test foraging efficiency under different circumstances, each with a different grid composition:

- Test A - grid of 36 familiar baits
- Test B – grid of 18 familiar baits and 18 familiar-coloured, fake baits

- Test C – grid of 9 familiar baits and 9 familiar-coloured, fake baits, and 9 novel baits and 9 novel-coloured, fake baits. This tested foraging efficiency in a complex environment with familiar and unfamiliar prey of different profitability available.

Test A aimed to establish a baseline foraging rate for all individuals when familiar, profitable prey are abundant and easy to find. In test B, familiar, profitable prey are spread amongst familiar-coloured, unprofitable mimics. This test examined whether individuals with different foraging strategies differed either in their foraging efficiency when familiar, profitable prey were slightly rarer, harder to find among unprofitable mimics, and "mistakes" (i.e., handling an unprofitable mimic) were possible. Test C examined whether robins differed in foraging efficiency in a more complex environment where familiar prey were rarer still, and novel, profitable prey existed as an alternative resource but were difficult to identify among and novel-coloured, unprofitable mimics. Tests were carried out sequentially with each robin receiving 3 trials of each test before moving onto the next.

Where applicable, fake and pastry baits were arranged alternately in the grid. In test C, familiar-coloured and novel-coloured baits (whether fake or not) were further arranged such that each row and column in the grid contained 3 baits of each colour, and no row or column had more than 2 baits of the same colour in sequence.

All tests were video recorded using either a digital video camera (GoPro Hero 7) or smartphone cameras (iPhone or Samsung Galaxy s22 Ultra). Video recordings were analysed to determine two metrics of foraging efficiency: foraging rate (number of baits eaten per second) and – for tests B and C – number of mistakes birds made.

Following test C, robins were presented with a final test (test D) to determine their foraging strategy. Once per day for four days, robins were presented with 4 familiar and 4 novel baits. Robins were permitted to forage for up to three minutes and the order in which baits were taken was recorded. Foraging strategy was determined based on the stage of novel food incorporation (Marples and Kelly, 1999) robins had reached on day 4.

5.4 Data analysis

5.4.1 Constraining test parameters

There was considerable variation in the number of baits eaten across different trials, with some birds consuming very few baits before leaving and some consuming half or more baits before moving on. These differences might disproportionately affect both foraging rate and number of mistakes. A bird that only eats one or two baits and immediately leaves has less opportunity to make mistakes than another bird that stays to eat 10 baits and is hungry enough to investigate many baits before leaving. The first bird may also experience an inflated foraging rate, as it is possible to eat two baits from the edge of the board in under a second, while (in addition to mistakes taking more time) eating 10 baits requires additional time to move around the board while observing surroundings. Additionally, as more baits are eaten the density of baits available decreases (particularly when mixed among fake baits), leading to longer times between each bait taken and lower foraging rate overall.

To control for these effects, foraging rate and number of mistakes were calculated based only on the performance of birds during the first 6 baits eaten. Consumption of 6 baits was chosen as the cut off as it maximized the number of trials that could be included in the analysis (127/135) while requiring that birds to forage for long enough to reasonably estimate their foraging rate. Any trials during which fewer than 6 baits were eaten were excluded from the analysis. Foraging was considered to "start" when a bird made first contact with the board in a trial and ended when the sixth pastry bait was eaten. Any time birds left the board and returned was subtracted from the total time, so that only time spent foraging on the board (and not e.g., feeding young or retreating to hide) was counted. Dividing this time into 6 (number baits eaten) gave foraging rate in baits per second. Number of mistakes was simply the number of mistakes made before the sixth bait was eaten.

Constraining foraging efficiency calculations to performance within a certain number of baits standardizes the foraging test for birds with different temperaments, hunger states, and in different circumstances that might affect how long they choose to forage. However, it is worth noting that this may exclude important differences in foraging behaviour that might impact efficiency. For instance, taking only one or two baits before moving to other foraging patches may be a way of maximizing food intake rate. Effectively investigating how different

approaches to exploiting foraging patches impacts overall efficiency would require a larger sample size and a longer experimental time, so was not carried out here. Nevertheless, it should be recognized that different patch exploitation behaviours may covary with foraging strategy, which would substantially alter foraging efficiency.

5.4.2 Determining foraging strategy

Four stages of novel food incorporation have been identified (Marples and Kelly, 1999):

1. Visual Inspection Only
2. Sampling
3. Regular Acceptance when Familiar Unavailable
4. Full Incorporation/Recruitment

By day four of test D, all robins were observed to fall into either stage one, stage three, or stage four. Robins in stage one (still refusing to eat the novel food) were classed as DC, while those in stages three or four were classed as AC. A Fisher's exact test was used to determine whether the distribution of AC and DC individuals was significantly affected by familiar colour.

5.5.2 Statistical Analysis

All analyses were carried out using R studio using R program version 4.2.1 (R Core Team 2022).

The "glmer" function (lme4 R package) was used to construct mixed effect global models for both foraging rate and number of mistakes. Each model included foraging strategy, test (A, B, or C), trial number, and familiar colour, as well as all interaction terms between foraging strategy, test, and trial, as explanatory variables. Robin identity was included as a random effect in both models. From these, the best-fitting models were extracted using the "dredge" function (MuMIn package) based on AIC values.

Models relating foraging rate to the explanatory variables used a Gamma error distribution. Models of number of mistakes used a negative binomial error distribution with a square root link and theta parameter set to 5000 to achieve the best possible fit for standardised residuals. Despite this, the distribution standardised residuals remained distinctly non-normal.

5.5 Results

5.5.1 Foraging Strategies

Of the 15 robins in our experiment, 7 were identified as AC and 8 as DC (Table 5.1). The proportion of DC individuals was higher than typically seen in bird populations (e.g., Marples *et al*. 1998; Marples and Mappes, 2011; McMahon *et al*. 2014) though considerably less than the highest proportion of DC birds seen in a sample McMahon and Marples, 2017). Familiar colour appeared not to affect foraging strategy expression. While the ratio of AC birds to DC birds differed considerably with familiar colour – a 2:1 ratio among green-familiar robins and a 1:2 ratio among yellow-familiar robins (Table 1) – this distribution was not significantly different from random (Fisher's exact test: p-value = 0.315).

5.5.2 Foraging Rate

Foraging rate was lower when fake baits were present (tests B and C) than when only pastry baits were offered in test A (Figure 5.1). The best fitting model confirmed this relationship, retaining only test as a significant explanatory variable for foraging rate (Table 5.2). This suggests that foraging rate is unaffected by foraging strategy. However, among the 3 models with the lowest AIC values (Table 5.3), two retained both foraging strategy and the interaction between foraging strategy and trial as significant explanatory variables, and one also retained the interaction between foraging strategy and test as a non-significant variable. All four models were within 2 AIC units of one another, making them statistically indistinguishable, though the most parsimonious model was accepted as the best fitting.

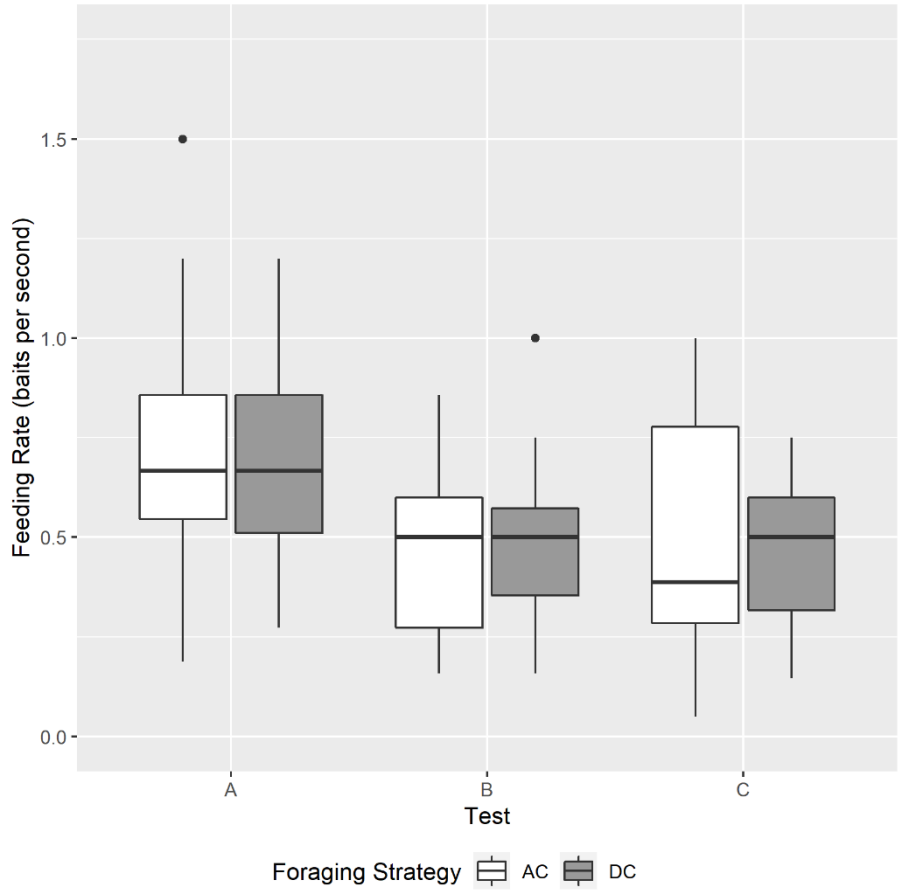

Figure 5.1. Feeding rate of AC and DC birds across each test type. Test A) only real, familiar baits present; B) real and fake, familiar-coloured baits present; C) real and fake baits of both familiar and novel colours present.

Table 5.2. Fixed effect estimates for best fitting model relating foraging rate to explanatory variables. (For full model output including random effect estimates see Table A3.1).

	Formula: rate ~ test + (1 | robinID)			
	Estimate	Std. Error	t value	Pr(>|z|)
(Intercept)	0.72742	0.06237	11.663	< 2e-16 ***
testB	-0.21974	0.05459	-4.026	5.68e-05 ***
testC	-0.26201	0.05218	-5.021	5.14e-07 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

Table 5.3. Models with the lowest AIC values (within 2 AIC) returned from the global model of foraging rate. Note fam.colour is the colour with which birds were familiar and AC.DC is foraging strategy.

Model Formula	AIC	BIC	logLik	deviance	df.resid
rate ~ test + (1 | robinID)	-17.0769	-2.9353	13.5384	-27.0769	120
rate ~ c.AC.DC + test + trial + (1 | robinID) + c.AC.DC:test.no	-18.4295	9.8536	19.2148	-38.4295	115
rate ~ test + trial + (1 | robinID)	-16.4743	3.3239	15.2371	-30.4743	118
c.fam.colour + test + trial + (1 | robinID) + c.fam.colour:trial	-17.4046	10.8785	18.7023	-37.4046	115
rate ~ c.AC.DC + test + trial + (1 | robinID) + c.AC.DC:test + c.AC.DC:trial	-17.6162	16.3236	20.8081	-41.6162	113

Comparing foraging rate across trials for different foraging strategies (Figure 5.2), different patterns are visible in each test. In test A (Figure 5.2a), foraging rate is variable but neither increases nor decreases across tests for either foraging strategy. When fake baits are introduced in test B (Figure 5.2b), foraging rate remains stable across trials among DC birds but increases with trial number among AC birds. In test C (Figure 5.2c), DC birds again show stable foraging rates across trials while AC birds show a slight increase in trial 3 and greater variation in foraging rate throughout. These patterns together with the suboptimal models suggest that an interaction between foraging strategy and trial may exist – whereby DC individuals are initially faster foragers than AC individuals when unprofitable prey are present, but AC individuals rapidly learn to overcome this difference - but could not be effectively detected in the small sample size of this study.

