

Chapter 7

General Discussion



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“With habitat and food selection - behavioural phenomena - playing a major role in the shift into new adaptive zones, the importance of behaviour in initiating new evolutionary events is self-evident.”

Ernst Mayr (in *Animal Species and Evolution*, 1963, p. 604)

This thesis examines the details of the foraging ecology of a single species at a few selected sites. In the previous chapters, the relevance of the findings for ecology in general is only briefly, and usually quite implicitly, addressed. In an attempt to place the results presented in wider contexts, in this last chapter I will allow myself more speculative reasoning. Firstly, I will discuss how and when the active diet selection observed in red knots and presented in Chapters 2 and 3 may contribute to ecosystem stability. Secondly, I will discuss the implications of the observed relationships of diet choice with gizzard mass (Chapters 4 and 5) and with habitat selection (Chapters 5 and 6) for the ontogeny of individual foraging specializations and the development of individual phenotypic variations more generally. Finally, in an attempt to contribute ideas for future research, I will discuss the potential contribution of the interaction between behavioural decisions and development to the processes that generate heritable variation (Piersma 2007). To what extent do behavioural decisions contribute to the phenotypic divergence between populations?

PREY CHOICE AND ECOSYSTEM STABILITY

We (humans) have had enormous impacts on the way that our world looks today. Ecological investigations make us aware that our activities change the Earth's biodiversity. The observation that so many species now face the danger of extinction does not come as a surprise to most ecologists, although it is often difficult to explain which of the many potential causes is most important. Rather, we have been wondering at least since Aristotle why so many species do *not* go extinct, and instead persist in coexistence (Hutchinson 1959).

This question has been difficult to answer. Ecosystems are complex: they consist of many (and often non-linear) intra- and interspecific interactions in ever changing environments. Therefore, one of the more appealing answers to this question is that the complexity itself stabilizes ecosystems. The idea that "complexity begets stability" was formalized by Robert MacArthur (1955), who suggested that the opportunities for stable coexistence between species increases as the number of interactions between species increases. Eighteen years later, this argument was convincingly destroyed by Robert May (1973) who showed that the stable parameter space of a given population actually decreases when more species are added to a model system of linear differential equations. One of the mechanisms that might explain the apparent contradiction between this prediction and the observed natural world, is that foragers adjust their behaviour to changing conditions, and particularly to changing prey populations.

Behavioural responses of foraging red knots

In this thesis, we have investigated the behavioural responses of foraging red knots to the complex environment offered by the seagrass-covered intertidal mud- and sandflats of the Banc d'Arguin. Firstly, we described how the main mollusc prey types cause red knots at Banc d'Arguin to choose a mixed diet, instead of taking the single most preferred prey (Chapters 2 to 4). These so-called partial preferences comprise a reaction to the different

constraints that different prey types pose to their consumers. These different constraints are expected to lead to individual differences in food preferences as a consequence of physiological differences among individuals (Chapter 4). An analysis of the arrows of causality underlying the correlation between behaviour and physiology, suggested that individual food preferences actually determine individual differences in gizzard mass and movement decisions rather than the other way around (Chapter 5). We proposed that individual red knots at Banc d'Arguin learn specialized foraging strategies to individually optimize prey consumption rates locally.

Diet choice and population dynamics

Theoretical studies have investigated the long-term consequences of consumers foraging on multiple prey species (focussing on indirect interactions between prey species known as apparent competition; Holt 1977). They find that such systems are generally unstable (Holt 1977; Holt 1984; Bonsall & Hassell 1997), leading to the extinction of one or several (prey) species. As the authors acknowledge, this is not what we see in nature, as there are many examples where coexisting prey species share a single species that consumes them. Many additions have been suggested by which such interactions could be part of a stable food web. These include the presence of spatial and temporal heterogeneity of the environment (Tilman 1994) and niche differentiation among prey and consumer species (Hutchinson 1959). I wondered whether individual diet choice could also be one of them. After all, our findings sketch a picture of behavioural adjustments in foraging red knots that allow for more complex individual diets in reaction to a more complex resource landscape. When habitat complexity increases, so may the variety of potential prey and also the variety of constraints on prey intake. The diet of individual consumers may not just include more species, but also the number of considerations that influence dietary decisions may increase. Could this have qualitative consequences on the population dynamics of prey species?

The history of prey choice in population models

The emergence of studies on habitat- and diet selection was sparked by the idea that they should provide a deeper understanding of population dynamics and species interactions (MacArthur & Levins 1964; MacArthur & Pianka 1966). Murdoch (1969) was the first to formalize population dynamic consequences of diet choice. He proposed 'switching' as a potential explanation for stable coexistence of species. This involves a consumer that prefers the most common of two prey types so that as their availabilities change, the consumer may switch its focus from one prey type to the other (assuming that searching for one prey type precludes finding the other). Since then, a rich body of work on adaptive diet choice has been built over several scientific generations (for reviews see eg. Stephens & Krebs 1986; Houston & McNamara 1999). This work has primarily focused on foraging behaviour itself, sometimes on the short-term consequences of the feedback between prey density and adaptive foraging behaviour, but rarely on long-term ecological consequences. Only the most basic ideas of optimal diet choice have been implemented in population models. These models typically assume a single axis of prey quality, usually

profitability (energy over handling time), and model the optimal acceptance probabilities after encounter (p_i) of both the 'main' and an alternative prey type that is less profitable, as a function of their densities (Fryxell & Lundberg 1994; Křivan 1996). A 'partial preference' may occur ($0 < p_i < 1$), but never for more than a single prey type. These modelling studies conclude that adaptive foragers can in some cases stabilize multi-prey systems that would be unstable with a *rigid* forager, but only under specific conditions in a small parameter range. Fryxell and Lundberg (1994) therefore conclude that adaptive diet selection is unlikely to be a ubiquitous stabilizing factor in trophic interactions.

Some studies underline that animals may not have necessary information to make perfect choices (Kotler & Mitchell 1995). This justifies the use of slightly sub-optimal feeding strategies, which introduces the possibility of partial preferences for more than one prey species (Fryxell & Lundberg 1994; van Baalen *et al.* 2001; Abrams & Matsuda 2003). It turns out that dynamics are especially sensitive to these model changes. As the prey densities at which partial preferences occur for two prey species increases, the parameter range of stable coexistence of the two prey species also increases (Fryxell & Lundberg 1994; Abrams & Matsuda 2003).

