Chapter 4

The effect of digestive capacity on the intake rate of toxic and non-toxic prey in an ecological context

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ABSTRACT Digestive capacity often limits food intake rate in animals. Many species can flexibly adjust digestive organ mass, enabling them to increase intake rate in times of increased energy requirement and/or scarcity of high-quality prey. However, some prey species are defended by secondary compounds, thereby forcing a toxin limitation on the forager's intake rate, a constraint that potentially cannot be alleviated by enlarging digestive capacity. Hence, physiological flexibility may have a differential effect on intake of different prey types, and consequently on dietary preferences. We tested this effect in red knots (*Calidris canutus canutus*), medium-sized migratory shorebirds that feed on hard-shelled, usually mollusc, prey. Because they ingest their prey whole and crush the shell in their gizzard, the intake rate of red knots is generally constrained by digestive capacity. However, one of their main prey, the bivalve *Loripes lucinalis*, imposes a toxin constraint due to its symbiosis with sulphide-oxidizing bacteria. We manipulated gizzard sizes of red knots through prolonged exposure to hard-shelled or soft foods. We then measured maximum intake rates of toxic *Loripes* versus a non-toxic bivalve, *Dosinia isocardia*. We found that intake of *Dosinia* exponentially increased with gizzard mass, confirming earlier results with non-toxic prey, whereas intake of *Loripes* was independent of gizzard mass. Using linear programming, we show that this leads to markedly different expected diet preferences in red knots that try to maximize energy intake rate with a small versus a large gizzard. Intra- and inter-individual variation in digestive capacity is found in many animal species. Hence, the here proposed functional link with individual differences in foraging decisions may be general. We emphasize the potential relevance of individual variation in physiology when studying trophic interactions.

INTRODUCTION

Constraints on food intake rate determine the shape of the functional response, an equation that is fundamental in population dynamical theory as it relates a forager's intake to the density of its prey (Holling 1959; MacArthur & Pianka 1966). The nature of these intake constraints also determines food preferences (i.e. the proportion of a prey type in the diet when not limited by availability of prey) (Westoby 1974; Belovsky 1978). Many animals appear to be constrained by internal processing of the prey (Jeschke, Kopp & Tollrian 2002). In these animals, flexibility in stomach- and/or gut size is often observed, allowing them to adjust their digestive capacity to changes in requirements and/or food availability (Secor & Diamond 1995; Starck 1999; Dekinga *et al.* 2001; Olsson *et al.* 2007; McWilliams & Karasov 2014). However, not all food-processing pathways may be equally dependent on digestive organ size. For example, the maximum intake rate of prey with high ballast-mass may be dependent on stomach size, whereas the intake of toxic prey may be constrained by other processes that are independent of stomach size, such as the removal of toxic compounds from the body. Consequently, changing digestive organ size may not only change maximum food intake rate, but also the relative aversion for prey containing toxic compounds.

The relations between organ size, digestive capacity, prey intake rates and diet preferences have been studied step by step in experiments with red knots (*Calidris canutus*) (Table 4.1). Red knots are medium-sized migratory shorebirds that feed on different species of mollusc prey which they ingest whole and crush in their gizzard (Dekinga & Piersma 1993; Piersma, Koolhaas & Dekinga 1993; Buehler & Piersma 2008). Gizzard size in red knots is highly variable both between and within individuals (van Gils *et al.* 2003a; van Gils *et al.* 2005a), and is related to the digestive quality of the diet, calculated as ash-free flesh mass over dry ballast mass (Piersma, Koolhaas & Dekinga 1993; van Gils *et al.* 2005b). In captivity experiments, gizzard size can increase or decrease by 50% within one week by offering a diet of hard-shelled or soft prey items, respectively (Dekinga *et al.* 2001). The intake rate of bivalve prey is limited by its shell-mass content as shown by van Gils *et al.* (2003a), who found that shell-mass processing rate relates linearly to squared gizzard mass. Since then, only two exceptions have been found to this 'rule'. The first one is in red knots staging in the Yellow Sea, *C. c. rogersi* and *C. c. piersmai* (Battley *et al.*

Table 4.1 Experimental studies on gizzard size and diet in red knots.

Study result	Reference
Gizzard size is related to diet	Piersma, Koolhaas & Dekinga (1993)
Gizzard size responds to changes in diet	Dekinga *et al.* (2001)
Shell-mass processing rate is a function of gizzard size	van Gils *et al.* (2003a)
Shell-mass processing rate explains diet preferences	van Gils *et al.* (2005b)
Shell-mass processing rate is higher on easy-to-crush prey	Yang *et al.* (2013)
Maximum intake on toxic prey not set by shell-mass processing rate	Chapter 2

2005), which digest the bivalve *Potamocorbula laevis* faster than expected from their gizzard size, probably because the force needed to crush this species is very small (Yang *et al.* 2013). The second exception was found in Banc d'Arguin, Mauritania, the main wintering area of the red knot subspecies *C. c. canutus* (Buehler & Piersma 2008; Leyrer *et al.* 2013). There, the most abundant mollusc prey, *Loripes lucinalis*, is easy to digest due to its thin shell. However, *Loripes* contains high levels of sulphur, which is produced by endosymbiotic bacteria in their gills (Johnson, Diouris & Lepennec 1994). Sulphur content of *Loripes* in Mauritania was estimated at 2–4% of dry flesh mass (van der Heide *et al.* 2012), and in such concentrations may be toxic to any animal species (Hall 2007).

In Chapter 2, we showed experimentally that red knots foraging *ad libitum* on *Loripes* are limited by the presumably toxic concentration of sulphur rather than by shell-mass processing rate. This toxic effect also explained the observed prey preferences, both in the laboratory (Chapter 2) and in the field (Chapter 3). Whereas red knots *C. c. islandica* in the Wadden Sea are solely limited by shell-mass processing rate and always preferred the prey with the highest digestive quality (van Gils *et al.* 2005b), *C. c. canutus* in Mauritania preferred a mixed diet of toxic but easy-to-digest *Loripes* and *Dosinia isocardia*, the latter which is harder to digest but not toxic (Chapters 2 and 3). The preferred proportion of *Loripes* in the diet appeared to depend on the strength of the toxin constraint relative to the digestive constraint. Hence, if gizzard size changes digestive capacity but not detoxification rate, the preference for *Loripes* is expected to be higher in birds with a small gizzard than in birds with a large gizzard.