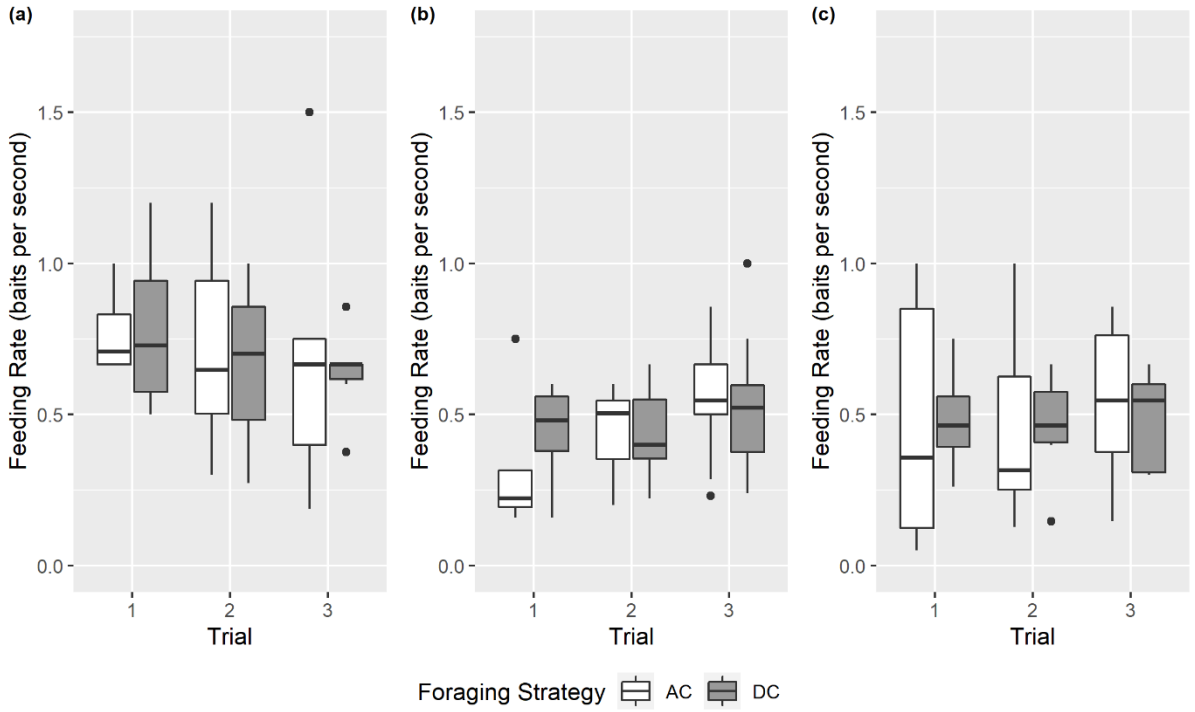

Figure 5.2. Feeding rate of AC and DC birds across trials in (a) test A, only real, familiar baits present (b), test B, real and fake, familiar-coloured baits present (c) test C real and fake baits of both familiar and novel colours present.

5.5.3 Number of Mistakes

Number of mistakes showed a distinctive pattern across test and trial number. Birds made fewer mistakes over time both across different tests and trials (Figure 5.3). This can probably be attributed to birds learning to identify the fake baits more accurately over time. Again, this was confirmed by the best fitting model, which retained both test and trial number as significant explanatory variables (Table 5.4). As with foraging rate, however, two of the models with the lowest AIC retained foraging strategy (Table 5.5), this time as a non-significant variable, and the model with the lowest AIC retained the interaction between foraging strategy and trial 3 as a marginally non-significant variable (p=0.0581).

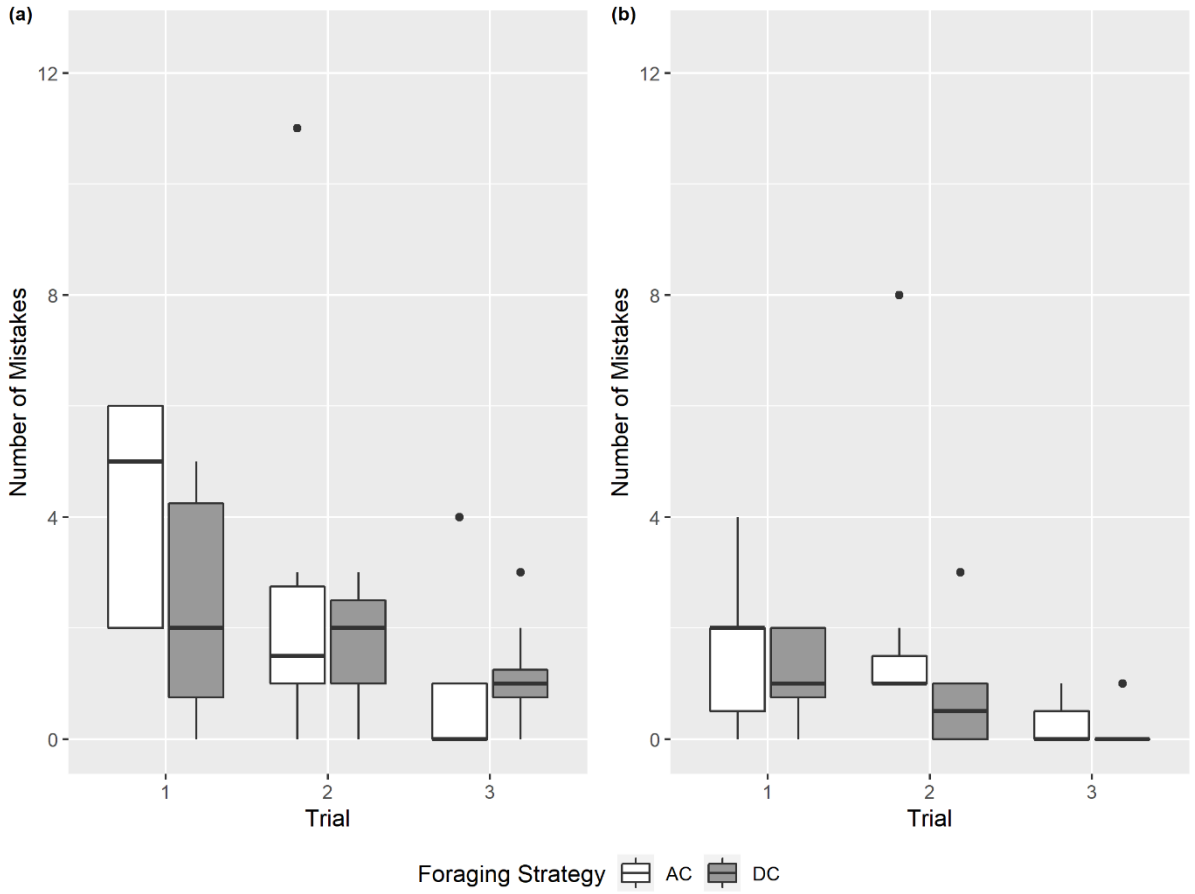

Figure 5.3. Number of mistakes (attempts to eat or investigate fake baits) by AC and DC birds over successive trials. Left, a) mistakes in test B with real and fake, familiar-coloured baits present. Right, b) mistakes in test C with real and fake, familiar-coloured, and novel-coloured baits.

Table 5.4. Fixed effect estimates for best fitting model relating number of mistakes to explanatory variables. (For full model output including random effect estimates see supplementary Table A3.2).

	Formula: mistakes6 ~ test + trial + (1 | robinID)			
	Estimate	Std. Error	t value	Pr(>|z|)
(Intercept)	1.6926	0.1318	12.839	< 2e-16 ***
testC	-0.4818	0.1146	-4.203	2.63e-05 ***
trial2	-0.1423	0.1405	-1.013	0.311
trial3	-0.8011	0.1384	-5.788	7.13e-09 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 5.5. Models with the lowest AIC values (within 2 AIC) returned from the global model of number of mistakes made. Note AC.DC is foraging strategy of individual birds.

Model Formula	AIC	BIC	logLik	deviance	df.resid
mistakes6 ~ c.AC.DC + c.test + trial + (1 | robinID) + c.AC.DC:test.no	273.9400	295.8173	-127.9700	255.9400	75
mistakes6 ~ c.test + trial + (1 | robinID)	275.3941	289.9790	-131.6971	263.3941	78
mistakes6 ~ c.AC.DC + c.test + trial + (1 | robinID)	275.7316	292.7474	-130.8658	261.7316	77

Examining the number of mistakes made over successive tests, a similar pattern of efficiency is seen as with foraging rate. When fake baits were initially introduced in test B (Figure 3a), DC individuals appeared to be slightly more efficient, making fewer mistakes than AC birds. AC birds quickly improved over trials 2 and 3, however, matching or beating DC birds in terms of mistakes made. In test C (Figure 3b), both AC and DC individuals continued to reduce their mistakes over successive trials, with DC birds appearing to make slightly fewer mistakes overall, however by this stage all birds were substantially better at distinguishing fake from real baits and generally made fewer than 2 mistakes in any trial.

5.6 Discussion

Compared to AC individuals that rapidly incorporate novel foods into their diet, DC individuals that refuse to do so appear to be severely underutilizing a valuable, potential resource (Marples *et al*. 1998). Why the DC foraging strategy evolved and how it coexists in populations with the AC foraging strategy is an unsolved problem in animal behaviour. The efficiency hypothesis – that DC individuals are more efficient foragers than AC individuals when familiar food is available – offers a possible explanation for the advantage of being DC (Preston and Marples, in prep a). This study is the first to test the hypothesis explicitly, by examining the foraging efficiency of wild birds in different foraging scenarios.

The results presented here do not offer conclusive support for the efficiency hypothesis. No relationship was found between foraging strategy and either foraging rate or mistakes made. Rather, the significant factors determining foraging efficiency were the nature of the foraging test and experience over successive trials. Foraging rate decreased in tests with unprofitable, fake baits, while the number of mistakes (fake baits examined) decreased over successive trials and tests. Neither result is surprising. The presence of fake baits introduces the possibility of mistakes which add to the total time spent foraging and thus reduce the number of (real) baits eaten per second. Additionally, with half of the baits replaced by fakes in the latter two foraging scenarios, the density of real baits decreased across test types, which might also be expected to increase foraging time. The decrease in number of mistakes over time can be attributed to birds' rapid learning to distinguish between the real and fake baits, leading to reduced sampling. This is also expected given their very different profitability (Sherratt, 2011), and birds' ability to attend to even very small differences in cues to distinguish profitable from unprofitable prey (Marples, 1993).

It is tempting, then, to conclude that foraging strategy and foraging efficiency are entirely independent of one another, and that foraging efficiency is merely a matter of learning, discrimination, and context (Hughes *et al*. 1992; Ishii and Shimada, 2010; Sherratt, 2011). However, while it was not found to be a significant variable by either of our models, certain results suggest that a relationship between foraging efficiency and foraging strategy may exist, but the effect did not reach statistical significance in this experiment. That foraging strategy (and its interactions with time) was retained as a variable in many of the models with

the lowest AIC is intriguing, however patterns in foraging rate and number of mistakes over time are also worth considering.

The largest difference in median foraging rate and number of mistakes made between AC and DC birds is seen following the introduction of fake baits (Figures 5.2b and 5.3a). This suggests that DC individuals may have been both faster foragers and less likely to make mistakes than AC individuals in this trial. However, high variation meant that even within this trial the difference was found to be not statistically significant. This difference is rapidly lost in subsequent tests as birds easily learnt to distinguish between real and fake baits, and consequently made fewer mistakes and showed less variation in the number of mistakes made. By the third trial in test B most birds made fewer than 2 mistakes and most birds had learnt to completely ignore the fake baits by the third trial in test C. If a difference existed in how likely AC and DC strategies were to make mistakes while foraging, the ease with which all birds could identify the fake baits could have masked the difference.

Handling time and predators' ability to learn how to efficiently manipulate different prey types are important determinants of foraging efficiency (Pyke, 1984; Persson 1985). Interestingly, while number of mistakes decreased over time, we detected no change in foraging rate over successive trials. This suggests that the handling cost (in time) of making mistakes was negligible for the birds and makes it unlikely a difference in foraging rate would have been detected even if a difference in accurate foraging is present.