There may be other, larger, and more fundamental mechanisms that cause partial preferences for multiple prey types. The described studies all modelled prey choice as the result of a fixed preference hierarchy (prey type A is preferred over prey B is preferred over C). Not only humans, but also red knots and several insects (Mayntz *et al.* 2005; Jensen *et al.* 2012) are shown to make more complex diet choices than to have a fixed hierarchy of prey preferences. In previous chapters (Chapters 2, 3 and 4) we have shown elaborately that partial preferences for two prey species are actually the optimal strategy for energy-maximizing red knots in Banc d'Arguin, and in other cases where multiple constraints act on a forager's food intake simultaneously. Hence, modelling of partial preferences by red knots as exhibited in Banc d'Arguin may change the predicted population dynamic interaction between red knots and their bivalve prey.

A population model of red knots and their main prey

In our study system, at least in the years investigated, mortality of adult red knots takes place mainly in winter (Leyrer *et al.* 2013). This implies that their survival may be largely determined by the abundances and condition of their bivalve prey in Banc d'Arguin. In Chapter 3 we show that annual survival is indeed correlated with the availability of food there, but not with the abundance of their most abundant prey species (not even when assuming a type-II functional response taking into account search, handling and digestion time). Only when adding prey choice to the equation as a function of prey toxicity and digestive quality, a correlation between food availability and yearly survival emerges. This implies that prey choice is an essential asset in the response of red knot population size to the density of their bivalve prey. However, to show the significance of prey choice not only to red knots themselves, but also to their prey (and via their prey to the rest of the food web), a change in the long-term population dynamic interaction between red knots and their bivalve prey should be demonstrated. A simple comparison between annual total prey intake by red knots and yearly secondary production of bivalves does not suffice,

because they tend to be highly interdependent (van der Meer, Piersma & Beukema 2001). To arrive at a more relevant expectation, this interdependency should be made explicit; it demands a population dynamical model.

An important aspect when modelling this system is the occurrence of discrete events in the annual cycle of both red knots and their bivalve prey, causing at least partial decoupling between their population dynamics. Red knots breed in the High Arctic. This has two important consequences. Firstly, their reproductive success should be - at least partly - independent of food conditions in the Banc d'Arguin, and determined by the situation in the Arctic (van Gils *et al.* 2016). Secondly, the temporary absence of red knots during the summer months means a strong decrease in predation pressure for bivalves. Bivalve reproduction at Banc d'Arguin is also a discrete event, in the case of *Loripes lucinalis* happening twice a year (in January/February and in July/August; van der Geest *et al.* 2014), with the second one being more significant for the two main prey species of red knots, *L. lucinalis* and *Dosinia isocardia* (Ahmedou Salem *et al.* 2014). Hence, the large majority of juvenile bivalves become available to red knots within a short time period, just before or after their return from the breeding grounds.

However, the annual number of juvenile bivalves that survives to the size at which they become available to red knots (at approximately at 2 mm length) is only to a certain extent determined by the abundance of reproductively active adults. Food limitation, availability of suitable sediment and predation of larvae influence their survival (Ólafsson, Peterson & Ambrose 1995) and diffuse the relation between the extent of predation of bivalves by red knots over the winter and their recruitment in the following autumn (van der Meer, Beukema & Dekker 2001; van der Meer, Piersma & Beukema 2001). Obviously, the nature of this stock-recruitment relation is key in determining the effect of prey choice by red knots on the long-term interaction between red knots and their bivalve prey.

In a preliminary modelling exercise, I circumvented the lack of data on the stock-recruitment relation by modelling two extreme scenarios. In the first scenario *Loripes* and *Dosinia* recruit to a fixed population size in one discrete event annually at 1 October. The system is modelled with a series of discrete difference equations in which the total per capita prey intake by red knots is calculated each day. Daily red knot survival decreases below a standard survival probability when the per capita energy intake rate falls below the minimum requirements. A mortality term is then added that increases linearly towards a maximum when no food is consumed. All adult red knots (older than 1 year), and half of the juveniles, are assumed to leave the system each year on 1 June and return on 1 October. A new juvenile cohort then also arrives, that is 30% of the adult population size.

Obviously, a long-term effect on bivalve populations is absent in this scenario. What this model does allow though, is a consideration of the effect of diet selection on the equilibrium density of a dynamic red knot population. When red knots optimize the acceptance probabilities of *Dosinia* or a *Loripes* according to the predictions of the diet choice model (see Appendix 7.1), the predicted equilibrium density of red knots is expected to be roughly 1.5 times larger than when red knots do not distinguish between them and maximize prey intake rate indifferent of the prey type (T. Oudman and V. Hin, unpublished

data). In this scenario, equilibrium densities of red knots strongly depend on the timing of red knot arrival in autumn relative to the recruitment date of the bivalve populations, which were here both assumed to be on 1 October.

In the second scenario, I determined whether the bivalve populations either benefit or suffer from optimal diet choice by a constant red knot density. The populations of *Loripes* and *Dosinia* are now assumed to exhibit continuous logistic population growth. Now the red knot population is assumed to be constant, and hence independent of the bivalve populations. The system is modelled by a set of two continuous differential equations, describing the population dynamics of *Loripes* and *Dosinia*. Per capita growth rates r_l and r_d (individuals/s), fitted to result in a realistic equilibrium densities at an intermediate density of red knots, and K_l and K_d are set at maximum observed densities of *Loripes* and *Dosinia*, roughly 2000 and 400 individuals/m² (J.A. van Gils, unpublished data). The per capita intake rates of *Loripes* and *Dosinia* by red knots are the same as in the previous scenario (see Appendix 7.1). Equilibrium densities of *Loripes* and *Dosinia* as a function of red knot density were determined by numerical continuation of bifurcations, using the Matcont package in Matlab (Dhooge, Govaerts & Yu 2003). In this scenario, the range of red knot densities resulting in stable non-zero populations of *Loripes* and *Dosinia* is roughly 1.3 times larger when assuming optimal prey choice instead of an indifferent forager (T. Oudman and V. Hin, unpublished data). At red knots densities where both optimal and indifferent diet choice lead to stable bivalve populations, adaptive diet choice generally leads to higher equilibrium densities of *Loripes*, but lower densities of *Dosinia* than in case of no diet choice.