In this study we tested (1) whether the maximum intake rate of sulphur-containing *Loripes* is indeed independent of gizzard size, and (2) whether maximum intake rate of *Dosinia* matches the earlier observed linear relation with squared gizzard size. This was done by manipulating gizzard sizes of 6 captive red knots in Mauritania through prolonged diets of either soft or hard-shelled prey, and afterwards measuring intake rates on both prey species in separate trials. Subsequently, the procedure was repeated with the soft- and hard-shelled diets reversed. In the discussion section we extend the linear programming model (Westoby 1974; Belovsky 1978; Belovsky & Schmitz 1994) that is described in Chapter 2, to quantify the expected diet preferences as a function of gizzard size.

METHODS

Birds and gizzard manipulation

The experiment was performed at the Iwik research station located on the peninsula of Iwik in the Banc d'Arguin, Mauritania. Six adult red knots were caught using mist nets on the night of 20 January 2012, and ringed with unique combinations of colour-rings for identification. Birds were held in an indoor cage (1.5 × 1 × 0.5 m) in a room with windows, and temperatures varying between 18 and 22°C. Food availability outside experimental trials was adjusted to maintain a low but not unnatural body mass, between 100 and 110 g (Leyrer *et al.* 2012). Together with food deprivation for at least 2 h before each trial, this

ensured that all birds were motivated to feed during the experimental trials. Gizzard masses were non-invasively measured regularly using ultrasonography (Dietz *et al.* 1999; Dekinga *et al.* 2001) (for more details see Appendix 4.1).

Birds were randomly divided into two groups of three birds with each group receiving a different gizzard-manipulating food regime outside the experimental trials. Initial differences in gizzard mass between groups were not significant ($F_{1,4}$ = 3.9, P = 0.12). Group 1 received hard-shelled prey to maintain a large gizzard. Prey for this group were collected on the sandy beach near the research station and consisted mainly of *Dosinia isocardia* but also small *Senilia senilis* and *Bittium reticulatum*. Additionally, flesh of large *Senilia senilis* was provided because not enough hard-shelled prey could be collected to satisfy the energy demands of the birds. Group 2 was provided only with flesh of *Senilia senilis*, which is a food type that decreases gizzard mass (Dekinga *et al.* 2001). Outside the experimental trials, birds had constant access to freshwater. Fourteen days after the birds had been caught, a first series of experimental trials was performed, spread over a period of ten days. After this period, the food regimes outside the trials were reversed between the groups, now with group 1 being provided soft food and group 2 a mixture of hard-shelled prey and soft food. Seven days after the reversal, a second series of experimental trials was performed over a period of eight days.

Experimental design

The experiment comprised a total of 60 trials. The first series of trials (thus before the gizzard-manipulation reversal) consisted of 39 trials, measuring intake rate of isolated birds either on *Dosinia isocardia* (3 or 4 trials per bird, 19 in total) or *Loripes lucinalis* (3 or 4 trials per bird, 20 in total). In the second series (thus after the gizzard-manipulation reversal), two *Dosinia* trials and two *Loripes* trials were performed with each bird (24 trials in total). During the second series of trials, one bird in group 2 started showing general signs of illness such as improper preening, ruffled feathers, reduced feeding and docile behaviour. The trials of this bird after the onset of illness (3 trials: 2× *Loripes* diet, 1× *Dosinia* diet) were removed from the intake-rate analysis as well as from the gizzard-mass analysis. *Dosinia* and *Loripes* were gathered daily in a sieve (mesh size 2 mm) from a sandy beach and a seagrass bed, respectively. Bivalves were offered alive, one day after gathering. During each trial, food (either *Dosinia* or *Loripes*) and seawater was provided *ad libitum* for 2 h, during which total intake was measured.

We estimated the number and size distribution of the eaten prey items by counting and measuring shell lengths of a sub-sample of each species to the nearest 1 mm at the start and at the end of each trial. Each sub-sample consisted of 100 prey items, or all prey items if less than 100 prey were left after the trial. Size distribution was estimated in length classes of 1 mm. To determine length-specific dry mass of shell (DM_{shell}) and ash-free dry mass of flesh ($AFDM_{flesh}$), 100 individuals of each prey species were stored in 4% borax-buffered formalin before analysis at the NIOZ Royal Netherlands Institute for Sea Research. Length of each individual was measured to the nearest 0.1 mm, after which flesh and shell were dried separately at 60°C for 3 days, weighed, incinerated at 560°C for 5 hours (only the flesh) and weighed again. The estimated number of ingested prey items

in each size-class was multiplied by its estimated DM_{shell} to arrive at an estimation of total ingested DM_{shell}. These estimates were compared with measured dry-mass of the faeces produced from the start until 4 h after the end of each trial. Pooling all before-trial shell measurements per species and setting negative estimations of the eaten number of prey in a size class to zero (which occurred only in the rare length classes) improved the correlation with dry faeces mass from 0.81 (Pearson's coefficient, $t = 11.7$, df = 69, $P < 0.001$) to 0.84 ($t = 13.0$, $P < 0.001$).

Statistical analysis

Statistics were performed in R version 3.1.0 (R Development Core Team 2013). The effects of group (group 1 or group 2) and diet (soft prey or hard-shelled prey) on gizzard mass during the experimental trials were tested by AICc comparison (function "ipv" in package "ipv") of linear mixed-effects models (function "ipv" in package "ipv"), estimating parameter values by maximizing log-likelihood (Burnham & Anderson 2002). Bird ID was included in each model as a random effect. Trends in the rate of change in gizzard mass from catch until the end of the experiments were analysed by local regression (function "ipv", span = 0.5) on 13–16 measurements for each bird, spread over the whole period. These regressions were used to estimate gizzard mass during each particular experimental trial.