Fundamentally, these are limitations of our ability to design tests that simulate with sufficient accuracy a natural foraging scenario. They do, however, offer valuable insights for the design of future studies to test the efficiency hypothesis of the DC foraging strategy. First, when testing birds' ability to find profitable, familiar prey, identifying or finding profitable prey must provide a substantial challenge to birds. If finding food is very easy, then foraging rates will be universally close to the maximum possible rate and errors close to the minimum, resulting in similar results for all individuals. Bounded at one end, metrics of foraging efficiency would also have little scope for variation. Thus, detecting a difference in foraging ability in a single instance would be extremely difficult. Detecting a small difference that persists over time would become all but impossible, as individuals learn and improve their foraging techniques and their foraging performance becomes even more perfect.

One way to create a genuinely challenging foraging test would be to test robins' ability to identify multiple fake designs and then use the most convincing design in a test on foraging efficiency. Another approach would be to change the background against which the familiar prey are presented to make them more or less cryptic. Again, this would require using sufficiently cryptic baits to reveal variation over time but could also be combined with the fake baits method to test multiple aspects of foraging (searching vs recognition of profitable food) to provide a more nuanced and robust test of potential differences among foraging strategies. Introducing a greater handling cost for baits, for instance by covering with non-food items (McMahon *et al*. 2014), may also reveal differences in foraging efficiency associated with individuals' ability to develop techniques to manipulate their prey.

In any case, a larger sample size may be necessary to detect a short-lived difference. Our sample size of 15 birds in this experiment was considered sufficient to detect small, persistent differences in efficiency over time, but overcoming the challenges posed by the experimental design in a single day effectively meant trying to detect a difference in one instance. A larger sample size would also address limitations mentioned earlier by allowing effective investigation of how birds' behaviour during tests (e.g., staying to eat many baits or leaving after only a few) might have impacted their overall efficiency in exploiting a single foraging patch.

While this study found no support for the hypothesis that DC individuals are more efficient foragers than AC individuals, it provides useful data and methodology for informing further tests of this hypothesis among birds. The results are also hint at where a difference in foraging efficiency may lie – in searching for profitable prey. Considering the challenges that foraging tests must overcome, it would be ill-advised to conclude that the results presented here effectively refute the hypothesis. Rather, they are tantalizingly suggestive of the merit of the efficiency hypothesis, but further work is needed to demonstrate what role foraging efficiency plays in the advantage of different foraging strategies that cope with novel foods.

Chapter 6

General Discussion

Despite their widespread prevalence, alternative foraging strategies that deal with novel foods remains an understudied topic of animal behaviour. There are many outstanding questions and a need to consolidate current knowledge to facilitate future research as discussed in the review (chapter 1). This thesis brings together published research and makes available for the first time evidence for the existence of alternative foraging strategies in two amphibian species and one mammal. Previously unpublished data from studies of AC and DC behaviour in several invertebrate species are also presented, including the first potential example of a DC individual in an invertebrate species.

Addressing some of the gaps identified in our current understanding of individual foraging strategies (chapter 1), this thesis explored aspects that determine foraging strategy expression. Proximate (chapters 2, 3, and 4) and ultimate (chapters 4 and 5) causes of AC and DC foraging strategies were investigated, and recommendations for a robust framework for future study of alternative foraging strategies were discussed throughout.

6.1 Cognitive mechanisms underlying foraging strategies

Understanding cognition is vital to understanding responses to novelty. Whether an animal considers something as familiar or novel is a matter of learning, memory, discrimination, and generalization (Greggor *et al*. 2015). AC and DC responses towards novelty must therefore involve some difference in cognitive function or how information about different foods is used. Despite this, studies of alternative foraging strategies have only explored the role of cognition to a limited degree. Marples *et al*. (2007) showed that domestic chicks (*Gallus gallus domesticus*) could learn that novelty itself is not aversive and generalize their responses appropriately, and that a negative experience was rapidly associated with novelty and reversed incorporation of that food. However, the foraging strategies of individual birds was not determined in these experiments. McMahon and Marples (2017) did assess the foraging strategies of individual blue tits (*Cyanistes caeruleus*) and showed that DC birds used the foraging behaviour of AC individuals to inform their own foraging decisions. Here, I investigated how familiarity and novelty are perceived by individuals with different foraging

strategies (chapters 2 and 3), aiming to discover what the underlying mechanisms are which underpin the differences between foraging strategies.

AC and DC individuals used experience with a food type in different ways (chapter 2). For DCs, a single exposure to a food elicited a sustained preference for that food, and preference was strengthened by greater experience. AC individuals, however, showed no sustained preference for a familiar food except when they had substantial prior experience with it. Even with substantial experience, ACs' preference for a food type was transient, lasting longer than would be expected from a purely neophobic response but waning more quickly than DCs' preference with less experience.

Differences in the familiarisation process could form a basis for how DC individuals form strong and lasting preferences. This has particular relevance for behaviour in the wild where extensive training on a specific prey type is unlikely. Limited exposure to prey of a particular type generates a strong preference in DC individuals and, if the prey is common enough to be encountered again, subsequent exposures ensure the preference is maintained over a long period.

In contrast, individuals of both foraging strategies were qualitatively similar in how they perceived and generalized across foods along a novelty gradient (chapter 3). AC and DC individuals agreed about where in the gradient foods became sufficiently novel to be treated differently from the familiar and from other baits in the gradient. However, AC and DC individuals did differ in their relative preference for their familiar food, with DCs showing a higher preference for their familiar food than ACs (and a lower preference for novel foods). Thus, provided novel foods are sufficiently different from familiar to be considered novel, how novel they are beyond that does not change the foraging strategy individuals express.

Taken together, these results suggest several conclusions:

- Familiarity is not all-or-nothing, but relative and dependent on experience with a food and the foraging strategy of the individual
- Novelty is perceived in a qualitatively similar way by both foraging strategies, and responses to novelty generalized similarly by both AC and DC individuals
- Different responses towards novel foods are driven primarily by DCs' relationship with their familiar food, not the degree of novelty of novel foods

As the affinity DC individuals have for their familiar food is related to experience, it is likely that the cognitive basis for the difference between foraging strategies involves some combination of learning, memory, and how information about the familiar is used. Explicit tests of these functions are a useful next step in determining why AC and DC individuals behave differently. In particular, further testing of search image use as an underlying mechanism (which has received some attention (e.g., Thomas *et al.* 2004, Marples and Mappes, 2011; and also chapter 5) is a promising line of investigation to better understand how alternative foraging strategies operate.

6.2 Plasticity of foraging strategy with hunger

Foraging strategies are genetically determined (Marples and Brakefield, 1995) but their expression can be remarkably plastic. Social circumstances have been shown to make DC individuals more AC (McMahon and Marples, 2017) while predation risk is suggested to make all individuals behave more DC (McMahon, 2014). However, until now no study has assessed whether hunger (or any internal driver of behaviour) alters expression of foraging strategies.

AC and DC individuals were observed to become more conservative in their foraging choices (i.e., show greater preference for familiar foods) when hungry (chapter 4). That hunger can make animals less likely to accept novel foods is a discovery with substantial practical implications. Hunger can make animals take greater risks (e.g., Godin and Crossman, 1994; Koivula *et al*. 1995; Killen *et al*. 2011), but when familiar foods are available or when animals have the opportunity to search for familiar food elsewhere - for instance, when releasing a population back into the wild - this finding suggests that depriving animals of food may make them less likely to effectively exploit or accept novel food resources.

It is possible that the effects of reducing hunger of novel food utilization are partially responsible for the success of “soft release” reintroduction programs. Reintroduced animals are faced with a novel environment and unfamiliar potential prey which they must learn to exploit. By providing supplementary food and shelter, soft release reintroductions typically result in greater survival, breeding, or weight gain of individuals compared to hard releases that received little or no supplementary resource provision (e.g., Bright and Morris, 1994; Mitchell *et al*. 2011; Liu *et al*. 2016; and see Tetzlaff *et al*. 2019 for a recent review and meta-

analysis). If soft releases provide animals with sufficient time and energy to explore new foods because of their maintenance diet, it would substantially affect their likelihood of incorporating novel foods into their diets in their new environments. To my knowledge, no comparative study of hard-released and soft-released animals has attempted to quantify the dietary breadth of released individuals, which would be an interesting test of resource use in new habitats following introduction.

Becoming more dietarily conservative with hunger also suggests that there may be temporal variation in foraging strategy expression in the wild. When food is generally scarce (e.g., winter) or nutritional demand high (e.g., in the breeding season), animals may become more DC in their behaviour at a population level. It might be worthwhile to test this idea directly, as if there are predictable patterns to when it pays to be novel, we can expect to see ecological and evolutionary trends that reflect this. Seasonal variation in predator understanding of aposematic signals changes the relative advantage of being conspicuous and has been proposed to explain temporal variation in prey signalling (Mappes *et al*. 2014). It is conceivable that hunger's effect on foraging strategy expression may lead to similar variation in selection pressures on prey populations.

6.3 Towards standard experimental design

Many of the experiments presented here were borne from a need to develop methodologies for testing foraging strategy expression. A particular concern was the comparability of results from different studies and a need to develop standard methodologies (chapter 1).

Results presented here identify two important variables that can influence the expression of foraging strategies:

- Duration of exposure to familiar foods in training (chapter 2)
- Hunger level of subjects (chapter 4)

The need to keep these elements constant has been largely overlooked in designing experiments and animals have been trained on or deprived of food to varying degrees in different studies e.g., 7 days exposure to familiar food and no deprivation in fish (Richards *et al*. 2014) compared to up to 4 weeks exposure and an hour of deprivation in blue tits

(McMahon and Marples, 2017). Often, this is a limitation of what can be achieved with a given design. For instance, in experiments here on wild birds (chapters 2 and 5), controlling for hunger was virtually impossible. Even ensuring similar experience with familiar food is problematic with wild animals as they may not appear consistently for training, and within training sessions may vary how much they eat. To mitigate this, a high minimum number of training sessions (7) was used when working with wild birds so that individuals that maintained a preference for familiar would do so for at least the length of the experiment. Nevertheless, some of the variance in proportions of DC individuals across different experiments is likely to be a consequence of differences in these design elements.

Consistency in training and hunger levels (where possible) should be added to a standard experimental design for testing foraging strategies (outlined in chapter 1). Fortunately, variation in novel foods used across experiments is unlikely to affect foraging strategy expression (chapter 3), provided of course the novel food is sufficiently novel and there are no other biases towards the stimuli involved.

6.4 Preference scores

Two methods to determine individuals' foraging strategies have been extensively used in studies on the behaviour: latency tests and evolution tests (chapter 1). However, a third method exists that has previously received little attention: preference scores. Originally developed to explore the gradual acceptance of novel foods in wild birds (Marples and Kelly, 1999; Kelly, 2001), preference scores assume that animals will tend to eat preferred foods before other foods. Individuals that maintain a higher preference for familiar over novel foods over an extended period are considered DC, while those with lower scores for familiar foods (and correspondingly higher scores for novel foods) are considered AC.

Here, preference scores were used almost exclusively to assess foraging strategies and to examine factors that alter novel food acceptance. This is the first time preference scores have been applied to fish (chapters 2 and 4), to large "prey" populations (greater than 8 baits, chapters 2, 3, 4) and to prey communities with more than two morphs (chapter 3). These experiments show that preference scores can be a robust way to identify an individual's foraging strategy. Individuals were remarkably consistent in their relative preference scores

for familiar and novel foods across multiple time points (chapters 2 and 3), i.e., individuals identified as DC at one time point behaved DC at other time points. In particular, preference scores at one time point successfully predicted consistent behaviour at future time points (chapter 3).

This preference score method of assessing foraging strategies combines many of the advantages of the latency and evolution tests. It is relatively quick to perform, can be applied to virtually any species and to test foraging in a variety of scenarios, and provides an unambiguous, numerical description of different stages of novel food incorporation over time. Undoubtedly, preference scores should be added to the standard repertoire of researchers studying foraging strategies. However, preference scores would benefit greatly from further development as a statistical tool. For instance, here relative difference in preference was assessed by comparing across individuals, but it could be useful to know how much a single animal's preference score must differ from "no preference" to suggest a significant preference for a particular food.

