Digestive Capacity and Toxicity Cause Mixed Diets in Red Knots That Maximize Energy Intake Rate

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ABSTRACT: Among energy-maximizing animals, preferences for different prey can be explained by ranking the prey according to their energetic content. However, diet choice also depends on characteristics of the predator, such as the need to ingest necessary nutrients and the constraints imposed by digestion and toxins in food. In combination, these factors can lead to mixed diets in which the energetically most profitable food is not eaten exclusively even when it is abundant. We studied diet choice in red knots (*Calidris canutus canutus*) feeding on mollusks at a West African wintering site. At this site, the birds fed primarily on two species of bivalves, a thick-shelled one (*Dosinia isocardia*) that imposed a digestive constraint and a thin-shelled one (*Loripes lucinalis*) that imposed a toxin constraint. The latter species is toxic due to its symbiotic association with sulfide-oxidizing bacteria. We estimated experimentally the parameters of a linear programming model that includes both digestive and toxin constraints, leading to the prediction that red knots should eat a mixture of both mollusk species to maximize energy intake. The model correctly predicted the preferences of the captive birds, which depended on the digestive quality and toxicity of their previous diet. At our study site, energy-maximizing red knots appear to select a mixed diet as a result of the simultaneous effects of digestive and toxin constraints.

Keywords: diet choice, toxin, mixed diet, constraints, *Calidris canutus*.

Introduction

Understanding the mechanisms that govern diet preference is a fundamentally important issue in ecology, as these mechanisms will determine habitat selection, predator-prey interactions, and overall population dynamics (e.g., Holt and Kotler 1987; Duffy 2003; Finke and Denno 2004; Piersma 2012). In ecology, diet selection questions are often interpreted in the light of optimal foraging theory (MacArthur and Pianka 1966; Stephens and Krebs 1986).

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Energy-maximizing animals must ingest diets that maximize energy intake without stepping out of the bounds imposed by nutritional, digestive, and toxin constraints (Westoby 1974; Pulliam 1975; Jeschke et al. 2002; Piersma and van Gils 2011). We used a linear programming model (Westoby 1974; Belovsky 1978) in which energy intake rate is maximized but digestive and toxin constraints are also recognized to explain the diet selection of red knots (*Calidris canutus*). We parameterized and tested the model on the subspecies *Calidris canutus canutus* (Buehler and Piersma 2008) in the birds' main wintering area, Banc d'Arguin in Mauritania, West Africa (Leyrer et al. 2006).

Red knots are shorebirds that feed on mollusks and commonly face a digestive constraint (van Gils et al. 2003; Piersma 2007; Buehler and Piersma 2008). They are known to select for thin-shelled prey, thereby minimizing ballast intake and, thus, processing time (van Gils et al. 2003, 2005*a*; Quaintenne et al. 2010). In Banc d'Arguin, red knots forage on the extensive intertidal seagrass beds, where the bivalve *Loripes lucinalis* (Lucinidae, Bivalvia) is their most abundant prey, followed by the bivalve *Dosinia isocardia* (Veneridae, Bivalvia; van der Geest et al. 2011, 2012; M. V. Ahmedou Salem et al. 2014). Together, *Loripes* and *Dosinia* constitute most of the potential prey available (Honkoop et al. 2008; van Gils et al. 2013). If the diet of Banc d'Arguin red knots was indeed governed by the ballast-processing constraint, then the birds should be eating the thin-shelled *Loripes* and ignoring the thicker-shelled *Dosinia.* They are not. Field studies show that a large part of their diet consists of prey other than *Loripes*, while densities are high enough to provide a pure *Loripes* diet (van Gils et al. 2012, 2013; Onrust et al. 2013). Thus, Banc d'Arguin red knots seem to actively select a mixed diet of *Loripes* and other prey species, in contrast to what would be predicted by the customary foraging models.

Mixed diets can result from different nutritional requirements (Westoby 1974; Pulliam 1975; Simpson et al. 2004),

but the flesh of different mollusk species are nutritionally similar (e.g., Zwarts and Wanink 1993). Alternative explanations for mixed diet selection include the need to regularly sample the quality of different prey types and the inability of a forager to distinguish between prey types (e.g., McNamara and Houston 1987). These arguments cannot, however, explain the consistently low fraction of *Loripes* in the red knots' diet when availability is high.