The effects of gizzard size (large or small gizzard) and prey species (either *Dosinia* or *Loripes*) on intake rate in the experiment were tested by AICc comparison of linear mixed-effect models, including Bird ID as a random effect. A variance structure was incorporated to correct for different variances in *Dosinia* and *Loripes* intake rates. *Dosinia* had a larger size range (3–15 mm) than *Loripes* (4–12 mm), and as larger bivalves contained exponentially more shell and flesh, estimations of DM_{shell} eaten from larger size classes gave exponentially larger variances. For *Loripes* as well as for *Dosinia*, the relation between DM_{shell} and shell length was estimated with a local regression function (function "ipv", span = 0.6), as non-linear regression did not give a satisfying fit (Bijleveld *et al.* 2015a) (for details see Appendix 4.2).

Ethics statement

The experiment was performed under full permission by the authorities of the Parc National du Banc d'Arguin (PNBA). No animal experimentation ethics guidelines exist in Mauritania. However, the experiment was carried out in strict accordance with Dutch animal experimentation guidelines. The NIOZ Royal Netherlands Institute for Sea Research has been licensed by the Dutch Ministry of Health to perform animal experiments under license number 80200. This license involves capture and handling of animals, and performing experiments, which nonetheless should be individually approved by the Animal Experimentation Committee (DEC) of the Royal Netherlands Academy of Arts and Sciences (KNAW). The DEC does not provide permits for experiments in foreign countries, but provided approval for equivalent experiments in the Netherlands by the same persons under permit number NIOZ 10.05, involving the capture of red knots, performing non-invasive experiments consisting of prolonged diets of

natural food types (i.e. foods that regularly occur in the diet of wild red knots) and repeated gizzard size measurements, and includes permission to release healthy animals in the wild after the experiment.

All possible efforts were made to minimize physical and mental impact on the experimental animals. Each bird was weighed and visually inspected for general condition daily, and removed from the experiment when not healthy (one bird). The reasons for the experiment to take place in Mauritania were purely scientific and by no means to avoid ethics guidelines. All experimental animals were released in the wild in healthy condition after the experiment.

RESULTS

The diet treatments successfully resulted in gizzard mass changes in the experimental red knots (Fig. 4.1, model comparison in Table 4.2, see Table A4.1 for model estimates). Although all birds initially reduced gizzard mass, a diet of hard-shelled prey resulted in larger gizzards (estimate ± SE: 8.3 ± 0.3 g) than soft prey (6.1 ± 0.3 g). Group (group 1 or group 2) had no significant effect on gizzard mass. However, the hard-shelled diet led to a larger rate of gizzard mass increase in group 2 than in group 1 (significant interaction between diet and group, see Table 4.2 and model estimates in Table A4.1), presumably because the birds were less eager to increase gizzard mass soon after the catch (see also Fig. 4.1). The diet-induced rates of change in gizzard mass were comparable to those found earlier (Dekinga *et al.* 2001) (for details see Appendix 4.3).

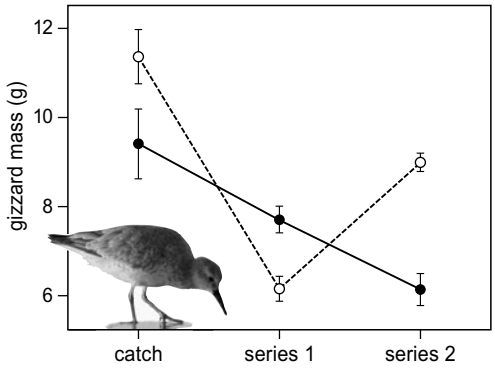

Figure 4.1 Mean gizzard mass of birds directly after catch, during the first and second series of trials. Directly after catch, the 6 red knots were randomly divided into two groups (group 1 and group 2). Both groups received different diets outside the experimental trials (soft or hard-shelled prey) to manipulate gizzard size. Initial differences in gizzard mass between groups were not significant ($F_{1,4}$ = 3.9, P = 0.12). After catch, all birds decreased gizzard mass, but Group 1 had larger gizzards than group 2 during the first series of trials, and smaller gizzards during the second series (Table 4.2, models 1.1 to 1.5), showing that the manipulation of gizzard size was successful. Each group consisted of three birds. However, data collected on one bird from group 2 after it became sick during series 2 was omitted from the graphs and the analysis. Error bars show standard error.

Gizzard mass manipulations had an effect on intake rate (expressed as DM_{shell}), dependent on prey species (model 2.1 in Table 4.2, see Table A4.1 for model estimates). As expected, DM_{shell} intake of toxic *Loripes* did not change with an increase in gizzard mass (estimated change from 1.25 to 1.31 mg/s, $t = 0.65$, $P = 0.52$), whereas intake of non-toxic *Dosinia* did increase with gizzard mass (estimated change from 2.00 to 3.12 mg/s, $t = 3.73$, $P < 0.001$). DM_{shell} intake on a *Loripes* diet was lower than on a *Dosinia* diet for small gizzard birds (estimated difference 0.75 mg/s, $t = 3.21$, $P = 0.002$) as well as for large gizzard birds (estimated difference 1.81 mg/s, $t = 8.37$, $P < 0.001$). These results are depicted in Figure 4.2, where gizzard masses are also shown on a continuous scale. The results indicate that the shell-mass processing constraint was alleviated with an increase

Table 4.2 Second-order Akaike's information criterion (AICc) comparison of statistical models.