6.5 The why of DC

Perhaps the crucial, outstanding question of alternative foraging strategies is the evolutionary drivers of AC and DC traits. Being DC appears to be distinctly disadvantageous, so this question can be conveniently framed as "why does the DC foraging strategy exist?".

Two ideas have been proposed to explain the existence of DC individuals: the toxin avoidance and the efficiency hypotheses (chapter 1). Of these, neither has received much support and (effective) direct tests are lacking. Here, the first explicit test of efficiency hypothesis was conducted (chapter 5), but firm conclusions cannot be drawn from the results. AC and DC individuals appeared to have equal rates of prey capture, and neither was more likely to attack non-food items by mistake than the other. However, the ease with which all birds could distinguish profitable baits from the unprofitable, non-food fakes suggested a flaw in the experimental design. Effectively, the foraging task was too simple, and variation in foraging efficiency was too rapidly diminished across all birds once they learnt about the setup, resulting in universally high foraging efficiency.

Why the DC foraging strategy exists therefore remains an open question. It is possible that both avoiding unprofitable prey and enhanced foraging efficiency by specializing on the familiar both play a role in allowing DC individuals to compete with AC neighbours. Interestingly, that animals preferred familiar food more when hungry (chapter 4) suggests some support for the avoidance hypothesis. When physiologically stressed, the cost of consuming defended or otherwise unprofitable prey is likely to be higher, in which case it makes sense to stick with your usual diet and not consume something new.

Ultimately, there is a need for further, explicit tests of both hypotheses. Despite the inconclusive results presented here, the observation that the difference in foraging efficiency between foraging strategies was greatest immediately after introducing a more complicated foraging scenario is suggestive of an effect. The efficiency hypothesis is therefore worthy of further study, and lessons learnt here will be useful in designing an effective test to provide a conclusive answer.

6.6 General application of this research

The experiments presented here (chapters 2-5) were conducted on two model species from very different taxa: robins (Aves) and three-spined sticklebacks (Actinopterygii). However, AC and DC foraging strategies appear to be universal among vertebrates (chapter 1). As such, it is reasonable to assume that the conclusions regarding foraging strategies drawn here are likely to be relevant to a wide range of species. Nevertheless, it would be prudent for future research to perform similar tests using species of different taxa and/or ecological niches to confirm the general validity of these results.

Alternative foraging strategies that deal with novelty are the focus of this thesis, and most of the conclusions drawn relate to understanding AC and DC behaviours and how to test them. However, many of the techniques used can find application wherever it is necessary to train animals on a particular food or measure responses to a novel one. For instance, when introducing a new feed to livestock, maximizing the acceptance of the new food may be aided by mixing with the old feed (to reduce the relative novelty of the mixture – chapter 3), allowing animals time to acquire familiarity with the new composition (chapter 2), and introducing the new feed at or immediately after established feeding times to minimize hunger levels (chapter 4).

AC and DC foraging strategies remain largely unrecognized in the field of dietary ecology. Consequently, their true impact on individuals and ecosystems remains unknown. Because they are so widespread and have dramatically different consequences for behaviour, understanding individuals' foraging strategies in response to novel food has profound potential to impact our model of how animals interact with their prey. As a final goal, this thesis aims to inspire researchers to incorporate foraging strategies into their interests by highlighting their relevance to many areas of behaviour (from cognition and generalization to physiological state and optimal foraging) and make accessible experimental designs that can be applied to virtually any species.

7. Bibliography

Balogh, A. C. V., Gamberale-Stille, G., Tullberg, B. S., & Leimar, O. (2010). Feature theory and the two-step hypothesis of Müllerian mimicry evolution. *Evolution*, *64*(3), 810-822.

Barnett, C. A., Bateson, M., & Rowe, C. (2007). State-dependent decision making: educated predators strategically trade off the costs and benefits of consuming aposematic prey. *Behavioral Ecology*, *18*(4), 645-651.

Barnett, C. A., Bateson, M., & Rowe, C. (2014). Better the devil you know: avian predators find variation in prey toxicity aversive. *Biology Letters*, *10*(11), 20140533.

Barnett, S. A. (1958). Experiments on 'neophobia' in wild and laboratory rats. *British journal of psychology*, *49*(3), 195-201.

Bartoń K (2022). _MuMIn: Multi-Model Inference_. R package version 1.47.1, <https://CRAN.R-project.org/package=MuMIn>

Blough, P. M. (1972). Wavelength generalization and discrimination in the pigeon. *Perception & Psychophysics*, *12*(4), 342-348.

Bridges, C. M. (2002). Tadpoles balance foraging and predator avoidance: effects of predation, pond drying, and hunger. *Journal of Herpetology*, 627-634.

Bright, P. W., & Morris, P. A. (1994). Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. *Journal of Applied Ecology*, 699-708.

Brodie III, E. D., & Janzen, F. J. (1995). Experimental studies of coral snake mimicry: generalized avoidance of ringed snake patterns by free-ranging avian predators. *Functional Ecology*, 186-190.

Brooker, R. W., Travis, J. M., Clark, E. J., & Dytham, C. (2007). Modelling species' range shifts in a changing climate: the impacts of biotic interactions, dispersal distance and the rate of climate change. *Journal of theoretical biology*, *245*(1), 59-65.

Brown, G. E., Demers, E. E., Goldman, J. A., Singh, A., Chivers, D. P., & Ferrari, M. C. (2020). Unpredictable risk enhances induced neophobia in northern red-bellied dace. *Animal Behaviour*, *168*, 121-127.

Brown, M. J., & Jones, D. N. (2016). Cautious crows: Neophobia in Torresian crows (Corvus orru) compared with three other corvoids in suburban Australia. *Ethology*, *122*(9), 726-733.

Burrows, M. T., Schoeman, D. S., Richardson, A. J., Molinos, J. G., Hoffmann, A., Buckley, L. B., ... & Poloczanska, E. S. (2014). Geographical limits to species-range shifts are suggested by climate velocity. *Nature*, *507*(7493), 492-495.

Camín, S. R., Martín-Albarracín, V., Jefferies, M., & Marone, L. (2016). Do neophobia and dietary wariness explain ecological flexibility? An analysis with two seed-eating birds of contrasting habits. *Journal of Avian Biology*, *47*(2), 245-251.

Carle, T., & Rowe, C. (2014). Avian predators change their foraging strategy on defended prey when undefended prey are hard to find. *Animal Behaviour*, *93*, 97-103.

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical population biology*, *9*(2), 129-136.

Coleman, S. L., & Mellgren, R. L. (1994). Neophobia when feeding alone or in flocks in zebra finches, Taeniopygia guttata. *Animal Behaviour*, *48*(4), 903-907.

Crane, A. L., & Ferrari, M. C. (2017). Patterns of predator neophobia: a meta-analytic review. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1861), 20170583.

Crane, A. L., Brown, G. E., Chivers, D. P., & Ferrari, M. C. (2020). An ecological framework of neophobia: from cells to organisms to populations. *Biological Reviews*, *95*(1), 218-231.

Dawkins, M. (1971). Perceptual changes in chicks: another look at the 'search image'concept. *Animal Behaviour*, *19*(3), 566-574.

Domjan, M. (1975). Poison-induced neophobia in rats: Role of stimulus generalization of conditioned taste aversions. *Animal Learning & Behavior*, *3*(3), 205-211.

Donner, K. O. (1951). The visual acuity of some passerine birds.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting LinearMixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01

Dubois, L., Diasparra, M., Bédard, B., Kaprio, J., Fontaine-Bisson, B., Tremblay, R., ... & Pérusse, D. (2013). Genetic and environmental influences on eating behaviors in 2.5-and 9-year-old children: a longitudinal twin study. *International Journal of Behavioral Nutrition and Physical Activity*, *10*(1), 1-12.

Fildes, A., van Jaarsveld, C. H., Cooke, L., Wardle, J., & Llewellyn, C. H. (2016). Common genetic architecture underlying young children's food fussiness and liking for vegetables and fruit. *The American journal of clinical nutrition*, *103*(4), 1099-1104.

Freire, R., Rogers, L., Creece, D., & Shamsi, S. (2022). Neophobic behavioural responses of parasitised fish to a potential predator and baited hook. *Applied Animal Behaviour Science*, *254*, 105722.

García, L. F., Viera, C., & Pekár, S. (2018). Comparison of the capture efficiency, prey processing, and nutrient extraction in a generalist and a specialist spider predator. *The Science of Nature*, *105*(3), 1-10.

Ghirlanda, S., & Enquist, M. (2003). A century of generalization. *Animal Behaviour*, *66*(1), 15-36.

Gibb, J. A. (1962). L. Tinbergen's hypothesis of the role of specific search images. *Ibis*, *104*(1), 106-111.

Godin, J. G. J., & Crossman, S. L. (1994). Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (Gasterosteus aculeatus) under predation risk. *Behavioral Ecology and Sociobiology*, *34*, 359-366.

Gotceitas, V., & Godin, J. G. J. (1991). Foraging under the risk of predation in juvenile Atlantic salmon (Salmo salar L.): effects of social status and hunger. *Behavioral Ecology and Sociobiology*, *29*(4), 255-261.

Greenberg, R. S. (2003). The role of neophobia and neophilia in the development of innovative behaviour of birds. *Animal innovation*.

Greenberg, R., & Mettke-Hofmann, C. (2001). Ecological aspects of neophobia and neophilia in birds. In *Current ornithology* (pp. 119-178). Springer, Boston, MA.

Greggor, A. L., Thornton, A., & Clayton, N. S. (2015). Neophobia is not only avoidance: improving neophobia tests by combining cognition and ecology. *Current Opinion in Behavioral Sciences*, *6*, 82-89.

Guilford, T. (1990). Evolutionary pathways to aposematism. *Acta Oecologica-International Journal of Ecology*, *11*(6).

Halpin, C. G., Skelhorn, J., & Rowe, C. (2014). Increased predation of nutrient-enriched aposematic prey. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1781), 20133255.

Ham, A. D., & Osorio, D. (2007). Colour preferences and colour vision in poultry chicks. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1621), 1941-1948.

Hanski, I., Hansson, L., & Henttonen, H. (1991). Specialist predators, generalist predators, and the microtine rodent cycle. *The Journal of Animal Ecology*, 353-367.

Hanson, H. M. (1959). Effects of discrimination training on stimulus generalization. *Journal of experimental psychology*, *58*(5), 321.

Harris, C. E., & Knowlton, F. F. (2001). Differential responses of coyotes to novel stimuli in familiar and unfamiliar settings. *Canadian Journal of Zoology*, *79*(11), 2005-2013.

Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Progress in retinal and eye research*, *20*(5), 675-703.

Hill, R. A., & Dunbar, R. I. M. (2002). Climatic determinants of diet and foraging behaviour in baboons. *Evolutionary Ecology*, *16*(6), 579-593.

Hughes, R. N., Kaiser, M. J., Mackney, P. A., & Warburton, K. (1992). Optimizing foraging behaviour through learning. *Journal of Fish Biology*, *41*, 77-91.

Ishii, Y., & Shimada, M. (2010). The effect of learning and search images on predator–prey interactions. *Population ecology*, *52*, 27-35.

Johnston, A. N., Burne, T. H., & Rose, S. P. (1998). Observation learning in day-old chicks using a one-trial passive avoidance learning paradigm. *Animal Behaviour*, *56*(6), 1347-1353.

Kacelnik, A. (1984). Central place foraging in starlings (Sturnus vulgaris). I. Patch residence time. *The Journal of Animal Ecology*, 283-299.

Kelly, D. J. (2001). *Dietary conservatism in passerines: the influence of novel odour and novel colour* (Doctoral dissertation, Trinity College (Dublin, Ireland). Department of Zoology).

Kelly, D. J., & Marples, N. M. (2004). The effects of novel odour and colour cues on food acceptance by the zebra finch, Taeniopygia guttata. *Animal Behaviour*, *68*(5), 1049-1054.