The preliminary analyses suggest that optimal prey choice by red knots is expected to have a positive influence on prey persistence when red knot density is constant, and on red knot population persistence when annual initial prey densities are constant. This implies that prey choice in this system may indeed have a positive effect on the coexistence of red knots and their bivalve prey in Banc d'Arguin. However, whether these positive effects of adaptive prey choice on population sizes will be maintained when they are allowed to interact in a fully dynamic model remains to be tested. It should be clear that the density-dependent rates of reproduction and growth in *Loripes* and *Dosinia*, as well as alternative prey types, need to be carefully described to arrive anywhere near an accurate prediction of the population dynamics.

INDIVIDUAL VARIATION: A DEVELOPMENTAL PERSPECTIVE

One of the most eye-catching features of the red knot body can actually not be seen with the bare eye for as long as the bird is alive. It is its muscular stomach, the gizzard. Gizzard masses can vary between healthy red knots from 4 to 15 g on a total body mass of ca. 120 g, even in a single mist net catch (e.g. Chapter 5). Red knots are not alone in showing plasticity in gizzard mass (Piersma, Koolhaas & Dekinga 1993), but the variation that is observed between individuals within one population at the same time is remarkable. This makes red knots particularly suited to study individual variation. Since variation in

gizzard size was first considered, the question has been whether diet choice is either a cause or a consequence of gizzard size (Piersma, Koolhaas & Dekinga 1993; Piersma 1994; van Gils 2004; Bijleveld 2015). This thesis is largely an extension of these studies, showing how diet preferences are expected to change with gizzard mass (Chapter 4), and considering individual diet preferences as a cause of variation in gizzard size (Chapter 5). Foraging experiments and optimal foraging theory have proven their value as tools to gain insight in the relation between gizzard size and diet, and the interactions between the environment and red knot behaviour. But can these tools be used to infer the causes of individual variation?

The limitations of optimal foraging theory

In attempts to lift ideas from “story telling” to explicit, quantitative hypotheses that allow an examination of the logical implications and a testing of the congruence between theory and observation, optimal foraging models are used to formalize expectations (Stephens & Krebs 1986). In the diet choice models (Chapter 2 to 4) as well as in the movement models (Chapters 5 and 6) we have assumed that foraging red knots optimize their diet choice with the objective to maximize their energy intake rate, and have been explicit on the different constraints that they face (limited time for searching and handling prey, a digestion limitation, and a toxin limitation on the intake of *Loripes lucinalis*). These comprise specifications of the general assumption of optimal foraging theory, which states that the animal under study has evolved to choose the “optimal diet”, that is the diet that is expected to maximize the currency that determines fitness, given the constraints that the animal faces (Stephens & Krebs 1986).

Stephens & Krebs (1986) see this approach as a respectable attempt to move beyond the classic ‘adaptationist approach’, which acts on the false idea that each trait is separately adapted by evolution (Gould & Lewontin 1979). I agree, although optimal foraging theory cannot be entirely excused either, because it fundamentally assumes a strict distinction between traits that are constraints and traits that are to be optimized. It may be true on the short time scale on which a single decision is made (to accept or to reject a prey, to stay or to move elsewhere), but on a slightly longer time scale, the forager may actually not just adjust its diet to fit its constraints, but also adjust these constraints to better fit its diet.

I will provide two examples for red knots. The first is that in the optimal diet models that we used, search efficiency and handling time are assumed to be fixed parameters. In reality, they will at least partly be the result of the diet, because individual experience in foraging on a specific diet increases the foraging efficiency on that diet (Davis & Stamps 2004; Villalba, Provenza & Han 2004). Hence, the optimal diet is not simply a consequence of handling time and search efficiency, the diet feeds back to increase search efficiency and decrease handling time on that specific diet. This would increase the ‘optimality’ of that diet. Similarly, and this is the second example, as discussed in Chapters 4 and 5, gizzard mass is not a fixed constraint either. To large degree, even the first studies on gizzard size in red knots have suggested it to be adjusted to the previous diet (Piersma, Koolhaas & Dekinga 1993; Dekinga *et al.* 2001). The diet is not just limited by digestive

capacity, but there is a feedback by the diet to adjust the limit set by digestive capacity towards its requirements for that diet (see also Bijleveld 2015). Consequently, the assumption that underlies the optimal diet tests in red knots, namely that red knots maximize their energy intake rate given the digestive capacity of their gizzard, is somewhat self-evident if gizzard size is on the long term adjusted to the realized intake rate. If that is the case, red knots will appear to be maximizing their energy intake rate with respect to their gizzard mass even when their actual strategy is to reduce intake rate to the minimum required energy intake (the so-called ‘satisficer’ strategy).

These two examples show that the difference between optimized traits and constraining traits is in fact arbitrary on a longer timescale. Many (if not all) traits to some extent interact with other traits, and should ideally be incorporated in the trade-offs that determine the theoretical optimal phenotype. Although the response rate may be slower as we move from behavioural traits such as prey preferences, via physiological traits such as digestive capacity, to structural traits such as bill size (page 197 in Bijleveld 2015), this does not imply any order in how different traits are to be prioritized when optimizing the phenotype to the environment. It also does not imply the extents to which plasticity is involved in the expression of these traits (Piersma & van Gils 2011; Stamps 2015). Before an optimization model can predict individual variation in the phenotype, it must include the limitations in the plasticity of each trait, and the costs that are involved in its adjustment. To determine these limitations, it must be acknowledged that traits adjust at different paces (Bijleveld 2015) and may be plastic during certain ages but not at others (Bateson 1979; Desai & Hales 1997).

Studying the limits of plasticity is necessary when wanting to infer the role of behaviour in the origin of individual variation in a wild population. This begs for experimental studies over longer timescales, at all ages (Stamps 2003), and in the context of the natural environment (Gilbert 2001; Senner, Conklin & Piersma 2015).