Model	Fixed effects[a]	K[b]	ΔAICc	AICc weight	Cumulative weight	LL[c]
Response variable: Gizzard mass						
1.1	Diet × group	6	–	0.69	0.69	–81.2
1.2	Diet	4	2.90	0.16	0.85	–85.2
1.3	Diet + group	5	3.00	0.15	1	–84.1
1.4	1	3	44.23	0	1	–107.1
1.5	Group	4	44.89	0	1	–106.2
Response variable: DM_{shelld} intake rate of either *Loripes* or *Dosinia*						
2.1	Gizzard × species	7	–	0.96	0.96	–40.4
2.2	Species	5	7.45	0.02	0.98	–46.7
2.3	Gizzard + species	6	7.76	0.02	1	–45.6
2.4	1	4	35.52	0	1	–61.9
2.5	Gizzard	5	37.09	0	1	–61.5
Response variable: log transformed DM_{shell} intake rate						
3.1	Log(gizzard)	4	–	0.85	0.85	–17.07
3.2	Log(gizzard) + species	6	4.34	0.10	0.95	–16.86
3.3	Log(gizzard) × species	8	5.65	0.05	1	–14.96
3.4	species	5	20.53	0	1	–26.17
3.5	1	3	22.11	0	1	–29.26

Model selection based on AICc, with a penalty of 2 per added parameter (Burnham & Anderson 2002). Models are ordered by adequacy, starting with the minimum adequate model. Model 1.2 is competitive with model 1.1. Model 2.1 and 3.1 do not have competitors. All models are linear mixed models with a Gaussian error structure, and contain bird ID as a random effect. Models 2.1 to 2.5 contain a variance structure based on prey species.

[a] In model 1.1 to 1.5, factor "diet" refers to the diet outside the experimental trials, being either soft or hard–shelled. Factor "group" refers to the order of these diet treatments (group 1 or group 2). In models 2.1 to 2.5, factor "gizzard" refers to gizzard size during the trial, which was either small or large; "species" refers to the prey species being offered, which was either *Dosinia* or *Loripes*. In models 3.1 to 3.5 log(gizzard) is a continuous variable that refers to the logarithm of estimated gizzard mass during the trial; species refers to prey species, which was either *Dosinia isocardia*, *Cerastoderma edule* or *Macoma balthica*. The symbol × means that the main terms as well as their interaction are fixed effects in the model. Models 1.4, 2.4 and 3.5 contain only an intercept, no fixed effects.

[b] The number of parameters in the model.

[c] Log likelihood.

[d] Dry ballast mass.

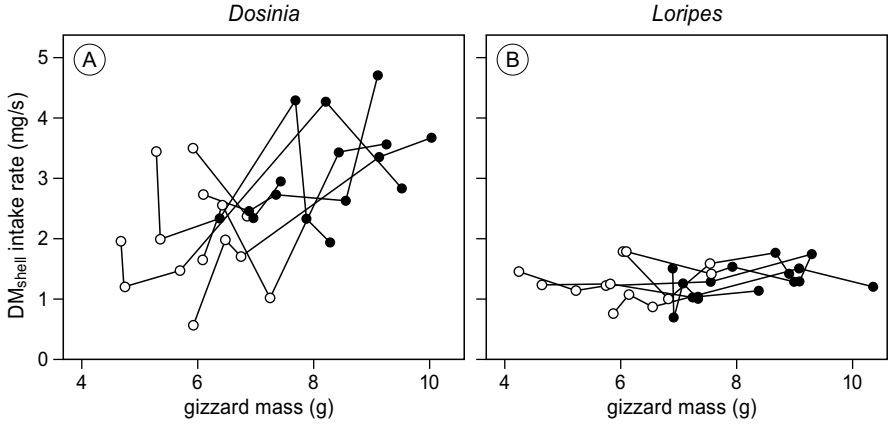

Figure 4.2 Dry shell mass (DM_{shell}) intake rate on a *Dosinia* diet (A) and on a *Loripes* diet (B). Lines connect all trials of the same bird when it was in the small gizzard group and in the large gizzard group. Intake of *Dosinia* was higher for birds with large gizzards, whereas intake of *Loripes* was not affected by gizzard size (model 2.4 in Table A4.1). *Loripes* intake rate was generally lower than *Dosinia* intake rate. These results confirm that intake of *Dosinia* is limited by a digestive constraint, whereas intake of *Loripes* is limited more stringently, presumably by its toxic load, and independent of gizzard mass.

in gizzard mass, as predicted, and that the toxin constraint was independent of gizzard mass. To test if morphological characters of individual birds other than gizzard size influenced intake rate, body mass, bill length, tarsus length and wing length of the individual birds were separately added as explanatory variables to model 2.1. None of these variables improved the statistical fit of the model (results not shown).

DISCUSSION

Maximum intake rate as a function of gizzard mass

To confirm that the relation between gizzard mass and dry shell-mass (DM_{shell}) intake rate on *Dosinia* agreed with the relations earlier observed by van Gils *et al.* (2003a), we compared the two outcomes. Van Gils *et al.* measured maximum DM_{shell} intake rates in 6 captive red knots (*C. c. islandica*) in the Dutch Wadden Sea on two non-toxic bivalve species, *Cerastoderma edule* and *Macoma balthica*. Similar to the present study, they manipulated gizzard masses by placing birds randomly in one of two groups, one with a soft prey diet and the other with a hard-shelled diet. They estimated gizzard mass in each bird as the mean of a series of gizzard measurements in the course of the experimental trials. By comparing linear models, they concluded that DM_{shell} intake was independent of bird individual, prey species and prey size. They found a linear relationship with gizzard mass on log-transformed data ($R^2 = 0.48$, $P < 0.001$, Fig. 4.3).

The effect of gizzard mass on prey intake rate, and a potential difference between the two studies on this relation was tested by combining both datasets, and comparing AICc values of linear mixed-effect models on log-transformed data (models 3 in Table 4.2),

containing bird ID as a random effect. As expected, the model that best explained DM_{shell} intake rate did not include prey species (*Dosinia*, *Cerastoderma* and *Macoma*; model 3.1 in Table 4.2, see Table A4.1 for model estimates), but did contain gizzard mass in the following way:

$$c = 10^{-1.244} G^{1.9} \quad , \tag{4.1}$$

where c is DM_{shell} intake rate (mg/s) and G is gizzard mass (g). This estimated relation does not differ from $c = 10^{-1.293} G^{2.0}$ as found by van Gils *et al.* (2003a), as standard errors completely overlap (Fig. 4.3).