Killen, S. S., Marras, S., & McKenzie, D. J. (2011). Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. *Journal of Animal Ecology*, *80*(5), 1024-1033.

Koivula, K., Rytkonen, S., & Orell, M. (1995). WILLOW TITS. *Ardea*, *83*, 397-404.

Krebs, J. R., Stephens, D. W., & Sutherland, W. J. (1983). *Perspectives in optimal foraging* (pp. 165-216). Cambridge: Cambridge University Press.

Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015). DNA barcodes for ecology, evolution, and conservation. *Trends in ecology & evolution*, *30*(1), 25-35.

Launchbaugh, K. L., Provenza, F. D., & Werkmeister, M. J. (1997). Overcoming food neophobia in domestic ruminants through addition of a familiar flavor and repeated exposure to novel foods. *Applied Animal Behaviour Science*, *54*(4), 327-334.

Lendvai, A. Z., Barta, Z., Liker, A., & Bókony, V. (2004). The effect of energy reserves on social foraging: hungry sparrows scrounge more. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1556), 2467-2472.

Lenth R (2023). _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.8.4-1, <https://CRAN.R-project.org/package=emmeans>

Liu, B., Li, L., Lloyd, H., Xia, C., Zhang, Y., & Zheng, G. (2016). Comparing post-release survival and habitat use by captive-bred Cabot's Tragopan (Tragopan caboti) in an experimental test of soft-release reintroduction strategies. *Avian Research*, *7*(1), 1-9.

Maes, E., De Filippo, G., Inkster, A. B., Lea, S. E., De Houwer, J., D'Hooge, R., ... & Wills, A. J. (2015). Feature-versus rule-based generalization in rats, pigeons and humans. *Animal Cognition*, *18*, 1267-1284.

Manatunge, J., & Asaeda, T. (1998). Optimal foraging as the criteria of prey selection by two centrarchid fishes. *Hydrobiologia*, *391*(1), 221-238.

Mappes, J., & Alatalo, R. V. (1997). Effects of novelty and gregariousness in survival of aposematic prey. *Behavioral Ecology*, *8*(2), 174-177.

Mappes, J., Kokko, H., Ojala, K., & Lindström, L. (2014). Seasonal changes in predator community switch the direction of selection for prey defences. *Nature communications*, *5*(1), 5016.

Mappes, J., Marples, N., & Endler, J. A. (2005). The complex business of survival by aposematism. *Trends in ecology & evolution*, *20*(11), 598-603.

Marples, N. M. (1993). Do wild birds use size to distinguish palatable and unpalatable prey types?. *Animal Behaviour*, *46*(2), 347-354.

Marples, N. M., & Brakefield, P. M. (1995). Genetic variation for the rate of recruitment of novel insect prey into the diet of a bird. *Biological Journal of the Linnean Society*, *55*(1), 17-27.

Marples, N. M., & Kelly, D. J. (1999). Neophobia and dietary conservatism: two distinct processes?. *Evolutionary Ecology*, *13*(7), 641-653.

Marples, N. M., & Mappes, J. (2011). Can the dietary conservatism of predators compensate for positive frequency dependent selection against rare, conspicuous prey?. *Evolutionary Ecology*, *25*(4), 737-749.

Marples, N. M., Kelly, D. J., & Thomas, R. J. (2005). Perspective: the evolution of warning coloration is not paradoxical. *Evolution*, *59*(5), 933-940.

Marples, N. M., Quinlan, M., Thomas, R. J., & Kelly, D. J. (2007). Deactivation of dietary wariness through experience of novel food. Behavioral Ecology, 18(5), 803-810.

Marples, N. M., Roper, T. J., & Harper, D. G. (1998). Responses of wild birds to novel prey: evidence of dietary conservatism. *Oikos*, 161-165.

Marples, N. M., Speed, M. P., & Thomas, R. J. (2018). An individual-based profitability spectrum for understanding interactions between predators and their prey. *Biological Journal of the Linnean Society*, *125*(1), 1-13.

McMahon, K. (2014). *Plasticity of foraging strategies in the blue tit, Cyanistes caeruleus and the domestic chick, Gallus gallus domesticus* (Doctoral dissertation, Trinity College (Dublin, Ireland). Department of Zoology).

McMahon, K., & Marples, N. (2017). Reduced dietary conservatism in a wild bird in the presence of intraspecific competition. *Journal of Avian Biology*, *48*(3), 448-454.

McMahon, K., Conboy, A., O'Byrne-White, E., Thomas, R. J., & Marples, N. M. (2014). Dietary wariness influences the response of foraging birds to competitors. *Animal Behaviour*, *89*, 63-69.

Michálek, O., Petráková, L., & Pekár, S. (2017). Capture efficiency and trophic adaptations of a specialist and generalist predator: a comparison. *Ecology and Evolution*, *7*(8), 2756-2766.

Milinski, M., & Heller, R. (1978). Influence of a predator on the optimal foraging behaviour of sticklebacks (Gasterosteus aculeatus L.). *Nature*, *275*(5681), 642-644.

Mitchell, A. M., Wellicome, T. I., Brodie, D., & Cheng, K. M. (2011). Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. *Biological conservation*, *144*(5), 1382-1391.

Modlinska, K., & Stryjek, R. (2016). Food neophobia in wild rats (Rattus norvegicus) inhabiting a changeable environment—a field study. *PloS one*, *11*(6), e0156741.

Modlinska, K., Stryjek, R., & Pisula, W. (2015). Food neophobia in wild and laboratory rats (multi-strain comparison). *Behavioural processes*, *113*, 41-50.

Morelli, F., Benedetti, Y., Møller, A. P., & Fuller, R. A. (2019). Measuring avian specialization. *Ecology and Evolution*, *9*(14), 8378-8386.

Moretti, L., Hentrup, M., Kotrschal, K., & Range, F. (2015). The influence of relationships on neophobia and exploration in wolves and dogs. *Animal Behaviour*, *107*, 159-173.

Parthasarathy, B., Müller, M., Bilde, T., & Schneider, J. M. (2022). Hunger state and not personality determines task participation in a spider society. *Animal Behaviour*, *190*, 143-152.

Pavlov, I. P. (1927). Conditioned reflexes (translated by GV Anrep). *London: Oxford*.

Perone, P., Çınar, Ç., D'ursi, P., Durmuşoğlu, L. R., Lal, V., & Tybur, J. (2021). Examining the effect of hunger on responses to pathogen cues and novel foods. *Evolution and Human Behavior*, *42*(4), 371-378.

Persson, L. (1985). Optimal foraging: the difficulty of exploiting different feeding strategies simultaneously. *Oecologia*, *67*(3), 338-341.

Pierrel, R. (1958). A generalization gradient for auditory intensity in the rat. *Journal of the Experimental Analysis of Behavior*, *1*(4), 303.

Pliner, P., Eng, A., & Krishnan, K. (1995). The effects of fear and hunger on food neophobia in humans. *Appetite*.

Pyke, G. H. (1984). Optimal foraging theory: a critical review. *Annual review of ecology and systematics*, *15*(1), 523-575.

Quesenberry, N. J., Allen, P. J., & Cech Jr, J. J. (2007). The influence of turbidity on three-spined stickleback foraging. *Journal of Fish Biology*, *70*(3), 965-972.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Raboy, B. E., & Dietz, J. M. (2004). Diet, foraging, and use of space in wild golden-headed lion tamarins. *American Journal of Primatology: Official Journal of the American Society of Primatologists*, *63*(1), 1-15.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological reviews*, *82*(2), 291-318.

Reebs, S. G., & Saulnier, N. (1997). The effect of hunger on shoal choice in golden shiners (Pisces: Cyprinidae, Notemigonus crysoleucas). *Ethology*, *103*(8), 642-652.

Richards, E. L., Alexander, L. G., Snellgrove, D., Thomas, R. J., Marples, N. M., & Cable, J. (2014). Variation in the expression of dietary conservatism within and between fish species. *Animal Behaviour*, *88*, 49-56.

Rönkä, K., De Pasqual, C., Mappes, J., Gordon, S., & Rojas, B. (2018). Colour alone matters: no predator generalization among morphs of an aposematic moth. *Animal Behaviour*, *135*, 153-163.

Rowe, C., Lindström, L., & Lyytinen, A. (2004). The importance of pattern similarity between Müllerian mimics in predator avoidance learning. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1537), 407-413.

Schuler, W., & Roper, T. J. (1992). Responses to warning coloration in avian predators. *Adv Stud Behav*, *21*, 111-146.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., ... & Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature communications*, *8*(1), 1-9.

Sherratt, T. N. (2003). State-dependent risk-taking by predators in systems with defended prey. *Oikos*, *103*(1), 93-100.

Sherratt, T. N. (2008). The evolution of Müllerian mimicry. *Naturwissenschaften*, *95*(8), 681-695.

Sherratt, T. N. (2011). The optimal sampling strategy for unfamiliar prey. *Evolution: International Journal of Organic Evolution*, *65*(7), 2014-2025.

Shine, R., & Sun, L. X. (2003). Attack strategy of an ambush predator: which attributes of the prey trigger a pit-viper's strike?. *Functional Ecology*, *17*(3), 340-348.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: an integrative overview. *The quarterly review of biology*, *79*(3), 241-277.

Skelhorn, J. (2011). Colour biases are a question of conspecifics' taste. *Animal Behaviour*, *81*(4), 825-829.

Skelhorn, J., & Rowe, C. (2007). Predators' toxin burdens influence their strategic decisions to eat toxic prey. *Current Biology*, *17*(17), 1479-1483.

Skelhorn, J., Griksaitis, D., & Rowe, C. (2008). Colour biases are more than a question of taste. *Animal Behaviour*, *75*(3), 827-835.

Skelhorn, J., Halpin, C. G., & Rowe, C. (2016). Learning about aposematic prey. *Behavioral Ecology*, *27*(4), 955-964.

Spence, R., & Smith, C. (2008). Innate and learned colour preference in the zebrafish, Danio rerio. *Ethology*, *114*(6), 582-588.

Svádová, K., Exnerová, A., Štys, P., Landová, E., Valenta, J., Fučíková, A., & Socha, R. (2009). Role of different colours of aposematic insects in learning, memory and generalization of naive bird predators. *Animal Behaviour*, *77*(2), 327-336.

Takola, E., Krause, E. T., Müller, C., & Schielzeth, H. (2021). Novelty at second glance: a critical appraisal of the novel object paradigm based on meta-analysis. *Animal Behaviour*, *180*, 123-142.

Terrace, H. S. (1964). Wavelength generalization after discrimination learning with and without errors. *Science*, *144*(3614), 78-80.

Terraube, J., Arroyo, B., Madders, M., & Mougeot, F. (2011). Diet specialisation and foraging efficiency under fluctuating vole abundance: a comparison between generalist and specialist avian predators. *Oikos*, *120*(2), 234-244.

Tetzlaff, S. J., Sperry, J. H., & DeGregorio, B. A. (2019). Effects of antipredator training, environmental enrichment, and soft release on wildlife translocations: a review and meta-analysis. *Biological Conservation*, *236*, 324-331.

Thomas, R. J., Bartlett, L. A., Marples, N. M., Kelly, D. J., & Cuthill, I. C. (2004). Prey selection by wild birds can allow novel and conspicuous colour morphs to spread in prey populations. *Oikos*, *106*(2), 285-294.

Thomas, R. J., King, T. A., Forshaw, H. E., Marples, N. M., Speed, M. P., & Cable, J. (2010). The response of fish to novel prey: evidence that dietary conservatism is not restricted to birds. *Behavioral Ecology*, *21*(4), 669-675.

Thomas, R. J., Marples, N. M., Cuthill, I. C., Takahashi, M., & Gibson, E. A. (2003). Dietary conservatism may facilitate the initial evolution of aposematism. *Oikos*, *101*(3), 458-466.

Tinbergen, L. (1960). The natural control of insects in pinewoods. I. Factors influencing the intensity of predation by songbirds. Archives Neerlandaises de Zoologie 13: 265–336. *Ardea*, *68*, 11-30.