Development of diet preferences and gizzard size

The observation that adult red knots do not adjust their preferences after a change in gizzard mass (Chapter 5), shows that diet choice is not directly influenced by gizzard size. This does not reveal how individual diet preferences were formed in the first place, and whether digestive capacity played a role in it (Chapter 5; Mathot, Dekinga & Piersma 2017). Dietary preferences are influenced by individual experience early in life (Distel & Provenza 1991; Provenza & Cincotta 1993; Estes *et al.* 2003). In mammals, they are influenced by experiences even before birth through food particles that pass the placenta and during weaning by the mother milk (Nolte & Provenza 1991; Nolte *et al.* 1992), in that way being “softly” inherited. In red knots, conception and breeding takes place on the tundra where both parents and hatchlings feed on insects. Mother leaves the hatchling after the eggs have hatched, and father leaves after taking care for another 2–3 weeks. Juveniles migrate without their parents, so when they land on a mudflat for the first time, they do so without their parents. Although we cannot rule out the possibility that hatchlings entirely inherit their parents’ dietary preferences, it is likely that preferences are influenced by dietary experiences during their first attempts to forage on mudflats, along

the flyway or after arrival on the wintering grounds. If so, then temporal and spatial variation, both considerable on the mudflats of Banc d'Arguin and on mudflats in general, help to explain individual variation in diet preferences.

This individual variation in dietary experiences may be amplified by inherited individual variation in gizzard size, or differences in its plasticity, which limits the range of potential dietary experiences. Similarly, pre-existing variation in exploratory behaviour, as proposed by Bijleveld *et al.* (2014), will help to further divert individual differences. Because the resulting preferences in turn affect gizzard mass, and may also influence the development of personality (Dall *et al.* 2012), we cannot make a distinction between traits that cause, and traits that follow the development of the individual phenotype.

Developmental canalization

To clarify the developmental approach to the role of behaviour as a source of individual variation in red knots, I now turn to a widely used graphical metaphor introduced by Conrad H. Waddington (Waddington 1942; Waddington 1953). To clarify his ideas on the interaction between genes and the environment during development, he drew a sloping surface, the epigenetic landscape (Fig. 7.1A), that is shaped by the complex interactions between the genes (Fig. 7.1B). In that landscape a ball, representing the development of the individual, starts at the top and rolls down the slope. The rolling ball, and the continuum of locations where it may end up at the end of the slope, denote the continuum of different phenotypes. It is then proposed that selection has acted on the surface of the epigenetic landscape, such that canals have been formed that stabilize development; a single genetic mutation usually has only little or no impact on the surface. As a consequence, the ball does not roll down the slope at random. Development of the phenotype is *canalized*. The environment is brought into this metaphor as an external stimulus that may push the ball sideways, and alter the canal into which the ball rolls.

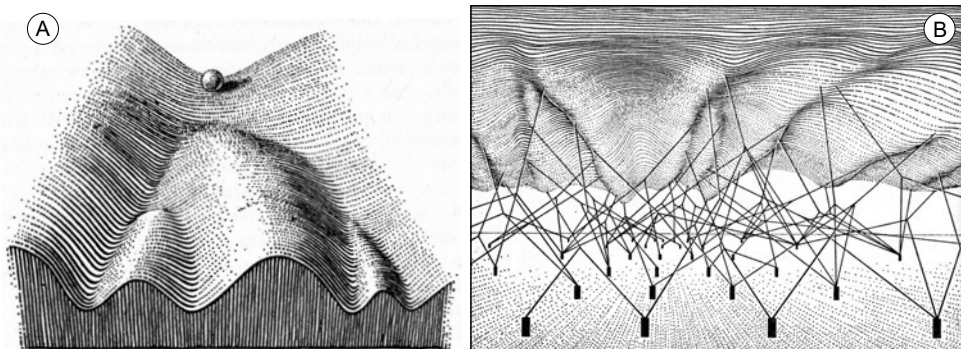


Figure 7.1 The original drawing of the epigenetic landscape as envisaged by Waddington viewed from above (A), showing a ball that is canalized (representing the phenotype) while rolling down a slope (representing development), and viewed from below (B) to show the complex interactions (the ropes) between genes (the pegs) that shape the landscape (Waddington 1957).

Behaviour as the sum of genes and environment

We can think about behaviour from a developmental perspective by asking how behaviour should be represented in the epigenetic landscape. One could argue that behavioural traits should be included in Waddington's landscape just like any other phenotypic trait: the expression of a behavioural trait is determined by the complex interaction between genes, including plasticity in the response to environmental circumstances. The relation between gizzard mass and diet preferences can then be reduced to a genetic coupling between the two. The genetic basis of behaviour is being studied with the help of another classic metaphor, the 'reaction norm' (Dingemans *et al.* 2010). In this metaphor, both the environment and some specific behavioural trait are represented by a one-dimensional gradient. The relation between the two, for a single genotype, is drawn by a curve in the plane spanned by the environment and the potential expressions of the behavioural trait. This curve can be drawn for any genotype, because it assumes that the expression of a behavioural trait is the result of a genetically coded plastic response to the environment. This implies that although behaviour may interact with other traits during development, individual variation in behaviour or any other trait can be decomposed into a genetic and an environmental part.

Behaviour as a force in itself

An alternative view is that the causes of individual variation cannot be reduced to its environmental and genetic components (Lewontin 2001; Bateson 2005; Jablonka & Lamb 2005; Laland *et al.* 2015). A main argument is that "the environment" is not a simple gradient, but the result of complex interactions between many environmental factors, very much like the genetic interactions that underlie the epigenetic landscape (Noble 2015). In addition, the environment will not be constant during development (Stamps 2003). During development, coincidental combinations of different environmental factors can lead to many, potentially novel, expressions of behavioural traits (Baldwin 1896; West-Eberhard 2003), which can be selectively incorporated by the organism (e.g. through learning) and have a major influence on development. As a consequence, behavioural responses to the environment often cannot be traced back to the genes, but should instead be considered as a primary source of variation (Jablonka & Lamb 2005). Another, and related, argument is that the environment cannot be defined without the animal that inhabits this environment (Lewontin 2001). Behaviour allows organisms to influence their own environment, either by habitat selection or by physically altering it (Darwin 1881; Waddington 1959; Lewontin 1983). Individuals can also influence each other's behaviour via social information (Avital & Jablonka 2000; Hoppitt & Laland 2013).