Here we study the hypothesis that a toxin in *Loripes* causes red knots to prefer a mixed diet. Captive red knots on a *Loripes* diet developed diarrhea within an hour after ingestion (T. Oudman, personal observation). Two captive red knots maintained on a *Loripes* diet for 2 weeks showed reduced feeding rates, low body mass, improper preening, ruffled feathers, drooping wings, and docile behavior (M. V. Ahmedou Salem and J. A. van Gils, unpublished data). This toxicity is most likely the result of the peculiar metabolic system of *Loripes*, which is largely dependent on the nutrients synthesized by symbiotic sulfide-oxidizing bacteria (van der Geest et al., forthcoming). A consequence of this symbiosis is the presence of sulfur-rich granules in the gills (Cary et al. 1989; Anderson 1995); in fact, van der Heide et al. (2012) found a relative sulfur content of 2%–4% (of total body dry flesh mass). Although monogastric animals are less susceptible to sulfur toxicity than ruminants (microbes in the rumen convert sulfur to toxic sulfide), high sulfur doses are toxic across species, resulting in diarrhea, dehydration, and lower feeding rates (Hall 2007). However, the precise physiological mechanism that causes *Loripes* to be toxic to red knots remains to be studied.

Belovsky and Schmitz (1994) argue that mixed diets can be optimal for herbivores when the intake rates on different food types are limited by different constraints, including feeding time (the sum of searching and handling times), digestion time, and toxicity (see Ritchie 1988 for an empirical example). Toxicity can play a decisive role in diet selection by herbivores (Johnson et al. 1993; Schmidt et al. 1998; Marsh et al. 2006) as well as other animals (Toft and Wise 1999; Skelhorn and Rowe 2007; Barnett et al. 2012). Hence, a combination of digestive and toxin constraints might explain mixed diet selection in red knots.

A Linear Model with Digestive and Toxin Constraints

We assume that a red knot has unlimited access to *Dosinia* and *Loripes* of fixed sizes, with negligible search times. Furthermore, we assume that all *Loripes* contain a fixed amount of toxin and that *Dosinia* contain no toxin. Following Belovsky and Schmitz (1994), a digestive constraint (c, maximum processing rate of ballast dry mass DM_{shell} in mg s^{-1}) is defined as

$$r_d k_d + r_l k_l \leq c, \tag{1}$$

where r_d and r_l are the intake rates (number of prey s^{-1}) on *Dosinia* and *Loripes*, respectively, and k_d and k_l are the ballast masses of their respective individual prey in milligrams. Similarly, the toxin constraint (q, maximum intake of toxin s^{-1}) is defined as

$$r_d s_d + r_l s_l \leq q. \tag{2a}$$

We scale the unit of toxin to the toxin content of an individual *Loripes*, so the toxin content of *Loripes*, s_l, is 1 (unitless). Since *Dosinia* is not toxic, s_d is 0, and the toxin constraint simplifies to

$$r_l \leq q. \tag{2b}$$

With e_d and e_l defined as the ash-free dry flesh masses AFDM$_{flesh}$ (scaling linearly with caloric content; see van Gils et al. 2005b) per individual *Dosinia* and *Loripes*, respectively, then total energy intake rate (Y, mg AFDM$_{flesh}$ s^{-1}) is defined by

$$Y = r_d e_d + r_l e_l. \tag{3}$$

The constraints limit the combinations of r_d and r_l that are possible for the forager (constraint lines in fig. 1). The optimal diet is thus the combination of intake rates that maximizes Y while obeying both constraints, denoted as $R^* = (r_d^*, r_l^*)$.
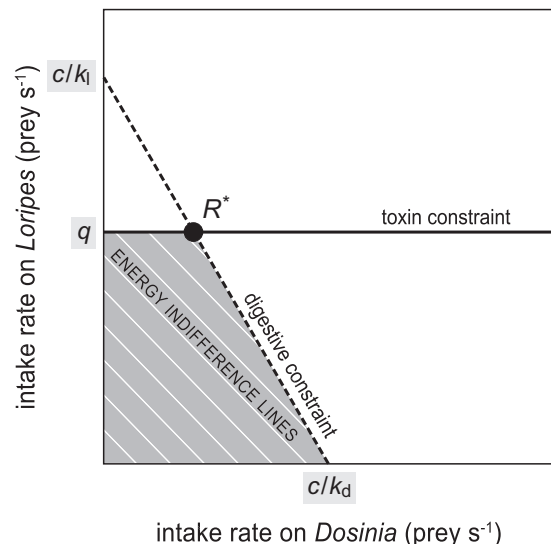
Figure 1: Optimal diet (R^*) under a digestive constraint (c, dashed line) and a toxin constraint (q, solid line). The gray area represents all possible combinations of intake rates on *Dosinia* (d) and *Loripes* (l), and the white lines are energy indifference lines that connect points of equal energy intake rate, with energy intake increasing toward the upper-right corner of the diagram. Value k represents the ballast mass of individual prey.