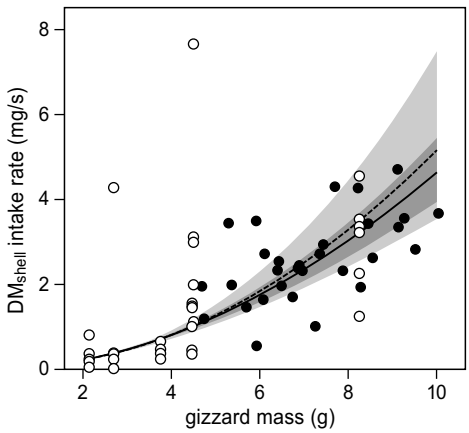

Figure 4.3 Linear regression on log-transformed DM_{shell} intake on non-toxic prey against log-transformed gizzard mass. Data from this study on *Dosinia* was combined with data from van Gils *et al.* (2003) on other non-toxic prey species. Adding the current data to the regression derived by van Gils *et al.* (2003) slightly changes the regression line (though not significantly; from dashed to solid line), but greatly reduces standard error (from light to dark grey area). Parameter estimates are shown in Table A4.1 (model 3.1). Note that van Gils *et al.* (2003a) averaged gizzard mass measurements per bird, whereas we estimated gizzard mass in each trial by interpolating measurements.

Within- and between-year variation in the toxin constraint

Maximum intake rate of *Loripes* in this study did not differ between large- and small-gizzard birds (Fig. 4.2). Because sulphur, presumably the toxic compound in *Loripes*, resides in the flesh and not the shell, we will from here on refer to the toxin constraint in terms of ash-free dry flesh mass ($AFDM_{flesh}$) instead of DM_{shell}. The best estimate of $AFDM_{flesh}$ intake rate is given by an intercept mixed-effect model on the *Loripes* data, with bird ID as a random effect, giving an estimate of 0.21 mg/s, with a within-individual variance of 0.002 and a between-individual variance of 0.0005 (Dingemanse & Dochtermann 2013). One year earlier, the intake constraint on *Loripes* was estimated at 0.12 mg/s (Chapter 2), with a within-individual variance of 0.0003 and a between-individual variance of 0.001 (T. Oudman, unpublished data). The large difference between the two years

in the intake constraint, despite small within- and between-individual variances within each year, is remarkable. This difference may be explained by yearly variation in the toxic load of *Loripes*, and/or by a difference in the capability or costs paid by red knots to deal with the toxic load of *Loripes*. The high consistency in *Loripes* intake between birds within years favours the first explanation. Differences in toxic load may relate to the mixotrophic life style of *Loripes* (van der Geest *et al.* 2014) and potentially has effects on the spatial distribution and population dynamics of *Loripes*, by influencing predation risk (van Gils *et al.* 2012; Curley, Rowley & Speed 2015).

(In)flexibility of the toxin constraint

Most of the mollusc biomass available to red knots in Banc d'Arguin consists of *Loripes* (van der Geest *et al.* 2011; van Gils *et al.* 2012; Ahmedou Salem *et al.* 2014; van den Hout *et al.* 2014), but its observed proportion in the diet is low (Chapter 3; van Gils *et al.* 2012; Onrust *et al.* 2013; van den Hout *et al.* 2014). Hence, releasing the toxin constraint would likely enable red knots to increase energy intake rate or decrease required foraging time in the field. The physiological processes that make *Loripes* toxic to red knots have not been studied, but may involve sulphide formation in the intestines during digestion. Most vertebrates can detoxify sulphide to a limited extent by oxidation to sulphate in the mitochondria of liver cells and red blood cells, and excretion by the kidney (Bagarinao 1992; Grieshaber & Völkel 1998). Energy investment in these detoxification pathways may enable red knots to increase their sulphur tolerance, but the consistent low fraction of *Loripes* in the diet and the low individual variation in the toxin constraint (this study; Chapter 2) suggests that sulphur tolerance either cannot be adjusted or is very costly to increase.

Diet preferences as a function of gizzard size

Gizzard masses of red knots caught in Banc d'Arguin are variable between individuals (mean = 9.89 g, SD = 1.30 g; van Gils *et al.* 2005a), ranging from 4 to 15 g (A. Dekinga, unpublished data). These differences in gizzard mass may accompany differences in diet preferences, as gizzard mass influences potential intake on *Dosinia*, but not on *Loripes*. Linear programming models can be used to quantify optimal diet preferences as a function of the constraints on intake rate under the assumption of energy maximization (Westoby 1974; Belovsky 1978; Belovsky & Schmitz 1994). In Chapter 2, we use a linear programming model to calculate expected diet preferences for energy intake maximizing red knots foraging on *ad libitum Loripes* and *Dosinia*. This model calculates which combinations of intake rates on *Dosinia* and *Loripes* are possible given both the shell-mass processing constraint and the toxin constraint on *Loripes*, and subsequently determines which of these combinations provides the highest energy intake rate. Based on measured values of the shell-mass processing constraint and the toxin constraint on *Loripes*, but without taking gizzard mass into account, it is deduced that the optimal proportion of *Loripes* in the diet is 39% in terms of dry shell mass, when both prey occur in *ad libitum* abundances. In Chapter 3, we show how this optimal proportion varies with densities of both prey types. Replacing a constant shell-mass processing constraint by the here

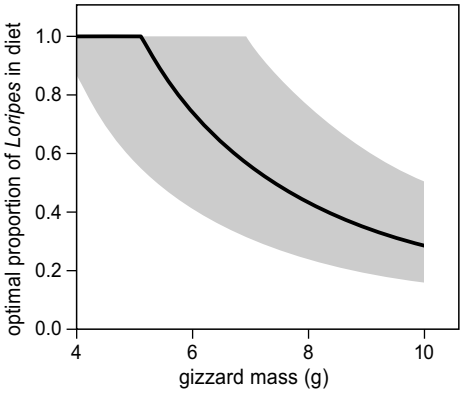

Figure 4.4 The predicted optimal proportion of *Loripes* in terms of dry shell mass in the diet of an energy intake maximizing red knot that has *ad libitum* access to both *Loripes* and *Dosinia*. Red knots with small gizzards are expected to feed exclusively on *Loripes*, whereas red knots with large gizzards are expected to have a large share of *Dosinia* in the diet. Grey area shows 95% prediction interval.