In this view, behaviour influences development in two ways. It is a force that (1) exploits coincidence and conspecific behaviour to create directional phenotypic variation, and (2) changes the environment in which development takes place. As such, behavioural differences may be a powerful source of individual variation in many traits (Stamps 2003; Piersma & van Gils 2011). Such behavioural forces, forces that may influence the path of development, are not explicit in Waddington's epigenetic landscape (Fig 7.1). It is hard to imagine how the path of the ball would influence external forces. To accommodate this

notion in the metaphor, we may turn it upside down (very carefully though, to respect the functionality of the original position for other purposes). Instead of the genetic interactions, we could imagine all factors shaping the environment as pegs; their complex interactions shape the environmental landscape, and shape the separate developmental canals in which the ball may roll. Behaviour can then be viewed as a force that influences the ball's sideways movement, perhaps from inside the ball. Genes and other sources of developmental constraint can be thought of as external forces, that limit the ball's sideways movement. Individual decisions may bring the ball in a direction that comforts the individual, although they must be allowed by the external forces, the developmental constraints. But the individual cannot see the future; it cannot look down the slope to see in which canal it will end up. Hence, sideways movements by the ball may be intentional, but this implies no knowledge of the canal in which it rolls. Rather, the decisions to move sideways may be guided by an evolved biased quality space (Dennett 2001); the organism can use its senses to provide it with a feeling about good or bad. For example, stomach ache may help an animal to judge a novel prey type, and gather "nutritional wisdom" (Richter 1943; Stephens & Krebs 1986). It may decide not to forage on foods that cause stomach pain, alter its searching behaviour and thereby change its environment; it may change the developmental canal in which it rolls. Similarly, the looks and behaviour of a stranger may help an animal to decide whether it should flee or not, or even copy its behaviour, and have "social wisdom". Of course the feeling will not necessarily be right, but it certainly makes novel behavioural variation in reaction to a new environment non-random (Jablonka & Lamb 2007).

Learning as a pathway of inheritance

If learning influences the development of behaviour, and that learned behaviour can be transmitted socially, it follows that learning is a non-genetic pathway of inheritance (Jablonka & Lamb 2005). Similarly, if individuals construct their environment, and with that influence the environment of the next generation, this can also be regarded as a pathway of inheritance (Lewontin 1983; Jablonka & Lamb 2005; Piersma & van Gils 2011). Just as in the evolution of human psychology, this complicates the question of which proportion of individual variation is of environmental origin and which of genetic origin, to the extent that the question itself becomes questionable (Bateson 2005). To explain the developmental origin of individual variation, is not enough to assess the statistical relationship between a behavioural trait and the level of genetic relatedness. Also the other pathways must be considered. How do individuals learn about food selection? How does the animal learn which habitat to select? How do these choices influence the environment to which the animal adjusts its phenotype?

That the ability to learn appears to be widespread especially among mammals, may be a consequence of our mammalian perception of the world (Laland & Hoppitt 2003). Researchers have questioned why songbirds and parrots, with their wonderful ability to imitate sound, did not evolve any sophisticated traditions in natural populations (e.g. page 174 in Jablonka & Lamb 2005). These researchers were thinking of symbolic language as we use it, but the cognitive abilities that these birds display may well be used in other

complex behaviours (e.g. Templeton, Laland & Boogert 2014). When certain habits are passed from generation to generation via social learning, we could call them traditions (Avital & Jablonka 2000). Examples of traditions across animal taxa are increasing exponentially, in vertebrates as well as invertebrates (Avital & Jablonka 2000; Hoppitt & Laland 2013). Also the effect of social learning on behavioural variation in wild populations is increasingly recognized (Healy & Rowe 2014). Studying traditions in the wild rather than in the laboratory may be a particularly fruitful exercise, because the expression and the function of complex behaviours may become apparent only when studied in the environmental setting in which the behaviour has actually evolved (Healy & Rowe 2014).

The next step is to show how socially induced behavioural variation interacts with physiological and structural traits in the course of development. What is the importance of the social inheritance pathway in producing the individual variation – not only in behaviour but in the phenotype in general - that we observe in wild populations? Is the red knot a suitable study species to answer this question?

RED KNOT TRADITIONS

Traditions are habits, passed from generation to generation by social learning (Avital & Jablonka 2000). The ecosystems in which red knots are studied, covering wintering and staging areas across the globe, provide a uniquely rich toolbox to study the causes and consequences of behavioural habits, and their relation with physiological and morphological traits. This is exemplified by the number of dissertations on the subject (Piersma 1994; van Gils 2004; van den Hout 2010; Leyrer 2011; Folmer 2012; Bijleveld 2015; de Fouw 2016). With the availability of this toolbox, the red knot may be an ideal model species to study how social learning affects development (Piersma 2011). May differences in red knot habits in between areas involve behavioural traditions? And might these traditions be involved in shaping other parts of the phenotype? Could behavioural traditions even influence the evolutionary divergence of these populations?

Traditions in habitat selection

Knowledge on the ontogeny of red knot behaviour and the role of social learning in the development of individual red knots is largely lacking. Nonetheless, the scientific literature on red knots does provide several clues to suggest that red knots use social information to decide where to forage. Firstly and most importantly, red knots are a classic example of a social foraging species (Goss-Custard 1970). Beyond doubt, habitat selection by red knots largely depends on habitat selection by conspecifics. More specifically, patch choice by captive red knots depended on the success of conspecifics in those patches, and the time to locate a food patch decreased with increasing group size (Bijleveld *et al.* 2015). Bijleveld *et al.* (2010) suggested that communal roosting enables red knots to gather public information on where to forage. Furthermore, (van den Hout *et al.* 2016) showed that juvenile red knots in Banc d'Arguin forage in more dangerous places than adults. The

authors suggest that this is due to a social hierarchy that prevents juveniles to forage where the adults do. This implies that habitat selection by red knots in the Banc d'Arguin involves a great deal of social interactions. In addition, red knots there show highly consistent foraging routines and are highly site faithful (Chapter 6), even over multiple years (Leyrer *et al.* 2006; T. Oudman, unpublished data).

The observed importance of social interactions for habitat selection opens up the possibility for the emergence of traditions. If juvenile red knots arrive in Banc d'Arguin after their first migration from the Arctic, the adults in general already have arrived and have come to a spatial distribution. The juveniles must then decide on where to forage, and it is likely that this decision is influenced by the distribution of the adult population. If juveniles are indeed 'forced' to feed at certain places by the adult population, and later as adults do the same to new juveniles, this means that social structures determine early-life experiences and the development of foraging routines. A tradition is passed from generation to generation.