Partial preferences are predicted when $r_d^* > 0$ and $r_1^* > 0$. Figure 1 shows that this is always and only true when the constraint lines cross and the slope of the energy indifference lines (lines that connect points of equal energy intake rate) is intermediate to the slopes of both constraint lines. In practice, this means that the prey type that is the higher quality prey type with respect to one constraint should be the poorer quality prey type with respect to the other constraint. If these conditions are satisfied, then R^* is calculated by equating both constraints (eqq. [1], [2b]):

$$R^* = (r_d^*, r_1^*) = \left(\frac{c - qk_1}{k_d}, q \right). \quad (4)$$

Model Parameterization and Testing

When using linear programming models, each constraint line must be estimated with data from a setting where the constraint is actually effective (Hobbs 1990; Owen-Smith 1993, 1996). To this end, the constraint lines were each determined independently in a separate experiment before being tested in another experiment whether they could explain the observed diet selection by red knots. In this first experiment, we separately measured maximum long-term intake rates of *Loripes* and *Dosinia*, the latter being a suspension-feeding bivalve that does not have a sulfur-based metabolism. Thus, the long-term maximum intake rate on *Dosinia* gives us an estimate of c, the digestive constraint (van Gils et al. 2005a). If the *Loripes* intake rate is limited by a toxin constraint, then the maximum ballast mass intake rate should be lower than c. The measured maximum intake rate on *Loripes* then provides an estimate of the toxin constraint, q. Other necessary parameters (k_d, k_1, e_d, and e_1) were directly measured on individual *Loripes* and *Dosinia*.

In a second experiment, we analyzed diet preferences of captive red knots with different dietary histories. For a period of 3 h, ad libitum amounts of either *Loripes* or *Dosinia* were offered to single birds, and directly afterward both prey types were offered simultaneously to give a choice between the two. Results from this experiment are compared with the predictions of the linear programming model. To evaluate the necessity of accounting for *Loripes* toxicity when predicting diet choice by red knots, models with and without a toxin constraint are considered.

The Effect of Water Availability on the Proposed Constraints

High temperatures and a lack of freshwater in Banc d'Arguin impose physiological stress on red knots with respect to water balance and salt excretion (Klaassen and

Ens 1990; Verboven and Piersma 1995; Gutiérrez et al. 2011b). Given the diarrhetic effect of *Loripes*, its toxicity may (partially) lie in its negative effect on water balance. In that case, the maximum ingestion rate of red knots on *Loripes* should be dependent on water salinity and availability. We tested for this by including three different water treatments in the first experiment, offering the birds either freshwater, seawater, or no supplementary water at all.

## Animals, Material, and Methods

The Birds

Experiments were carried out in January/February 2011 at the Iwik research station of Parc National du Banc d'Arguin, Mauritania, West Africa (lat. 19°53′N, long. 16°17′W). Six red knots were caught locally with mist nets, individually color-ringed for identification (for procedures, see Leyrer et al. 2006, 2013), and placed indoors in a 1.5 × 1.0 × 0.5-m holding pen under temperatures varying between 18° and 24°C. Three birds were juvenile (i.e., in their second calendar year, half a year old), and the other three were in their third calendar year or older.

For the experiments, the holding pen was divided into six transparent compartments of 0.5 × 0.5 m to isolate each bird. Ad libitum access to freshwater was given between experimental trials. Outside of the experiments, staple food consisting of live mollusks (mainly *Loripes lucinalis* and *Dosinia isocardia* but also, e.g., *Senilia senilis*, *Bittium reticulatum*, and *Abra tenuis*) was offered throughout the day. At night, the birds were offered the flesh of large *S. senilis* and trout pellets (Trouvit, Produits Trouw, Vervins, France).

Prey were collected daily in the field by sieving mud over a 2-mm sieve and were kept fresh by storing them in a refrigerator at 7°C the day before use. Birds were offered only live prey rinsed with seawater. To ensure a rate-maximizing feeding strategy during the foraging trials, the total amount of food was adjusted to maintain a constant low, but not unnatural, body mass (90–110 g; Leyrer et al. 2012). All birds were weighed every morning to the nearest gram, and their health status was monitored throughout each day.