Toscano, B. J., Gownaris, N. J., Heerhartz, S. M., & Monaco, C. J. (2016). Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. *Oecologia*, *182*(1), 55-69.

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox–a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution*, *6*(11), 1320-1331.

Turner, J. R., & Speed, M. P. (1999). How weird can mimicry get?. *Evolutionary Ecology*, *13*(7), 807-827.

van den Berg, C. P., Troscianko, J., Endler, J. A., Marshall, N. J., & Cheney, K. L. (2020). Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods in Ecology and Evolution*, *11*(2), 316-332.

Ward, A. J., Thomas, P., Hart, P. J., & Krause, J. (2004). Correlates of boldness in three-spined sticklebacks (Gasterosteus aculeatus). *Behavioral Ecology and Sociobiology*, *55*(6), 561-568.

White, K. P., Al-Shawaf, L., Lewis, D. M., & Wehbe, Y. S. (2023). Food neophobia and disgust, but not hunger, predict willingness to eat insect protein. *Personality and Individual Differences*, *202*, 111944.

Wolf, M., & Weissing, F. J. (2012). Animal personalities: consequences for ecology and evolution. *Trends in ecology & evolution*, *27*(8), 452-461.

Zaman, J., Chalkia, A., Zenses, A. K., Bilgin, A. S., Beckers, T., Vervliet, B., & Boddez, Y. (2021). Perceptual variability: Implications for learning and generalization. *Psychonomic Bulletin & Review*, *28*, 1-19.

Zaman, J., Yu, K., & Lee, J. C. (2022). Individual differences in stimulus identification, rule induction, and generalization of learning. *Journal of Experimental Psychology: Learning, Memory, and Cognition*.

Zimmermann, A., Stauffacher, M., Langhans, W., & Würbel, H. (2001). Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural brain research*, *121*(1-2), 11-20.

Appendix 1. Supplementary material for Chapter 1 – Novel Foods and the Foraging Strategies that Deal with Them

A1.1 Dietary Conservatism in Other Species

Here we present data from experiments investigating the existence of dietary conservatism in a range of species that have not been included in the published literature. These are arranged, below, into those experiments investigating dietary conservatism in vertebrates and those investigating the behaviour in invertebrates.

A1.2 Dietary Conservatism in Vertebrates

Four pilot experiments have explored the existence of the DC foraging strategy for the first time in three vertebrate species: smooth newts, common frogs, and laboratory mice. All experiments found support for the existence of dietary conservatism in their respective species, broadening the range of vertebrate taxa displaying the behaviour and further supporting dietary conservatism's ubiquity across the vertebrate clade.

A1.2.1 Dietary Conservatism in Smooth Newts.

Two experiments have investigated the existence of dietary conservatism in smooth newts (*Lissotritus vulgaris*). These experiments applied different methodologies, both of which support the existence of the AC and DC foraging strategy in this species.

Experiment 1

Five smooth newts were captured from suburban ponds in Dublin, Ireland in 2008. These were transferred to the laboratory where they were trained to accept either red- (three newts) or green- (two newts) dyed *Daphnia magna* as their familiar food. Training lasted for 10 days, with newts receiving 8 familiar-coloured *Daphnia* each day. Following training, newts were tested for dietary conservatism using an order-based latency test. Testing was carried out daily for 8 days. Newts were presented with four *Daphnia*, two red and two green, and allowed to forage. Thus, each newt was given 2 of its familiar colour and 2 of their novel colour. The order in which *Daphnia* were eaten was used to calculate a

preference score for each food type in each trial (see Box A1, below), with lower scores indicating a stronger preference for that food type.

Box A1.1. Calculating Preference Scores

Preference scores use the order in which prey are eaten to determine an individual's preference for one prey type over others. Preference scores are calculated by summing the ordinal position of each prey item eaten and dividing by the number of prey of that type available. E.g., If an animal is presented with 5 red and 5 green prey and consumes all prey in the following order: three red, one green, two red, four green, then its preference score for red prey can be calculated by

1+2+3+5+6/5 = 3.4

If an individual fails to consume all available prey any uneaten prey are assigned the mean of the uneaten ordinal positions. E.g., If 5 red, and 5 green prey are presented and the individual consumes in the following order: three red, one green, one red, three green and fails to eat one green and one red, the preference score for red is calculated by:

1+2+3+5+((9+10)/2)/5 = 4.1

Lower preference scores indicate a stronger preference for that food type.

Calculating preference scores assumes that preferred foods will be eaten before others, that individuals have a free and unbiased choice in their prey selection, and that all prey types are equally abundant. Where not all available prey are consumed, the preference score assumes that all remaining prey are equally likely to be consumed next.

Across all newts and all 8 testing days, preference scores for familiar coloured prey were lower than those for novel prey (median preference scores 3 and 7, respectively) and this difference was statistically significant (Wilcoxon signed-rank test, V = 64, p = 3.217e-05). Within individuals, significantly different novel and familiar preference scores were used to

infer foraging a DC foraging strategy. Three individuals had significantly lower familiar preference scores than novel preference scores (Wilcoxon signed-rank tests, all $p<0.05$; see table A1 below) and were identified as DC. The remaining two newts were identified as AC.

Table A1.1. Data for individual newts from Experiment 1. Familiar and Novel Preference Scores are the summed preference scores over 8 trials. V and p-values are for Wilcoxon signed-rank tests comparing difference in familiar and novel preference scores for each newt.

Newt ID	**Familiar Colour**	**Familiar Preference Score**	**Novel Preference Score**	**V score (Wilcoxon signed-rank)**	**p-value (Wilcoxon signed-rank)**
1	Red	30	50	2.5	0.03125
2	Red	27	53	0	0.01471
3	Red	40	40	5	1
4	Green	29	51	1	0.0239
5	Green	31	49	6	0.09694

The presence of AC and DC individuals in this small population suggests the existence of dietary conservatism in smooth newts. Individuals' preferences cannot be attributed to neophobia as preferences were maintained over 8 days of exposures and, more importantly, despite the newts eating the novel food each day (i.e., neophobia must have been overcome). That a slight majority of individuals (3 out of 5) were found to be DC is unusual. However, the small sample size in this experiment limits our ability to draw meaningful conclusions about the proportion of ACs and DCs among newts.

Experiment 2

Fifteen smooth newts were captured from suburban ponds in Dublin in 2009 and were transferred to the lab. Newts were trained to eat one of two colour morphs of their experimental food, black and white laboratory-bred strains of *Drosophila sp.* Training and testing were colour balanced, with one morph acting as the "familiar" food and the other as the "novel" food in each case (7 newts familiar with black *Drosophila*, 8 newts familiar with white *Drosophila*). Newts were trained with their familiar colour morph for 10 days, receiving 10 appropriately coloured prey each day. Following training, evolution tests (see

Box 1) were carried out with initial ratios of familiar to novel food of 9:1. Each testing session lasted a maximum of 90 minutes, and evolution tests concluded when one morph reached fixation in the population.

Of the 15 newts tested, 7 drove the novel prey to fixation in their prey populations and were identified as DC. The remaining 8 were identified as AC. Fixation of novel prey occurred within 1-8 days of testing (mean = 4.286 days; sd = 2.870 days). More black novel prey were sent to fixation than white novel prey (5 to 2, Table A2) but this difference was not significant (Fisher's exact test, $p = 0.1319$).

Table A1.2. Number of novel and familiar fixations during evolution testing among newts by familiar colour.

Familiar Colour	Number of Novel Fixations	Number of Familiar Fixations
Black	2	6
White	5	2

As 7 of the 15 newts were identified as DC by an evolution test (and the remainder identified as AC), this experiment provides robust evidence for the existence of dietary conservatism in this species. That neither prey colour was more likely to be sent to fixation suggests this preference is independent of prey characteristics.

The proportion of DC newts in this experiment was similar to that in Experiment 1 (close to 50%, although in this experiment AC individuals are in the majority). The two experiments suggest a higher proportion of DC individuals in newts than in other species (Table 2, main text), however further tests with larger sample sizes are needed to confirm the ratio of foraging strategies. Nevertheless, both experiments found substantial evidence for the existence of dietary conservatism in smooth newts.

A1.2.2. Dietary Conservatism in Common Frogs

One experiment has investigated the existence of dietary conservatism in the common frog (*Rana temporaria*). This experiment applied the evolution method of testing and found evidence for the existence of both AC and DC individuals in this species.

Twenty common frogs were collected from a suburban garden pond in Dublin in 2009. After transfer to laboratory housing, frogs were trained for 10 days on black and white laboratory-bred strains of *Drosophila* by presenting them with 10-15 flies per day and allowing the frogs to eat. Half of the frogs were trained on black and half on white *Drosophila* strains, as their familiar-coloured food.

Following training, an evolution test (see box 1, main text) with an initial familiar to novel ratio of 9:1 was used to test for dietary conservatism. Frogs were presented with 10 appropriately coloured *Drosophila* and allowed to forage for up to 90 minutes. Testing

continued until the novel morph reached fixation or extinction in the population, or for a maximum of 10 days in most cases. After 10 days of testing, two individuals had failed to drive either morph to fixation but appeared to be close to doing so and were given one additional day of testing.

Of the 20 frogs in this experiment, three sent the novel prey to fixation during evolution testing and were identified as DC, while and 15 sent the familiar prey to fixation and were identified as AC. Two individuals failed to send the novel prey to either fixation or extinction and were not identified as AC or DC. Of the two individuals given an additional day of testing, one failed to send the novel morph to fixation or extinction while the other sent it to fixation. Fixation of the novel prey only occurred among individuals familiar with black *Drosophila* (i.e., novel, white prey), but a Fisher's exact test failed to find a significant difference in the likelihood of novel fixation across groups ($p = 0.0867$). However, both individuals that failed to send the novel morph to either fixation or extinction were also familiar with black *Drosophila*; including these suggests that the likelihood of maintaining the novel morph in the population at any level is significantly different across colour groups, occurring more often when the white prey morph is novel (Fisher's exact test, $p = 0.0325$).

That three individuals were identified as DC and 15 identified as AC in this experiment supports the existence of dietary conservatism in *R. temporaria*. Additionally, two frogs could not be identified as AC or DC in this experiment. This may reflect an intermediate strength of DC expression by these individuals.

It is worth noting that the colour of the novel or familiar prey may have influenced prey choice, as fixation of novel prey only occurred among individuals familiar with black *Drosophila*. Curiously, this colour effect appears to affect prey choice in the opposite way to newts, above, where white *Drosphila* were sent to extinction slightly more often. The proportion of DC individuals found in this experiment (3/20) is similar to that seen in several studies of other species (Table 2, main text). However, colour bias in prey selection can affect expression of dietary conservatism. Consequently, the proportion of DC individuals detected in this experiment may not reflect their actual frequency in frog populations. Further exploration of dietary conservatism among frogs should take additional precautions to disentangle the effects of colour on dietary decision-making.

A1.2.3. Dietary Conservatism in Mice

Forty laboratory mice (*Mus musculus*) of strain C57BL/6J were housed in groups of 5 in 30cm x 20cm x 15cm cages. Groups were either entirely male or entirely female, with an overall sex ratio of 1:1. Mice were maintained in a 12:12 light/dark cycle at 20-24C and 40-65% humidity. Water was provided *ad libitum*. Throughout the experiment, mice were fed Picolab Rodent Diet 20 5053 (LabDiet, Missouri, USA) which was also available *ad libitum* except prior to, during, and immediately after testing.

Cocoa and vanilla odour cues were used to distinguish novel and familiar food. To ensure only odour (and not e.g., taste) was being used by mice to distinguish between food types, the odour source (3 teaspoons of cocoa powder or 0.8ml of vanilla extract on filter paper) was placed in an upturned petri dish lid and covered by the bottom half of the dish in which several holes approximately 3mm in diameter were drilled. This setup prevented physical contact with the odour source while allowing mice to associate the odour with the food placed on top. Familiar and novel odours were balanced across groups which were also sex balanced.