derived gizzard-mass dependent shell-mass processing constraint (eq. [4.1]) and parameterizing the model with the here obtained values (see Appendix 4.4 for a detailed model description) shows that this proportion changes considerably with gizzard mass (Fig. 4.4). The model predicts that energy maximizing birds with a gizzard mass below 5.2 g prefer an exclusive *Loripes* diet. Red knots with greater gizzard masses are expected to have a lower proportion of *Loripes* in the diet, which is less than 40% of total DM_{shell} intake rate in birds with a 10 g gizzard. Hence, model predictions show that, given the observed variation in gizzard sizes of red knots in the wild, considerable inter- and intra-individual variation in diet preferences can be expected. This result may translate to many other species, because flexibility in digestive organ mass is a general phenomenon (Piersma & Lindström 1997), being observed in mammals (Hammond *et al.* 1994), reptiles (Secor & Diamond 1995), fish (Olsson *et al.* 2007) and birds (McWilliams & Karasov 2014). Toxin constraints are observed widely too, especially in herbivores, (e.g. Rosenthal & Berenbaum 1992), but are not a prerequisite to explain a functional link between individual variation in physiology and diet preferences. For example, external handling constraints may also, in combination with digestive capacity, cause a mixed diet that depends on the strength of the digestive constraint (Belovsky & Schmitz 1994).

To experimentally test the here predicted link between digestive capacity and diet preferences comes with complications. If the animals adjust their preferences to gizzard mass in an experiment with gizzard manipulations, it is clear that they base their choice on physiological state. However, if the animals do not adjust their preferences, the here predicted link may still be correct, but the causality reversed; in that case, gizzard mass may be adjusted to individual differences in diet (see Bijleveld *et al.* 2014). Hence, the model cannot be proven incorrect in the experimental setting presented in this paper, but should be accompanied by field observations. This will be the subject of the next chapter.

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APPENDIX 4.1. Gizzard mass measurements

Gizzard size of each bird was measured within one day after catch, and every third day during both series of trials. They were measured non-invasively by AD and TO with an ultrasound apparatus (model Aquilla, Pie Medical Benelux, Maastricht, The Netherlands), according to the procedure described in Dekinga *et al.* (2001). The observer did not know to which experimental group each bird belonged. Height (H) and width (W) were always measured twice and both averaged. Average H and W were transformed to gizzard mass (G) by the formula $G = -1.09 + 3.78HW$, derived in a calibration study on 29 dead red knot bodies with variable gizzard masses (A. Dekinga, unpublished data). Gizzard mass estimations did not differ between AD and TO when repeated by both observers (n = 35). The slope of the major axis regression (function "ipv" in R package "ipv") did not differ from 1 (95%CI [0.96,1.66], $r = 0.28$, $P = 0.1$) and the elevation did not differ from zero (95%CI [–4.97,0.48], $t = -1.6$, $P = 0.1$). Gizzard mass on each day was modelled for each bird with a polynomial model, fitted to all measurements (function "ipv" in the basic package in R, span = 0.5).

APPENDIX 4.2. Estimating dry shell mass from shell length

Allometric relations are classically estimated as power functions of the form $Y = aX^b$ (Huxley 1932). When this method is applied to the relation between shell length and shell dry mass (DM_{shell}) in *Loripes* and *Dosinia*, DM_{shell} of individuals between 8 and 10 mm are underestimated (see Fig. A4.1). The exponent of the allometric equation appears to rise after 8 mm of length. This appears to be a general tendency in bivalves (Katsanevakis *et al.* 2007; Hendriks *et al.* 2012; Bijleveld *et al.* 2015a). Therefore, we expect the inflected curve to be a consequence of the ontogeny of bivalves. Fitting a loess function instead of a power function accounts for the changing exponent (Bijleveld *et al.* 2015a).

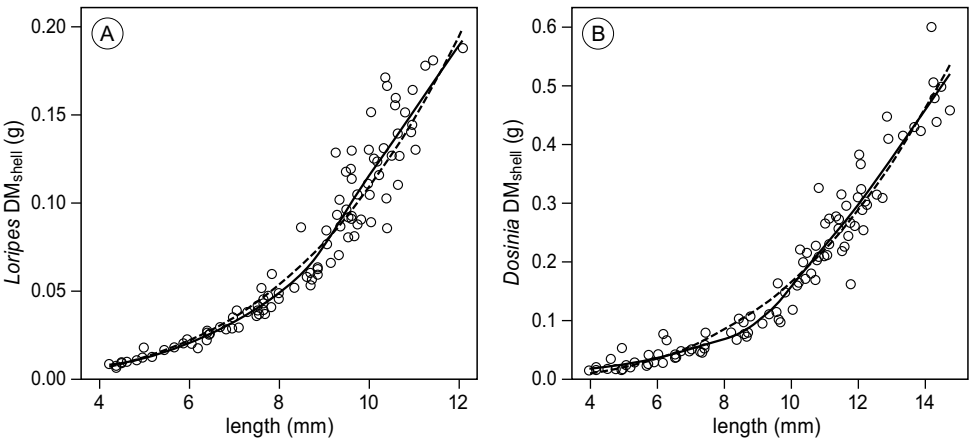

Figure A4.1 Dry shell mass (DM_{shell}) as a function of length for *Loripes* (A) and *Dosinia* (B). Fitting a power curve (dashed line) gives an overestimation of DM_{shell} in medium sized (8–10 mm) individuals, in both prey species. Fitting a loess curve (span = 0.6) solves this issue (solid line). Note the different scalings of the axes.

APPENDIX 4.3. Rates of change in gizzard mass

Initially, all birds decreased gizzard mass after catch (mean ± between-individual SE: 0.40 ± 0.09 g/day). After reaching a minimum around day 10 after catch, group 1 birds slightly increased gizzard mass again (0.23 ± 0.02 g/day), whereas group 2 on average remained stable (0.04 ± 0.08 g/day). After the diet switch at day 24, group 1 decreased gizzard mass (0.24 ± 0.02 g/day) whereas gizzard masses of group 2 increased (0.30 ± 0.06 g/day). The observed rate of diet-related gizzard mass increase was identical to the rate observed by Dekinga *et al.* (2001) who found a diet-induced rate of increase of 0.30 ± 0.05 g/day. The diet-induced rate of decrease was slightly weaker in this study than in Dekinga *et al.* (–0.38 g/day, SE not given), which however fits well with the here observed initial decrease rate after catch.