Parameterization of Prey Characteristics

We separated the flesh and shell material of 200 *Dosinia* and *Loripes* individuals of variable lengths. By measuring shell length (to the nearest 0.1 mm) and separately drying (3 days at 60°C), weighing (to the nearest 0.1 mg), incinerating (5 h at 560°C), and weighing each again, we determined ballast dry masses, DM_{shell}, and ash-free dry flesh masses, $AFDM_{flesh}$. Based on data presented by Zwarts and
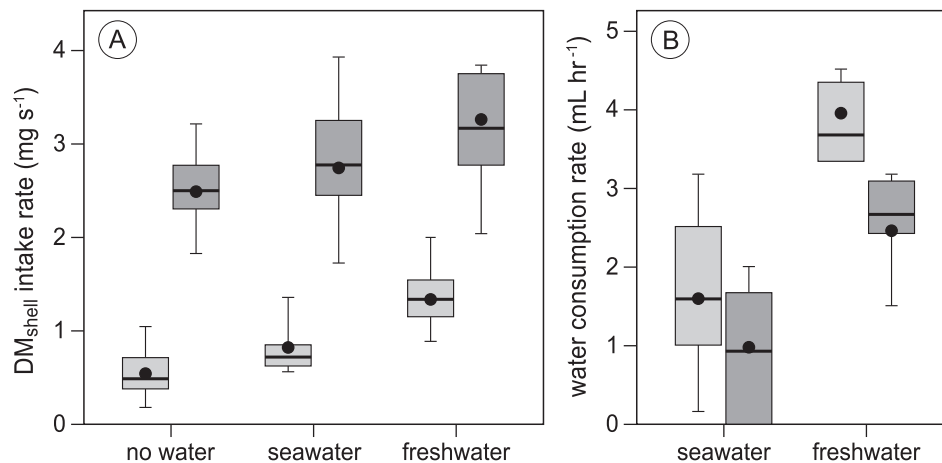
Figure 2: Long-term intake rate (*A*) and water consumption rate (*B*) of red knots feeding on *Loripes* (lightly shaded boxes) or *Dosinia* (darkly shaded boxes). Whiskers indicate the most extreme data points. The birds had ad libitum access to either freshwater or seawater or were provided no water at all. Circles reflect the values as estimated by the best statistical models based on these data (model 1a.4 in *A*, model 1b.4 in *B*; see table 1). Ballast dry mass DM_{shell} intake rates on seawater are used to parameterize the diet selection model.

Wanink (1993), it is reasonable to assume that metabolizable energy density in the flesh is similar in different prey species and sizes (estimated as 15.95 kJ/g in van Gils et al. 2005*b*). We therefore used $AFDM_{flesh}$ as our measure of energy content. The relationships between lengths and masses were used to estimate average DM_{shell} and $AFDM_{flesh}$ of 8–9-mm *Dosinia* (k_d and e_d, respectively, in eqq. [1]–[4]) and 8–9-mm *Loripes* (k_l and e_l, respectively).

Experiment 1: Parameterization of Digestive and Toxin Constraints

Individual birds that had been withheld food for 6 h were offered *Dosinia* (4–14 mm) or *Loripes* (4–11 mm) in separate trials ad libitum for 6 h. All prey items were counted before and after each trial to determine the number of prey items consumed. The lengths of subsamples were measured before and after each trial to infer size distributions. Both measures were combined to estimate total DM_{shell} intake in each trial and divided by the total time of the trial to arrive at long-term DM_{shell} intake rates.

The digestive constraint c equals the best estimate of the long-term DM_{shell} intake rate on *Dosinia*. The long-term DM_{shell} intake rate on *Loripes* is expected to be lower due to its toxicity. Then, because toxicity is measured in units of an individual *Loripes* of 8–9 mm, toxin constraint q equals the estimated long-term DM_{shell} intake rate on *Loripes*, divided by k_l.

To get the birds accustomed to captivity, a habituation period of 6 days preceded the experiment. In each trial, either *Loripes* or *Dosinia* was offered, combined with one of three drinking water regimes: ad libitum freshwater, ad

libitum seawater (salinity ≈40‰; Wolff and Smit 1990), or no water. First, all birds performed each combination of diet and water treatment once, resulting in 36 trials (6 birds × 2 prey types × 3 water treatments). Additionally, 10 randomly chosen trials were repeated (not all trials were repeated due to time limitations). Two trials where all prey items were eaten were removed from the analysis because this would give an underestimation of maximum intake rate. These trials were repeated on another day with more food. This resulted in a total of 46 trials for intake rate analysis (*Loripes*: 8 with freshwater, 7 with seawater, and 8 without water; *Dosinia*: 8 with freshwater, 8 with seawater, and 7 without water) performed on 12 different experimental days, with a day of rest between each experimental day.