Mice were trained on their familiar food in a transparent, plastic testing arena (approximately 35cm x 45cm x 20cm and lined with wood chip) by allowing them to forage from a petri dish laced with either cocoa powder or vanilla extract (as detailed above) once daily for 5 minutes. Mice were first trained in their groups of 5 for 10 days, after which they were trained in the same manner but in smaller groups of 2-3 mice for a further 3 days. Finally, mice were habituated to eating their familiar food alone in the arena. To reduce stress when foraging alone, their home cage would be placed adjacent to the arena wall so that the other group members were visible.

Following training, mice were tested for dietary conservatism using a latency test. Mice were allowing to forage alone in the arena for 5 minutes with two petri dishes approximately two thirds of the way down the arena, one containing their familiar odour and one containing the alternative, novel odour. The time taken to eat from each dish and the number of visits to each dish was recorded. Mice that failed to eat from the novel dish were tested again until they either ate from the novel dish or until 7 trials had been

conducted. Latency to consume the novel food was calculated in seconds across all trials for each mouse.

Figure A1.1 shows the latencies of individual mice to consume from the novel odour (NO) dish. The majority of mice (30/40) ate from the NO dish in the first trial (within 300s): these were classed as AC. Four individuals ate from the NO dish during the second trial (latencies between 300 and 600s). Six mice displayed greater reluctance to eat from the NO dish, taking between 3 and 6 trials to consume from the NO dish (latencies 600-1800), and one individual did not eat from the NO dish at all in 7 trials: these were classed as DC.

As this study did not measure latencies to incorporate the novel food but rather the time taken to begin consuming, it is arguable that food neophobia rather than foraging strategy was measured. However, the long duration of this latency coupled with the large number of exposures individuals classed as DC had before consuming any baits suggests a longer-term reluctance to eat food with a novel odour. Furthermore, 39 individuals visited the NO dish at least once during their first trial (the last individual did so in their second trial), suggesting that neophobia had been overcome enough to contact the source of the novel odour. While it is likely that the full picture of novel food incorporation is absent – leading to the classification of 4 individuals as "uncertain DC" – these data nevertheless provide the first support for the existence of individuals in a mammalian population.

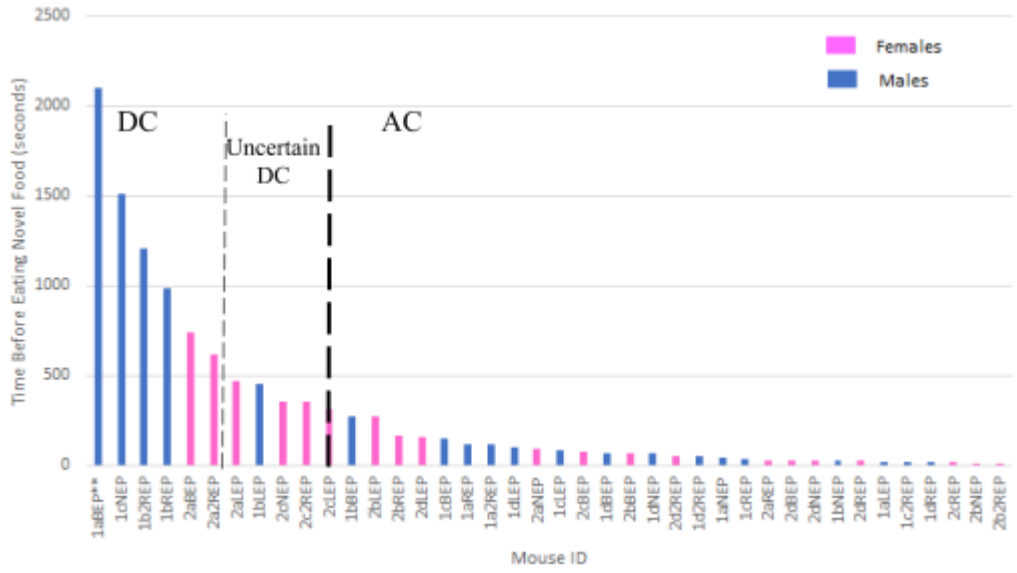

Figure A1.1. Latencies of individual mice to consume food from the novel odour dish.

A1.3 Dietary Conservatism in Invertebrates

Here we present data from three pilot experiments testing for the existence of dietary conservatism in invertebrates. The majority of these studies failed to find individuals with DC foraging strategies among invertebrate populations (but see 3. Dietary Conservatism in Ladybirds, below), however the preliminary nature of these studies means we cannot state with confidence that dietary conservatism does not exist among invertebrates or even among the species tested here. More rigorous experimentation is needed to build on these initial findings with larger sample sizes and a standardized methodology to cement our understanding of invertebrate foraging strategies and facilitate comparison of results across experiments.

A1.3.1. Dietary Conservatism in African Praying Mantids

One experiment has examined African praying mantids (*Sphodromantis lineola*) for dietary conservatism. A latency test for dietary conservatism was used and found no evidence for a DC foraging strategy in this species.

Twenty African praying mantids were purchased from a commercial supplier (Aquatic World, Cardiff) and housed in horticultural propagation trays (dimensions 38.0 x 24.0 x 17.5 cm) lined with white Plaster of Paris to provide a uniform background against which prey would be conspicuous. The experimental environment was kept constant for temperature (25±1◦C), relative humidity (35±5%), and light regime (18/6 light/dark cycle). Mantids were divided into two groups of 10 and trained on one of two prey morphs: naturally coloured bluebottle larvae (*Calliphora vomitoria*), a yellowish/beige colour, and red bluebottle larvae coloured by introducing red food dye into the maggots' diet. Mantids were presented with 10 appropriately coloured larvae once daily for 11 days to familiarise them with the colour morph. During training, two individuals familiar with naturally coloured larvae died leaving asymmetrical group sizes (8 and 10).

Following training, a latency test (see box 1, main text) was used to test for mantids' dietary conservatism. Mantids were provided with four larvae (two of the familiar colour and two of the novel colour) once daily for 16 days and the number of each colour eaten was recorded the following day. Neophobia was measured as the number of trials until the first novel

coloured food item was consumed, including only trials in which at least one food item was consumed. Dietary conservatism was assessed by the number of novel and familiar prey consumed during the experiment, as well as the recruitment time, defined as the number of test days until the novel morph had been consumed on three consecutive days, including only days on which at least one prey item was eaten. A GLM was used to determine whether the number of novel prey eaten increased with testing day, as individual mantids became more experienced with the novel prey.

All mantids ate at least two novel larvae during the experiment. There was no significant difference in duration of neophobia across groups (Wilcoxon-rank sum test; medians 1.0, 1.0; W = 40.5; p = 1). Mantids showed no preference for familiar over novel prey during the experiment. More novel prey were eaten than familiar prey, however there was no significant difference in the mean number of familiar and novel prey consumed (paired t-test of means; familiar = 5.94, novel = 7.16; t = -2.0103, df = 17, p-value = 0.061). An initial preference for familiar prey might be concealed if individuals developed a preference for novel prey during later stages of testing. A GLM found no significant relationship between number of novel prey eaten and testing day (Estimate = -0.03636; standard error = 0.38434; t-value = --0.095; p-value = 0.927).

Fifteen of the 18 mantids successfully recruited the novel prey during testing, however establishing recruitment was complicated by low numbers of prey eaten by some mantids. Of the 3 individuals that failed to recruit the novel prey, 2 only consumed any prey on 4 out of 16 testing day and showed no preference for the familiar prey, consuming 1 and 2 familiar and 4 and 2 novel prey, respectively. The third mantid that failed to recruit the novel prey ate on 7 of 16 days and ate the novel prey on 4 of those days, suggesting that it may have been dietary conservative. This individual also ate more familiar than novel prey (8 and 5, respectively), however an exact binomial test shows that eating only 5 of a possible 14 novel prey is not statistically significantly different from choosing at random (p = 0.424), thus evidence for dietary conservatism in this individual is weak at best.

Taken together, the lack of an overall preference for familiar prey, high rate of recruitment of novel prey, and lack of evidence of a preference for familiar prey among those individuals that failed to recruit, this experiment found no strong evidence for a DC foraging strategy, and thus dietary conservatism among African praying mantids.

A1.3.2. Dietary Conservatism in Spiders

Between 2006 and 2007, 31 spiders of three species were collected and tested for dietary conservatism in a laboratory setting. Jumping spiders (*Salticus* sp.) and house spiders (*Tegenaria domestica*) were collected from the wild in the Cardiff area, while wolf spiders (*Pardosa* sp.) were purchased from Blades Biological (Cowden, Edenbridge, Kent TN8 7DX).

Following transfer to the laboratory, spiders were maintained in boxes lined with tissue paper and deprived of food for 7 days prior to training. Ebony and yellow morphs of *Drosophila melanogaster* (purchased from Blades Biological) were used as the experimental food. Six spiders were set aside to test for an initial colour preference. These spiders were naïve to the experimental foods and were presented with equal numbers of each prey morph once daily for 8 days. The number of each prey type eaten was recorded. The remaining spiders were divided into two groups, each group receiving one *Drosophila* morph as their familiar prey and the other as their novel prey. During training, spiders were presented with 4 familiar-coloured prey daily for between 7 and 17 days. Following training with their familiar food, a latency test (see box 1, main text) was used to examine dietary conservatism expression: spiders were presented with equal numbers of familiar and novel prey once daily and allowed to forage, with the number of each prey type eaten at the end of the foraging session recorded. Partially consumed prey were considered eaten, while dead but intact *Drosophila* were not counted as eaten. Testing continued for between 1 and 15 days for each spider, and the number of prey presented varied from 4 to 10 of each morph.

The number of spiders in each colour group and the length of their training can be found in Table A1.3. Fifteen spiders died during the experiment (two during the initial colour preference test and 13 during training). Their data were excluded from the analysis leaving 4 spiders in the initial colour preference data set and 11 spiders in the final testing data set.

Table A1.3. Number of spiders in each group (those trained with ebony *Drosophila* and those trained with yellow) and at each training length.

	Number of Spiders	
Training Length (Days)	Ebony-familiar	Yellow-familiar
7	2	2
8	2	1
14	2	1
17	1	0

For each spider in the initial colour preference test, a preference score was calculated by subtracting the number of ebony prey consumed from the number of yellow prey consumed. More ebony *Drosophila* were consumed than yellow *Drosophila* (97 vs 77) resulting in a negative mean preference score (-0.625). However, this was not significantly different from zero, the expected score if both prey morphs were equally likely to be eaten ($t = -1.83$, p-value = 0.07663), suggesting that spiders had no underlying preference for either colour.

In the main experiment, testing for foraging strategy, most spiders (all but 3) ate at least 3 novel prey in their first trial, so the data were interrogated using a preference score rather than latency to consume 3 novel prey. Preference scores were calculated by subtracting the number of novel prey eaten from the number of familiar prey eaten in each test, with a negative score indicating more novel prey being eaten and a positive score indicating more familiar prey being eaten. To examine the possibility that a preference for familiar prey may be quickly overcome, we analysed preference scores on the first day of testing and across all days. No preference for either novel or familiar food was detected either on the first day of testing (t-test: mean = 0.33, $t = 0.69$, p-value = 0.504) or across all testing days (Wilcoxon-signed rank test: median = 0; $V = 2242.5$, p-value = 0.232).

As these data do not suggest any preference for familiar over novel prey, they offer no evidence of the DC foraging strategy or dietary conservatism being present in spiders.

A1.3.3. Dietary Conservatism in Ladybirds

Seven-spot ladybirds (*Coccinella septempunctata*) were collected from gardens and parks in Dundalk, Co. Louth during July 2007. Following transfer to the laboratory, ladybirds were maintained in petri-dishes on a diet of pea aphids (*Acyrthosiphon pisum*). Red and green aphid morphs were used as the experimental food; these were procured from existing breeding stock in Cardiff University and populations maintained on separate broad bean plants (*Vicia faba*) for the duration of the experiment.