Dietary traditions

Considering that mudflats are highly heterogeneous habitats (e.g. Chapters 5 and 6), habitat selection has far-reaching consequences on the diet. If the habitat selection of juveniles is influenced by adult habitat selection, so is the resource availability that juveniles encounter after their arrival in the wintering area. Hence, resource availability, perhaps the most important aspect of the environment (Piersma 2012), is not a given to which they must adapt; it is partly constructed by their conspecifics. Consequent diet choice will depend on this availability and is potentially influenced by genetic variation, but may also be influenced by the foraging habits of conspecifics. Although purely speculative, it is easy to imagine that dietary habits such as the consumption of toxic *Loripes lucinalis* in Mauritania, or the 'slurping' of *Hydrobia ulvae* in the Wadden Sea is transferred socially.

An observation to provide some credibility to this speculation is that red knots do not instantaneously start consuming artificial food items in captivity (Piersma, Koolhaas & Dekinga 1993). Captive red knots encounter trout pellets, *trouvit*, for the first time in their lives after it is presented to them in captivity, where it is often used as staple food. Virtually all red knots initially ignore this food and may continue to do so for several days before they sample it. Some will even starve without touching *trouvit*, but most red knots will try, and after some initial hesitation prefer pellets even over a bivalve diet (Piersma, Koolhaas & Dekinga 1993). Interestingly, captured red knots are more likely to start eating pellets when a conspecific is placed in their cage that is familiar with pellets (T. Piersma and A. Dekinga, unpublished data). Presumably, this is because their motivation to consume this new food type increases by watching another red knot do it. This implies that red knots may at least partly build up food preferences by learning through social information.

Another interesting case is the currently popular dietary habit by red knots in Mauritania to consume seagrass rhizomes. Isotope data from 2002 to 2015 (van Gils *et al.* 2016, van Gils *et al.* in prep) shows that this behaviour has increased in recent years (Figs

2A and C). Could it be that seagrass consumption is a recent discovery by red knots that is learned by social interactions? If so, the spread of seagrass foraging among the red knots of Banc d'Arguin is the mudflat equivalent of the famous example in blue tits and great tits near Southampton, England, that invented the behaviour to remove the caps from milk bottles, which was socially transmitted across the UK within a few years (Fisher & Hinde 1949; Aplin, Sheldon & Morand-Ferron 2013).

The proportion of seagrass in the diet has been proposed to be an adaptive dietary response to the combined changes in the density of bivalves and a change in the population distribution of a morphological trait (van Gils *et al.* 2016). The average bill size of juveniles has been reducing in recent years, a proposed consequence of an increasing mismatch between hatching date and the insect peak in the Arctic (van Gils *et al.* 2016). This may lead to a lower availability of bivalves in the diet, as a larger proportion is buried too deep to be reached. As predicted from this hypothesis, the proportion of seagrass in the diet is higher in years where the maximum intake rate of bivalves is low, due to their limited availability (Figs 2B and D, van Gils *et al.* in prep). Unfortunately, the annual predicted intake of bivalves is collinear with time (Pearson's coefficient -0.73). Therefore, their explanatory powers cannot be separated statistically.

A sudden increase in the expression of this behaviour without a change in densities of any prey type would have been a strong case for the birth of a new tradition. This does not mean that seagrass foraging cannot be explained as a tradition. The consumption of seagrass may be a behaviour that is expressed by all red knots when the availability of other prey is low, but that does not imply how the behaviour is inherited.

Traditions and developmental canalization in red knots

The possibility of foraging traditions in red knots is particularly exciting because we have so many clues already on the intricate relation between diet, physiology and morphology. Once it would be shown that traditions play a role in the diet of red knots, there is a rich literature to substantiate its potential influence on the development of the entire phenotype. Recalling my own version of Waddington's landscape, traditions in red knot diet choice can be represented as a path in the environmental landscape that was formed by earlier balls (other individuals), and a certain preference by the ball to follow that path. A tradition has the potential to lead the ball into directions that otherwise would not have occurred, even though in principle it would have been able to do so. The individual just wouldn't have thought of it, or it wouldn't have thought it to be a good idea. Red knot traditions may induce the development of phenotypes that otherwise would not occur.

Let me take the consumption of seagrass as a hypothetical example of a tradition. A behaviourally induced increase in this behaviour changes the requirements of the digestive tract, and is likely to have consequences for gizzard mass and gut morphology. Seagrass foraging may also influence the microbial content of the gut and particularly the caeca, which influence the digestibility of plant material. Red knots have well-developed caeca (P. Battley, unpublished data), which in many herbivore and omnivore birds contain high concentrations of gut bacteria, helping the digestion of plant material (McNab 1973). In humans, microbial communities in the gut make up an important phenotypic trait that