Water intake was measured in each trial by subtracting evaporated water in a reference water bowl (either freshwater or seawater) from water that disappeared from the water bowl in the trial. Seven trials involving three different birds were removed from the analysis because birds were observed bathing in the water bowl, resulting in 24 trials for water consumption analysis (*Loripes*: 5 with freshwater and 6 with seawater; *Dosinia*: 7 with freshwater and 6 with seawater).

Experiment 2: State-Dependent Preference Test

Either *Loripes* (4–11 mm) or *Dosinia* (4–14 mm) were offered ad libitum to a single bird for 3 h, preceded by 6 h of food deprivation. Directly afterward, a preference test was carried out in which the bird was simultaneously offered 40 *Loripes* and 40 *Dosinia* individuals, all of the same

Table 1: Model selection to find the best fits to the data obtained in each experiment by comparing weighted corrected Akaike Information Criterion (AICc) values

Experiment, model[a]	Fixed effects[b]	K[c]	ΔAICc	AICc weight	Cumulative weight	LL[d]
Experiment 1: Long-term DM$_{shell}$[e] intake rate:						
1a.4	Diet + water	6	0	.93	.93	−28.16
1a.5	Diet + water + diet : water	8	5.14	.07	1	−27.86
1a.2	Diet	4	11.78	0	1	−36.64
1a.1	1	3	76.53	0	1	−70.21
1a.3	Water	5	77.37	0	1	−68.17
Experiment 1: Long-term water consumption:						
1b.5	Diet + water + diet : water	5	0	.7	.7	−67.54
1b.4	Diet + water	4	2.11	.24	.94	−70.09
1b.3	Water	3	5.04	.06	1	−72.92
1b.2	Diet	3	53.68	0	1	−97.24
1b.1	1	2	55.2	0	1	−99.26
Experiment 2: Prey preference:						
2.5	Number + diet + number : diet	6	0	1	1	−220.3
2.4	Number + diet	5	21.88	0	1	−232.27
2.2	Number	4	34.46	0	1	−239.58
2.3	Diet	4	39.32	0	1	−242.01
2.1	1	3	52.29	0	1	−249.51

[a] Models 1a use the normal distribution; models 1b use the Poisson distribution. Both contain bird ID as a random factor. Models 2 are binomial and contain both bird ID and trial number as random factors.

[b] Diet refers to a diet of either *Dosinia* or *Loripes*, water refers to the different water treatments, and number refers to the choice number (1–20) in experiment 2.

[c] Number of parameters in the model.

[d] Log likelihood.

[e] Ballast dry mass.

length (8–9 mm) and arranged in separate piles on a white plastic tray (0.25 × 0.30 m, with three high edges of 0.2 m and one low edge of 0.05 m). The bird had to step over the low edge to reach the prey items, each an equal distance away. The species of each ingested prey item was recorded. The tray was removed after 20 ingestions or after 1 h (in one case, 13 ingestions). Each of the six birds was exposed to both treatments twice, yielding a total of 24 trials carried out over 4 days (in between the last four experimental days of experiment 1).

Statistical Analyses

Generalized linear mixed model selection was performed in R using the function lmer from library lme4 in R, version 3.0.1 (R Development Core Team 2013) to test the effect of treatments in both experiments. Bird ID and trial number (experiment 2 only) were included as random variables. For both experiments, a set of candidate models, including all combinations of explanatory variables and their second-order interactions, was ranked according to the likelihood of each model. Rank was determined by calculating the Akaike weight of the model, using second-order corrected Akaike Information Criterion (AICc) for

small sample sizes (Burnham and Anderson 2002). We tested for the potential effect of treatment on the previous day by adding previous-day treatment as a fixed factor and comparing the calculated AICc values.

Results

Verifying the Presence of a Toxin Constraint

In red knots, maximum DM$_{shell}$ intake rate depends on gizzard size (van Gils et al. 2003). However, measured DM$_{shell}$ intake rates for respective diets of *Dosinia* and *Loripes* were found to be distinctively different from each other (fig. 2*A*; model 1a.4 in table 1; data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5fp4g [Oudman et al. 2014]). The *Dosinia* DM$_{shell}$ intake rate resembled the expected intake rate of a digestively constrained red knot with a gizzard of 7.7 g (see van Gils et al. 2003 for calculations), which is 1.4 g lower than the mean gizzard mass found in free-living birds in the same period (A. Dekinga, unpublished data). The DM$_{shell}$ intake rate of *Loripes* was only 30% of the average DM$_{shell}$ intake rate of *Dosinia*, which shows that a factor other than shell-mass processing limited the intake rate of *Loripes*. Without exception, birds suffered from diarrhea