Table A4.1 Parameter estimates in fixed part of minimum adequate statistical models.

Parameter	Estimate	SE	DF[a]	*t*-value	*P*-value
Model 1.1: Gizzard mass ~ diet × group + (1|Bird)					
Intercept	6.09	0.38	49	16.03	<0.0001
Hard-shelled diet	1.62	0.39	49	4.16	0.0001
Group 2	0.06	0.52	4	0.11	0.91
Hard-shelled diet : group 2	1.23	0.53	49	2.32	0.02
Model 2.1: DM_{shell}[b] intake rate ~ gizzard × species + (1|Bird)					
Intercept	1.25	0.10	51	12.66	<0.0001
Large gizzard	0.07	0.10	51	0.65	0.52
Dosinia	0.75	0.23	51	3.21	0.002
Large gizzard : Dosinia	1.06	0.32	51	3.35	0.002
Model 3.1: log(DM_{shell} intake rate) ~ log(gizzard) + (1|Bird)					
Intercept	–1.21	0.17	53	-6.88	<0.0001
Log(gizzard)	1.87	0.24	53	7.83	<0.0001

NB: All models are linear mixed-effects models (function "lme" in package "nlme" in R), with bird-ID as a random effect. Parameters were estimated by maximizing the log-likelihood. In model 1.1, gizzard mass is measured in g, diet refers to either a soft or a hard-shelled diet, and group refers to experimental group (either 1 or 2, differing only in the order of the diet treatments). In model 2.1, DM_{shell} intake rate refers to dry shell-mass intake rate (mg/s), gizzard refers to the experimental treatment (being either small on a soft diet or large on a hard-shelled diet), and species refers to the prey species, being either *Loripes* or *Dosinia*. In model 3.1, log(gizzard) refers to the natural logarithm of gizzard mass (measured in g). A variance structure was incorporated in model 2.1 to correct for different variances in the *Loripes* and *Dosinia* trials.

[a] Degrees of freedom

[b] Dry shell mass

APPENDIX 4.4. Predicting diet preferences from gizzard mass: a linear programming model

A situation is assumed in which both *Loripes* and *Dosinia* are offered *ad libitum* to red knots that are maximizing energy intake rate. The idea of the linear programming model is to first derive all possible combinations of dry shell mass intake rates on *Dosinia* and *Loripes* (r_d and r_l, measured in mg/s; see Table A4.2 for a list of all used symbols) while respecting both the ballast-mass processing constraint and the toxin constraint (Fig. A4.2 A). Then it is determined which of all possible combinations of r_d and r_l provides the highest energy intake rate, denoted as $R^* = (r_d^*, r_l^*)$. In Chapter 2, we deduced that as long as *Loripes* is limited by a toxin constraint, R^* is found by drawing both constraints in a plane spanned by r_d and r_l. R^* is the point where both constraint lines intersect. It is calculated as:

$$(r_d^*, r_l^*) = (c - q, q)\,, \tag{A4.1}$$

where c is the digestive constraint (maximum dry shell-mass (DM_{shell}) intake in mg/s); q is the toxin constraint (maximum DM_{shell} intake *Loripes* in mg/s). The units differ from Chapter 2, where intake rates were measured in individuals per second. Instead, we measured intake rate in mg DM_{shell} per second to facilitate the implementation of the current experimental results, where prey of variable sizes were used. We can do so because no relation between prey length and the ratio of ash-free dry flesh-mass over dry shell mass ($AFDM_{flesh}$:DM_{shell}) was found, neither in *Dosinia* (R^2 = 0.006, P = 0.22) nor in *Loripes* (R^2 = 0.005, P = 0.22). Energy content of *Dosinia* and *Loripes* (r_d and r_l), measured as $AFDM_{flesh}$ per unit of DM_{shell}, was estimated for both *Dosinia* and *Loripes* by averaging all measured individuals without accounting for size, resulting in 0.057 ± 0.001 (mean ± SE) and 0.163 ± 0.005 mg $AFDM_{flesh}$ per mg DM_{shell}, respectively.

The optimization procedure can be performed graphically by drawing both constraints as lines in a plane spanned by r_d and r_l. In each point in this plane, total intake rate of ash-free flesh mass can be calculated by for each prey species multiplying DM_{shell} intake rate with energy content, and adding them up:

$$Y = r_d\, e_d + r_l\, e_l\,. \tag{A4.2}$$

The optimal combination of r_d and r_l (R^*) is found by maximizing Y, given that neither constraint line is crossed. Fig. A4.2 B shows that changing gizzard mass from 6 g to 9 g leads to an increase in the digestive constraint, but not the toxin constraint. Fig. A4.2 B shows the constraint lines both for a 6 and a 9 g gizzard in the plane spanned by r_d and r_l, showing a shift in r_d^* but not in r_l^*. Hence, the absolute amount of *Loripes* in the diet remains constant, but the proportion of *Loripes* in the diet decreases when gizzard mass increases (Fig A4.2 C).