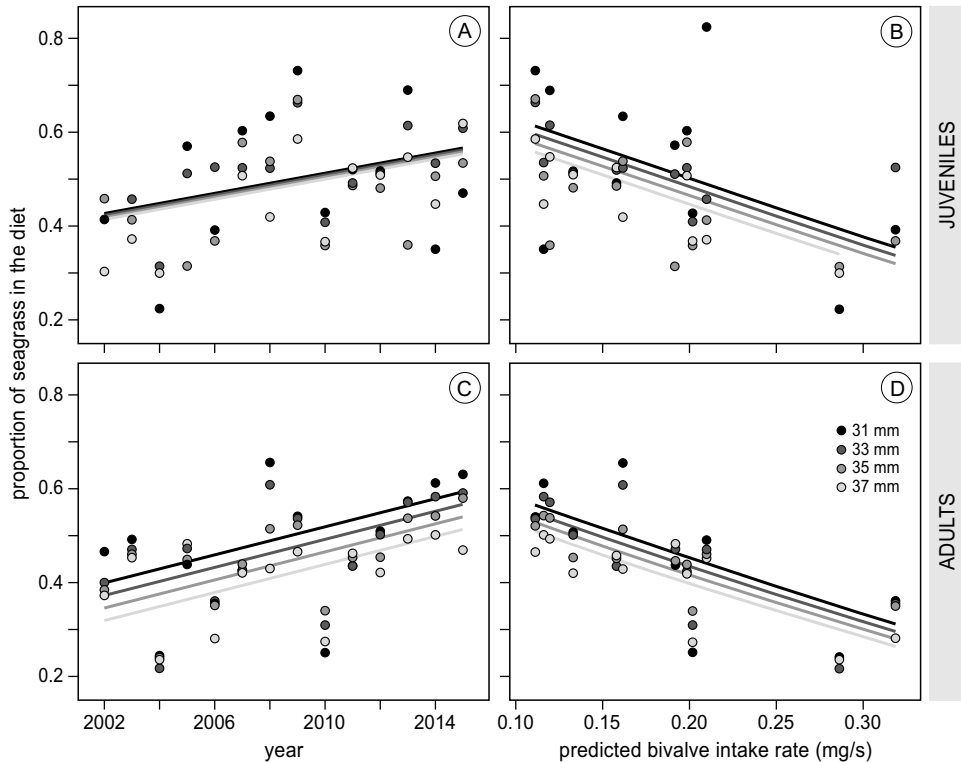


Figure 7.2 Mean proportion of seagrass in the red knot diet. The proportions of seagrass in the diet were estimated from N and C isotope levels of blood samples from 2,649 red knots, collected between 2002 and 2015 (van Gils *et al.* 2016). In panels A and C, each point shows the mean per year for juveniles and adults, separated in four classes of bill length. In panels B and D, the same isotope data is plotted as a function of the yearly average maximum predicted bivalve intake rate ($\text{mg ash-free dry flesh mass s}^{-1}$). This was calculated using a diet-choice model, based on the yearly average observed densities of *Loripes* and *Dosinia* (Chapter 3). Regression lines show the results of the best statistical model. AICc-values were compared of linear models with all combinations of explanatory variables ('Year', 'Age' and 'Bill length' for panels A and C, and 'Bivalve intake rate', 'Age' and 'Bill length' for panels B and D) and second order interactions. *Zostera* proportions were logit-transformed before analysis. The effect of 'Year' and 'Bivalve intake rate' could not be separated because they showed strong collinearity (Pearson's coefficient -0.73).

inherits non-genetically, with important and non-reversible consequences on behavioural and physiological development (Cox *et al.* 2014). Also in red knots, early experiences with toxic food, plant material and other specific food characteristics may permanently shape development. They may influence the development of the gut microbiome, the ability to detoxify, and the rate at which the gizzard muscle responds to training. They may also influence the development of the relation between physiological workloads and behavioural responses (e.g. the amount of ingested toxins before the animal refrains from eating more *Loripes*, or the level of gut fullness at which the animal considers it full).

It is possible that the gut bacteria involved in digesting plant material in red knots are also associated with the toxic effect of *Loripes* consumption. Sulphur consumption is

particularly dangerous for ruminants, due to the presence of specialized bacteria in the rumen that help digesting plant material and have the ability to convert dietary sulphur and sulphate to toxic sulphide (Kandylis 1984; Hall 2007). As a consequence, the evolved habit of seagrass foraging in *canutus* red knots may come at a cost of the ability to consume *Loripes*. If indeed the gut microbiome is formed partly by parental effects in juvenile knots and has lasting effects on physiology, then this may explain life-long and heritable differences in dietary preferences of non-genetic origin. Continued foraging traditions may, through behavioural and gut-microbial inheritance, lead to a change in the selective pressures on diet and physiology, and contribute to the evolutionary divergence of red knot subspecies.

Testing foraging traditions

The existence of foraging traditions in red knots is speculative. It is not easy to test these speculations. Unfortunately, the isolated breeding areas and dispersed breeding greatly hinder the study of inheritance in red knots. We cannot test whether diet preferences are influenced by genetic variation or other parental effects, because we have no information on (genetic) relatedness. However, the possibility to test diet preferences in juvenile and adult red knots from different subspecies on different prey types offers many possibilities for an inquiry into the existence of traditions. This may offer some logistic problems, but previous attempts at the Radboud University in Nijmegen to grow *Zostera noltii* from Banc d'Arguin in aquaria were successful (L.L. Govers, personal communication). Growing seagrass in the laboratory provides the potential to test at NIOZ whether there are differences in the propensity to consume seagrass rhizomes between juvenile and adult *canutus* red knots, caught in autumn in Poland. These juveniles have never been in the Banc d'Arguin and cannot have learned this behaviour socially (although both *islandica* and *canutus* juvenile red knots have been observed eating plant material, on the tundra and on the Poland shore, J.A. van Gils and J. Wilson, personal communication). Another experiment could test whether juveniles are more likely to pick up the habit of seagrass consumption when in the presence of experienced adults. Repeating the same experiment with *islandica* red knots would be interesting as well, as it might provide clues on whether evolution has led to differences between the subspecies in seagrass preference, handling time and propensity to learn this behaviour socially.

Until the 1930s, large beds of *Zostera noltii* existed also in the Dutch Wadden Sea. Seagrass beds spanned approximately 150 km², including subtidal seagrass (van der Heide *et al.* 2007). We can only speculate whether *islandica* red knots consumed rhizomes in these beds, and how this history has affected their current behavioural reaction if encountered with seagrass. Another prey type that is present in Banc d'Arguin but not in the Wadden Sea, and has not been common in the Wadden Sea since red knots have started to use it after the last ice age (Buehler, Baker & Piersma 2006), is *Loripes lucinalis*. The capacity to process toxic *Loripes* (Chapters 2 and 4) is likely to have been an important evolutionary pressure for red knots in Banc d'Arguin, as *Loripes* is their most common food source. It is therefore expected that the physiological pathways for detoxification are better developed in *canutus* red knots than in *islandica* red knots. Social and individual

learning may be involved in the behavioural response to the availability of *Loripes* as a food source. Do juvenile and adult *islandica* and *canutus* react differently? How do juvenile *canutus* red knots learn to adjust their diet choice to optimize their intake? Can *islandica* red knots learn it? How does the presence of experienced adult *canutus* red knots influence this process?