Ladybirds were arranged into groups and allowed to breed. Eggs laid were kept in isolation until hatching. Once hatched, 16 larvae were kept in groups until they were approximately 3mm long, when they were transferred to individual petri-dishes. Training began immediately after hatching, with 8 larvae assigned red aphids and 8 assigned green aphids as their familiar food. During training, larvae were fed exclusively on their familiar colour for 10 days.

Testing began the day after training ended. Eight larvae (4 familiar with red aphids, 4 familiar with green) survived to testing. Larvae were tested for dietary conservatism by offering them 2 familiar-coloured and 2 novel-coloured aphids and recording the order in which they were eaten. Tests lasted until all 4 aphids were eaten and were carried out daily until pupation occurred, between 3 and 13 days after testing began. Five adults (3 familiar with red aphids, 2 familiar with green) survived pupation and were tested using the same methods as they had been as larvae for 8 days following emergence. A further 2 adults died or escaped on days 4 and 5 of testing.

The order in which familiar- and novel-coloured aphids were eaten was used to generate preference scores for individuals at both life stages (Table A1.4), with lower scores indicating a preference for that food type. T-tests and Wilcoxon signed ranked tests were used to determine whether preference scores were significantly different from zero overall for larvae and adults, and for individuals as both larvae and adults (Table A1.5). Data from larvae that were only tested on 2 days (due to pupation on day 3 of testing) were excluded from analysis as preference scores over so few encounters cannot be distinguished from random consumption.

Neither larvae nor adults showed an overall preference for either novel or familiar food (t-value = -2.5704, p = 0.05001 and t-value = -1.2418, p-value = 0.2821, respectively), however the overall preference score, derived from all larvae, was only marginally non-significant (p = 0.05001). Examining the preferences of individuals, none of the 5 adults and 5 of the 6 larvae with sufficient data for testing showed a significant preference for either food type (Table A1.5), suggesting that they were not dietary conservative. One larva (Ladybird 5, Table A1.5) showed a significant preference for familiar prey (p=0.03054), suggesting it was displaying a DC foraging strategy. The remaining individuals, therefore, were displaying an AC foraging strategy.

Table A1.4. Preference scores for ladybirds as larvae and adults. Ladybird IDs are consistent across life stages; [1] denotes larvae that pupated on the 3rd day of testing – as these generated only 2 days of data, they were excluded from analysis; [2] denotes adult ladybirds that failed to complete testing due to death or escape. Difference is calculated by subtracting the Novel Preference from the Familiar Preference – a difference of 0 indicates no preference, a negative difference indicates preference for the familiar, and a positive difference indicates preference for the novel.

Ladybird	Familiar Preference	Novel Preference	Difference
Larvae			
1[1]	6	14	-8
2	67	63	4
3	14	26	-12
4	27	33	-6
5	30	50	-20
6	55	65	-10
7[1]	9	11	-2
8	12	18	-6
Adults			
1	39	41	-2
3[2]	17	13	4
4	39	41	-2
7	34	46	-12
8[2]	18	22	-4

Table A1.5. Results of statistical tests on preference score data. Ladybird ID is consistent across larvae and adults. All Larvae and All Adults indicates pooled preference scores tested to determine whether an overall preference exists for either food type within a life stage group. Test used was either t-test or Wilcoxon signed rank test (for non-parametric data). * indicates a significant p-value.

Ladybird ID	Test	t value/ V statistic	df	p-value
Larvae				
2	Wilcoxon	36.5	na	0.7762
3	Wilcoxon	0	na	0.1489
4	t-test	-1.1677	5	0.2956
5	Wilcoxon	0	na	0.03054*
6	t-test	-1.0467	11	0.3177
8	t-test	-1.7321	2	0.2254
All Larvae	t-test	-2.5704	5	0.05001
Adults				
1	t-test	-0.31399	7	0.7627
3	t-test	0.75593	2	0.5286
4	t-test	-0.22771	7	0.8264
7	Wilcoxon	0	na	0.09467
8	Wilcoxon	0	na	1
All Adults	t-test	-1.2418	4	0.2821

This is the first study to find possible evidence for the existence of dietary conservatism among invertebrates. While only a single, larval individual in this experiment showed a significant preference for familiar over novel prey (despite extensive experience of the novel, which was also eaten throughout testing), this observation raises the possibility that dietary conservatism may not be restricted to vertebrates. That only one individual here displayed a DC foraging strategy suggests a possible explanation for other studies' failure to detect dietary conservatism in invertebrates: if only a very small proportion of individuals within invertebrate populations employ a DC foraging strategy, then it will be difficult to detect with limited sample sizes. The small sample sizes of this experiment make it impossible to draw firm conclusions about the prevalence of dietary conservatism among invertebrates, so larger studies are needed to further support or refute this observation. It is

possible that the individual in this experiment preferentially preyed upon red aphids for reasons unrelated to its prior experience with that colour morph. Another possibility is that dietary conservatism among invertebrates may be restricted to particular life stages. Larval ladybirds have a high metabolic demand as they grow in preparation to pupate, and specific foraging strategies may be advantageous to maximising prey capture during this time. Unfortunately, the individual displaying the DC foraging strategy in this experiment did not survive pupation so it is unknown whether it would have continued to display its preference for familiar prey as an adult. It does, however, suggest that further studies should consider testing invertebrates at different life stages to avoid potentially missing DC behaviour which may not be apparent throughout an individual's life.

Appendix 2. Supplementary material for Chapter 2 – How Familiar is Familiar?

Below are tables of results for within-treatment models of preference for familiar food. See main text for full details.

Table A2.1. Model of preference score and explanatory variables for fish in treatment Ex1.

Family: Beta regression(3.501)
Link function: logit

Formula: Prefs2 ~ DC.class + factor(day) + DC.class:factor(day) + s(fish, bs = "re")

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.1853	0.4464	2.655	0.007928 **
DC.classdc	-0.1691	0.7241	-0.234	0.815304
factor(day)2	-2.2619	0.6147	-3.680	0.000234 ***
factor(day)3	-1.5680	0.6031	-2.600	0.009326 **
factor(day)4	-2.0751	0.6109	-3.397	0.000683 ***
DC.classdc:factor(day)2	2.4606	1.0032	2.453	0.014174 *
DC.classdc:factor(day)3	0.9697	0.9816	0.988	0.323225
DC.classdc:factor(day)4	0.1336	0.9948	0.134	0.893170

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value
s(fish)	0.9813	6	1.242	0.27

R-sq.(adj) = 0.427 Deviance explained = 62.1%
-REML = -13.129 Scale est. = 1 n = 32

Table A2.2. Model of preference score and explanatory variables for fish in treatment Ex4.

Family: Beta regression(6.459)
Link function: logit

Formula: Prefs2 ~ DC.class + factor(day) + DC.class:factor(day) + s(fish, bs = "re")

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.8437	0.5210	1.619	0.10537
DC.classdc	1.2810	0.7308	1.753	0.07962 .
factor(day)2	-0.3806	0.5892	-0.646	0.51823
factor(day)3	-1.4011	0.5914	-2.369	0.01784 *
factor(day)4	-1.5344	0.5417	-2.833	0.00461 **
DC.classdc:factor(day)2	-0.8632	0.7963	-1.084	0.27835
DC.classdc:factor(day)3	0.2288	0.7990	0.286	0.77459
DC.classdc:factor(day)4	0.6053	0.7672	0.789	0.43013

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value
s(fish)	5.172	7	18.96	0.000583 ***

R-sq.(adj) = 0.606 Deviance explained = 76.1%
-REML = -17.551 Scale est. = 1 n = 34

Table A2.3. Model of preference score and explanatory variables for fish in treatment Ex7.

Family: Beta regression(8.801)
Link function: logit

Formula: Prefs2 ~ DC.class + factor(day) + DC.class:factor(day) + s(fish, bs = "re")

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.7738	0.6590	2.692	0.00711 **
DC.classdc	0.5845	1.0083	0.580	0.56213
factor(day)2	-2.0496	0.4792	-4.278	1.89e-05 ***
factor(day)3	-2.3887	0.4822	-4.954	7.29e-07 ***
factor(day)4	-3.4418	0.4994	-6.891	5.53e-12 ***
DC.classdc:factor(day)2	0.8810	0.7435	1.185	0.23604
DC.classdc:factor(day)3	2.2871	0.8284	2.761	0.00577 **
DC.classdc:factor(day)4	3.0619	0.7838	3.906	9.37e-05 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value
s(fish)	6.346	7	63.1	<2e-16 ***

R-sq.(adj) = 0.844 Deviance explained = 91.3%
-REML = -27.852 Scale est. = 1 n = 35

Table A2.4. Model of preference score and explanatory variables for fish in treatment Ex10.

Family: Beta regression(13.29)
Link function: logit

Formula: Prefs2 ~ DC.class + factor(day) + DC.class:factor(day) + s(fish, bs = "re")

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.73580	0.68958	3.967	7.27e-05 ***
DC.classdc	-0.02163	0.92481	-0.023	0.981342
factor(day)2	-2.01268	0.50283	-4.003	6.26e-05 ***
factor(day)3	-4.25644	0.52582	-8.095	5.74e-16 ***
factor(day)4	-3.82334	0.51337	-7.448	9.51e-14 ***
DC.classdc:factor(day)2	1.08817	0.73624	1.478	0.139405
DC.classdc:factor(day)3	2.76311	0.69700	3.964	7.36e-05 ***
DC.classdc:factor(day)4	2.53258	0.69070	3.667	0.000246 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value
s(fish)	6.366	7	63.95	<2e-16 ***

R-sq.(adj) = 0.881 Deviance explained = 92.6%
-REML = -31.867 Scale est. = 1 n = 35

Appendix 3. Supplementary material for Chapter 5 – Can Efficiency Explain Foraging Strategies?

Below are the full outputs for best fitting models of foraging efficiency metrics and explanatory variables. Full model outputs are presented here due to size and to preserve clarity in main text. See text for full details.

Table A3.1. Complete best fitting model relating foraging rate to explanatory variables.

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']				
Family: Gamma (identity)				
Formula: rate ~ test + (1 | robinID)				
AIC	BIC	logLik	deviance	df.resid
-17.1	-2.9	13.5	-27.1	120
Scaled residuals:				
Min	1Q	Median	3Q	Max
-2.1945	-0.6533	-0.0547	0.6318	2.5163
Random effects:				
Groups	Name	Variance	Std.Dev.	
robinID	(Intercept)	0.009331	0.0966	
Residual	0.158955	0.3987		
Number of obs: 125, groups: robinID, 15				
Fixed effects:				
	Estimate	Std. Error	t value	Pr(>|z|)
(Intercept)	0.72742	0.06237	11.663	< 2e-16 ***
testB	-0.21974	0.05459	-4.026	5.68e-05 ***
testC	-0.26201	0.05218	-5.021	5.14e-07 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				
Correlation of Fixed Effects:				
	(Intr)	testB		
testB	-0.554			
testC	-0.599	0.642		

Table A3.2. Complete best fitting model relating number of mistakes to explanatory variables.

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']				
Family: Negative Binomial(5000) (sqrt)				
Formula: mistakes6 ~ test + trial + (1 | robinID)				
AIC	BIC	logLik	deviance	df.resid
275.4	290.0	-131.7	263.4	78
Scaled residuals:				
Min	1Q	Median	3Q	Max
-1.8920	-0.7144	-0.1521	0.4938	4.2588
Random effects:				
Groups	Name	Variance	Std.Dev.	
robinID	(Intercept)	0.07098	0.2664	
Number of obs: 84, groups: robinID, 15				
Fixed effects:				
	Estimate	Std. Error	t value	Pr(>|z|)
(Intercept)	1.6926	0.1318	12.839	< 2e-16 ***
testC	-0.4818	0.1146	-4.203	2.63e-05 ***
trial2	-0.1423	0.1405	-1.013	0.311
trial3	-0.8011	0.1384	-5.788	.13e-09 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				
Correlation of Fixed Effects:				
	(Intr)	testC	trial2	
testC	-0.423			
trial2	-0.495	-0.049		
trial3	-0.554	0.069	0.500	