The relation between gizzard mass and R^* can be formalized by inserting equation 4.1 from the main text, denoting c as a function of gizzard mass G (g), into equation A4.1. Contrastingly, q is constant and estimated as 1.29 mg DM_{shell} per second (linear mixed-

effect intercept model on *Loripes* data, containing bird-ID as random effect). Hence, R^* is dependent on gizzard mass in the following way:

$$(r_d^*, r_l^*) = (10^{-1.244} G^{1.9} - 1.29, 1.29) . \quad \text{(A4.3)}$$

When gizzard mass drops below 5.2 g, then *Loripes* intake rate is no longer limited by the toxin constraint, but becomes limited by the shell-mass processing constraint. In that case r_d^* becomes zero (see for details Chapter 2). The expected diet preferences, which we define as the optimal proportion of *Loripes* in the diet, is calculated by dividing r_l^* by total DM_{shell} intake:

$$\frac{r_l^*}{r_d^* + r_l^*} = \frac{22.6}{G^{1.9}} \qquad if \quad G > 5.2\text{g} \quad \text{(A4.4a)}$$

$$\frac{r_l^*}{r_d^* + r_l^*} = 1 \qquad otherwise \quad \text{(A4.4b)}$$

This relation is shown in Fig. S4.2 C. In conclusion, red knots with a gizzard below 5.2 g are expected to always prefer *Loripes* over *Dosinia*, and birds with larger gizzard sizes to include a proportion of *Dosinia* in their diet that increases with gizzard size. The uncertainty in the predicted preferred diet that results from the variances in the constraint measurements was relatively large (grey area in Fig. S4.2 C), as they are multiplied in the estimation. The prediction interval was calculated by drawing 100.000 values for each of a sequence of gizzard masses from simulated constraint values, which were assumed to follow the normal distribution.

Table A4.2 Variables and parameters used in the diet selection model.

Symbol	Value	Unit	Description
r_d	variable	mg/s	DM_{shell} Intake rate on *Dosinia*
r_l	variable	mg/s	DMshell Intake rate on *Loripes*
R^*	variable		Optimal combination of r_d and r_l
G	variable	g	Gizzard mass
c	variable	mg/s	Digestive constraint, i.e. the max. DM_{shell}[a] intake rate on non-toxic prey
q	1.29	mg/s	Toxic constraint, i.e. the max. DM_{shell} intake rate on *Loripes*
e_d	0.057	mg/mg	$AFDM_{flesh}$[b] per DM_{shell} in *Dosinia*
e_l	0.163	mg/mg	$AFDM_{flesh}$ per DM_{shell} in *Loripes*

[a] Dry shell mass
[b] Ash-free dry flesh mass

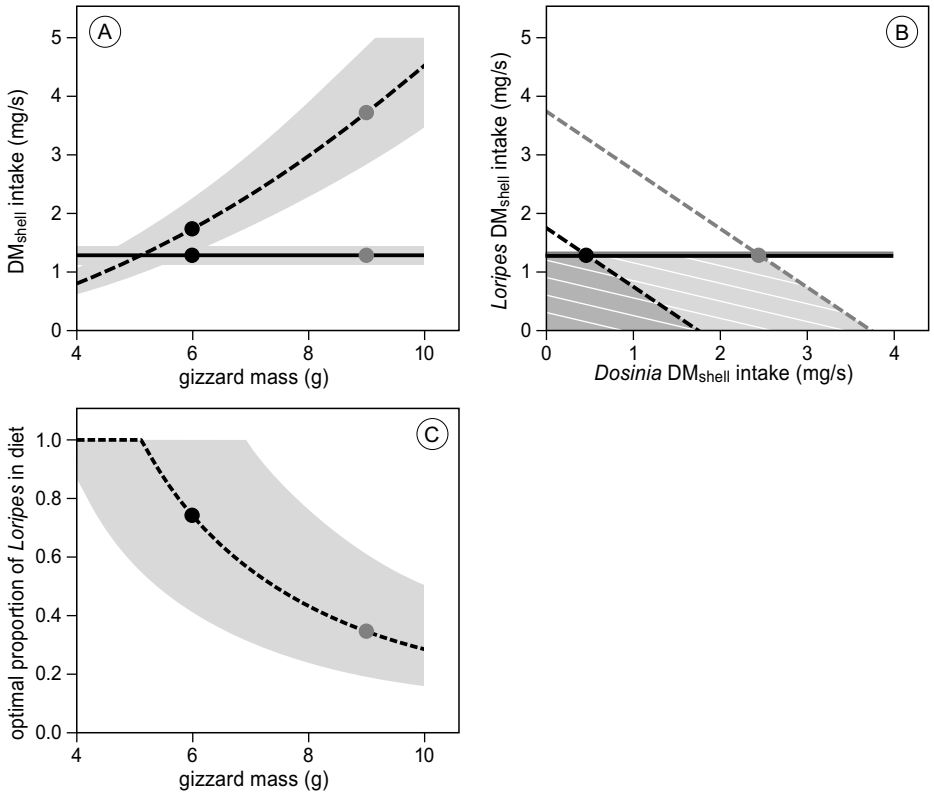

Figure A4.2 Graphical representation of the linear programming model. A) The observed relations between gizzard mass (G) and two intake constraints. Toxin constraint (q), represented by the solid line, only limits the intake of *Loripes* and is independent of gizzard mass. Digestive constraint (c), shown by the dashed line, limits the intake of both *Loripes* and *Dosinia* and increases exponentially with gizzard mass. Black dots show q and c at G = 6 g (comparable to small gizzard group), and grey dots show q and c at G = 9 g (comparable to the large gizzard group). Grey areas are estimated values ± SD. SDs were calculated as the square root of the sum of the fixed and random effect variances from the linear mixed-effect models (model 2.1 in Table 4.1 for *Dosinia*, intercept model on *Loripes* data for *Loripes*). B) Optimal diet choice when both *Dosinia* and *Loripes* are available *ad libitum* for a gizzard mass of 6 g (black dot and lines) and 9 g (grey dot and lines). Solid lines show q and dashed lines show c at levels corresponding to the dots in panel A. Dark grey area shows all possible combinations under both constraints for a 6 g gizzard, light grey area for a 9 g gizzard. White lines connect points of equal energy intake rate, calculated from e_d and e_l, with increasing energy intake to the right and up. The maximum energy intake is reached where constraint lines intersect (dots). Thus, when G changes from 6 to 9 g, the digestive constraint increases (from black to grey dashed line), whereas the toxin constraint remains unchanged (black and grey solid line), leading to an increased optimal intake on *Dosinia* but not on *Loripes*. C) Expected relation between gizzard mass and the optimal proportion of *Loripes* in the diet. Dotted line connects mean predicted proportions as calculated. Grey area encloses the 95% prediction interval. Black dot shows the expected proportion at G = 6 g, grey dot shows expected proportion at G = 9 g, corresponding to the predictions in panel B.