Table 2: Values to parameterize the diet selection model including a toxin constraint

Parameter	Value	Unit	Description
e_d	5.7	mg	$AFDM_{flesh}$ per individual *Dosinia*
e_l	9.7	mg	$AFDM_{flesh}$ per individual *Loripes*
k_d	102.7	mg	DM_{shell} per individual *Dosinia*
k_l	69.6	mg	DM_{shell} per individual *Loripes*
s_d	0	NA	Toxicity of individual *Dosinia*
s_l	1	NA	Toxicity of individual *Loripes*
c	2.75	mg s^{-1}	Maximum tolerable DM_{shell} intake rate
q	.012	s^{-1}	Maximum tolerable toxin intake rate

Note: *Dosinia* (d) and *Loripes* (l) are assumed to be of medium size (8–9 mm in length), as used in the preference test. The unit of toxin is chosen to be one individual *Loripes*, that is, $s_l = 1$. *Dosinia* is assumed to contain no toxin, that is, $s_d = 0$. Value q is calculated from the data as the maximum ballast dry mass (DM_{shell}) intake rate on *Loripes* (0.82 mg s^{-1}) divided by k_l, the fitted DM_{shell} per individual *Loripes* (69.6 mg); e = ash-free dry flesh mass, $AFDM_{flesh}$; k = ballast mass of individual prey; s = toxin content; c = digestive constraint; q = toxin constraint; NA = not applicable.

once being given a diet of only *Loripes*; they recovered within an hour after being offered different foods.

The Effect of Water Availability

The DM_{shell} intake rates for *Dosinia* and *Loripes* were highest when freshwater was provided as drinking water (estimate ± SE: 3.3 ± 0.14 mg/s and 1.34 ± 0.09 mg/s, respectively), lower when seawater was provided (2.75 ± 0.14 mg/s and 0.82 ± 0.1 mg/s, respectively), and lower still when no water was provided (2.49 ± 0.14 mg/s and 0.56 ± 0.09 mg/s, respectively). However, the availability of freshwater did not release birds from a toxin constraint, as the intake rate of *Loripes* was still lower than that of *Dosinia* (fig. 2A).

Birds drank more when *Loripes* rather than *Dosinia* was on offer and also drank more when freshwater was provided (2.88 ± 0.14 and 2.60 ± 0.14 mL/hr for *Loripes* and *Dosinia*, respectively) rather than seawater (1.19 ± 0.16 and 0.90 ± 0.17 mL/hr, respectively), as shown in figure 2B (see also models 1b in table 1). A comparison of these results with the long-term food intake rates shows that increased food intake (be it *Dosinia* or *Loripes*) coincides with increased water intake.

Parameterization of the Diet Selection Model

The DM_{shell} of 8–9-mm *Dosinia*, k_d, was 102.7 ± 18.2 mg (mean ± SD), and the DM_{shell} of 8–9-mm *Loripes*, k_l, was 69.6 ± 14.2 mg. The $AFDM_{flesh}$ of 8–9-mm *Dosinia*, e_d, and *Loripes*, e_l, were 5.7 ± 1.1 mg and 9.7 ± 1.8 mg, respectively (see also table 2). We parameterized the digestive and toxin constraints based on the estimated values with seawater (the only source of water for red knots on

Banc d'Arguin). Digestive constraint c equals the maximum long-term DM_{shell} intake rate on *Dosinia* (2.75 mg/s), leading to an energy intake rate of 0.15 mg $AFDM_{flesh}$/s. Long-term maximum DM_{shell} intake rate on *Loripes* (0.82 mg/s) was divided by k_l to arrive at a toxin constraint q of 0.012 individual *Loripes* per second, equivalent to an energy intake rate of 0.12 mg $AFDM_{flesh}$/s.