To investigate the extent to which juvenile experience and traditions determine adult routines in the wild, an experiment could be carried out where juvenile *islandica* or *canutus* red knots are offered different prolonged diet treatments, before being released in the wild with satellite tags. Currently, juvenile as well as adult *canutus* and *islandica* red knots are present in captivity at the NIOZ, and plans are indeed being made to use the latest satellite technology to record their locations after release in the wild (K. Mathot and E. Kok, personal communication). Together with additional observations of their behavior in the field (by telescope, camera and benthos sampling, such as described in Chapters 5 and 6), this exercise has the potential to offer insights in the extent to which foraging behavior by red knots, diet choice as well as the degree of site fidelity and aggregation, is influenced by social interactions and different pathways of inheritance. An exciting, but logistically even more challenging, potential experiment would be to transport juvenile and adult *islandica* and *canutus* red knots from the Wadden Sea to the Banc d'Arguin, and follow them after release with the TOA system or modern satellite tags.

Finally, the potential significance of an interaction between gut microbiota and diet can be tested by physiological investigation of casualties, including their gut bacteria, and isotopic analysis of the blood. The potential parental inheritance of the gut bacterial community can be tested by faeces and cloacal samples of parent and young at the Arctic breeding sites. Laboratory experiments with foraging red knots can further test the relation between the microbiota and relative preferences and maximum intake rates of *Loripes* and seagrass rhizomes, and could include an antibiotics treatment.

CONCLUSIONS

In this thesis, we have investigated which environmental factors influence the foraging decisions of red knots wintering in the Banc d'Arguin. I hope that the results are of interest in themselves, but they become especially interesting when comparing them to the factors that we know to influence red knots wintering in the Wadden Sea, or in other parts of the world (Piersma 2007). The different decisions that they make can be used as behavioral indicators, and hint on the different selection pressures that act on the subspecies of red knots in the different areas; the forces that underlie their evolution. To interpret these hints, it is essential to take individual development into account. We must acknowledge that red knots are not 'optimal machines', but that each individual is constructed under the confluence of information from genes and the environment in the broadest sense, and that each decision is a consequence of all previous ones. Despite the logistic difficulties to determine genetic relatedness between red knots and to study parents and their offspring, the knowledge that we now have on foraging decisions, and

the sophisticated study methods that are developed, may enable unprecedented detail in the study of the role of behaviour in the development of individual variation and evolution in a wild animal species.

I have also highlighted the potential of complex foraging decisions to affect population dynamics of red knots and their bivalve prey, and increase the potential for stable coexistence. This was done from an optimality approach. The subsequent discussion on the importance of development, and the potential existence of red knot foraging traditions, implies that also social interactions may affect the functional response on different prey types, and thereby population dynamics. This adds another layer of complexity to the functional response. Of course it is in no one's interest to make the functional response as complex as possible, and of course it must be made as simple as possible to answer the question that is asked. But not simpler. I think that the implications of complex behaviour, including social behavioural traditions, on population dynamics and ecosystem functioning deserves further thought.

The prime reason why red knot studies have been so successful in showing the intricate relationships between animals and their natural environment, is that these relationships still exist to be studied. When compared to most terrestrial ecosystems, intertidal areas all over the world are relatively untouched by humans. But all are currently under threat of destruction, some more than others (Ma *et al.* 2014; Piersma *et al.* 2016). A simple advice is to reduce human impact in any possible way. This thesis does not offer more concrete directions on how to preserve these areas and what it is exactly that must be protected within these areas. What this thesis does show, is that everything matters. Red knots are not static; the behavioural decisions that they make, and even the way that they look, are directly affected by their environment. The Banc d'Arguin may still harbour the greatest number and highest densities of wintering shorebirds of all intertidal areas in the world (van de Kam *et al.* 2004). The beautiful diversity and complexity in their behaviour, of which this thesis shows only a glimpse, exists only because the rest of the Banc d'Arguin ecosystem is so diverse and complex as well. We can be just as creative and diverse as our surroundings allow us to be. And therefore we must protect it.

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APPENDIX 7.1. The functional response of diarrhetic red knots

In previous chapters, we have modelled both the digestive constraint and the toxin constraint as fixed maximum rates that cannot be surpassed. Indeed, red knots in the experiments of Chapters 2 and 4 do seemed to maximize their intake of *Loripes* until reaching this threshold value, which justified the use of a fixed value for functional purposes. However, when specifically modeling energy intake rate as a function of *Loripes* intake rate, it may be necessary to consider the diarrhetic effect that *Loripes* consumption has on red knots, which appears to seriously impair energy assimilation efficiency (V. Hin and T. Oudman unpublished data).

With increasing consumption of *Loripes*, energy intake rate increases. On the other hand, energy assimilation efficiency decreases. As a consequence, the maximum energy assimilation rates may be at intermediate intake rates of *Loripes*. Thus, the apparent constraint by red knots on *Loripes* intake may actually not be a fixed threshold, but the result of optimizing the intake rate of *Loripes* to maximize the assimilated energy intake. If we assume that this is the case, and further assume that assimilation efficiency decreases linearly with the ash-free dry flesh (AFDM_{flesh}) intake rate of *Loripes*, then we can calculate the function that describes this decrease. Resource intake rate (Y , mg AFDM_{flesh} s⁻¹) can then be described as a function of the densities of *Loripes* D_l and *Dosinia* D_d (nr/m²), and acceptance probabilities p_l and p_d :

$$Y = a \frac{p_l D_l e_l + p_d D_d e_d}{1 + ah(p_l D_l + p_d D_d)} \text{Eff} \quad (\text{A7.1})$$

$$\text{where } \text{Eff} = \text{eff}_0 - q \frac{p_l a D_l e_l}{1 + p_l a D_l h} \quad (\text{A7.2})$$

$$\text{and } a \frac{D_l k_l + D_d k_d}{1 + ah(D_l + D_d)} \leq c \quad (\text{A7.3})$$

where a is the searching efficiency (m²/s), h is the handling time per prey item (s) and e_l and e_d are the energy contents of a single prey item (mg AFDM_{flesh}). Energy assimilation efficiency (Eff) is described by a linearly decreasing function, starting at eff_0 and decreasing at a rate proportional to the AFDM_{flesh} intake rate of *Loripes*. Shell mass intake rate was assumed to be limited by a digestive constraint c (mg/s), given the individual dry shell masses of *Loripes* and *Dosinia* (k_l and k_d in mg). The optimal acceptance probabilities of *Loripes* and *Dosinia* (p_l and p_d) can be analytically derived from the above equations. The solutions however are not straightforward, and to solve them we used the mathematical software Maple (Maple 9.0 Math & Engineering software).