We calculated predictions of the diet selection model for two different situations. In the first, we assumed that intake rate is limited only by a digestive constraint (fig. 3A). In the second, we assumed that the *Dosinia* intake is limited by a digestive constraint and that the *Loripes* intake is limited by its toxic effect (fig. 3B). In the first case, optimal diet R^* is given by $(r_d^*, r_l^*) = (0, c/k_l) = (0$ individuals s^{-1}, 0.040 individuals s^{-1}) (fig. 3A), resulting in an energy intake rate of 0.39 mg $AFDM_{flesh}$/s (eq. [3]). The model thus predicts that *Dosinia* is always rejected, resulting in a diet of only *Loripes* (fig. 3A). In the second case, where the model includes a toxin constraint, a mixed diet is predicted (fig. 3B), resulting in $R^* = (r_d^*, r_l^*) = (0.019$ individuals s^{-1}, 0.012 individuals s^{-1}), as calculated by equation (4), which gives an energy intake rate of 0.22 mg $AFDM_{flesh}$/s. Thus, partially accepting both *Dosinia* and *Loripes*, resulting in a diet of both *Dosinia* (61%) and *Loripes* (39%), should lead to a higher energy intake rate than eating only *Dosinia* (0.15 mg $AFDM_{flesh}$/s) or only *Loripes* (0.12 mg $AFDM_{flesh}$/s). Note that in addition to changing the expected preferences, the inclusion of the toxin constraint considerably lowers the maximum predicted energy intake rate.

Diet-Dependent Preference Test

Prey preference strongly differed between birds on a *Loripes* diet and birds on a *Dosinia* diet. When given the choice
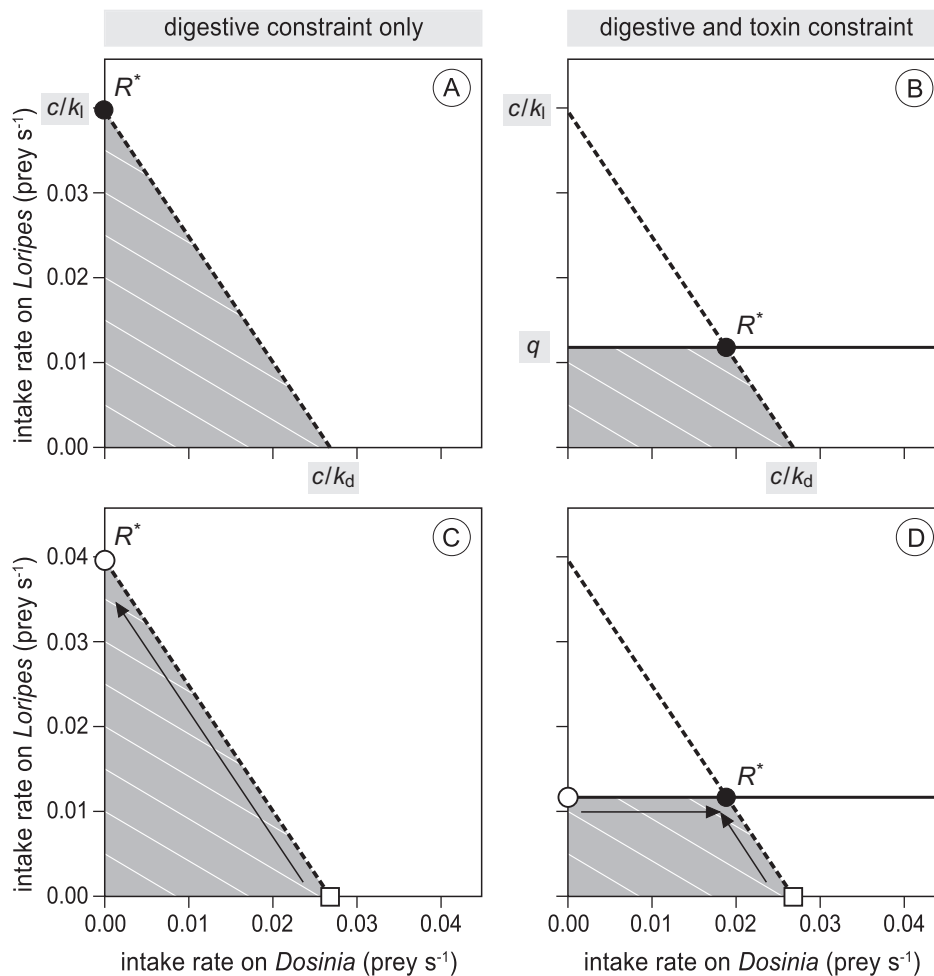
Figure 3: Optimal diet predictions for red knots foraging on *Dosinia* and *Loripes* under a digestive constraint (*A, C*) or under both a digestive constraint and a toxin constraint (*B, D*). Dashed lines indicate the maximum digestion rate, and solid lines indicate the maximum rate of toxin intake. The gray area represents all possible combinations of intake rates of *Dosinia* and *Loripes* given the constraint(s). Intake rates are expressed as individuals per second, referring to medium-sized prey (8–9 mm, as in experiment 2). White lines are energy indifference lines (cf. fig. 1). Slope of the lines is given by $-e_d/e_l$ (based on estimates obtained in this study; table 2). The point of highest possible energy intake rate within the gray area is denoted as R^*. The lower panels predict the outcome of experiment 2 under a digestive constraint (*C*) and under both a digestive constraint and a toxin constraint (*D*). Arrows show how the birds on either a *Dosinia* diet (open squares) or a *Loripes* diet (open circles) should arrive at R^* when both are offered. Slopes and intercepts of the constraint lines are based on the results of experiment 1 (see table 2).

between the two, the *Dosinia* diet birds strongly preferred *Loripes* and vice versa. However, this effect slowly disappeared over the course of 20 prey choices (fig. 4; model 2.5 in table 1; data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5fp4g [Oudman et al. 2014]). This strong initial difference in preference between the two diet groups and their convergence during the course of the trial toward a slight preference for *Dosinia* is predicted by the multiple constraint model (fig. 3D) but not by a model with only a digestive constraint, which predicts that all birds will always choose *Loripes* irrespective of previous diet (fig. 3C). The inclusion of treatment

during the previous day did not yield better fits in any statistical model (not shown in table 1). This suggests that there was no effect of *Loripes* consumption during the previous day on diet choice in the subsequent experiment.

Discussion

Experiment 1 confirmed that red knots feeding on *Dosinia* were limited by a digestive constraint, whereas red knots feeding on *Loripes* were limited by a toxin constraint. As *Loripes* have a higher digestive quality than *Dosinia*, red knots should prefer *Loripes* with respect to digestibility and
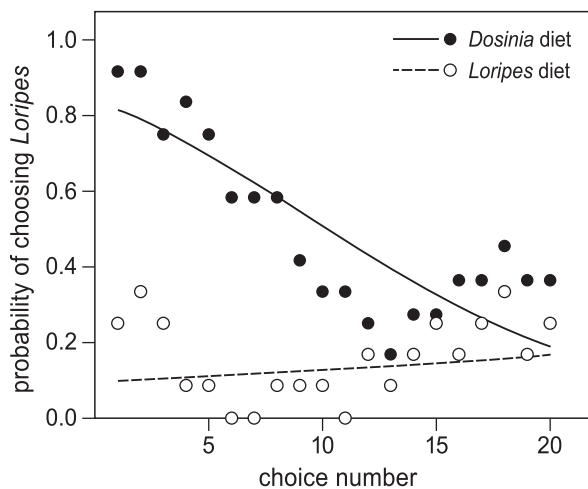
Figure 4: Probability of choosing *Loripes* (8–9 mm) over *Dosinia* (8–9 mm) after a 3-h exposure to ad libitum *Dosinia* (filled circles) or *Loripes* (open circles), calculated as the proportion of birds that chose *Loripes*. A total of 20 choices were made in succession by each bird in each trial (*n* = 24, balanced). Lines show estimated values of the best statistical model (model 2.5 in table 1) after a *Dosinia* diet (solid line) and after a *Loripes* diet (dashed line).

Dosinia with respect to toxicity. Consequently, the multiple constraint model predicts that partial acceptance of both *Dosinia* and *Loripes* yields the highest energy intake rate when both are offered ad libitum. The outcome of experiment 2 is consistent with these model predictions and demonstrated that red knots take into account their state with respect to both these constraints when selecting their diet (Whelan and Brown 2005). Red knots that were feeding on the limit of their digestive constraint but not their toxin constraint (i.e., when fed *Dosinia*) had increased preference for *Loripes*, whereas red knots that where feeding on the limit of their toxin constraint but not their digestive constraint (i.e., when fed *Loripes*) had increased preference for *Dosinia*. However, we also observed that through eating, the state of the animal changed and thereby so did the nature of the limiting constraint(s). In both treatments, the strong preference for the previously unavailable prey type decreased gradually as that prey type was included in the diet, and preference returned toward a mix of both prey types. Hence, our studies validate that red knots prefer a mix of *Loripes* and *Dosinia* over a diet of either one of them, and we have shown that this behavior can be explained by the constraints that limit their food intake.

Water Consumption and Food Intake

Birds with access to freshwater (i.e., without the burden of physiological salt removal) drank more water (fig. 2*B*)

and increased food intake independent of diet (fig. 2*A*). This is a common finding in contexts where freshwater is scarce (Winchester and Morris 1956; Hochman and Kotler 2006; Shrader et al. 2008; but see Druce et al. 2009). Apparently water slightly alleviates the toxic effect of *Loripes* and ameliorates the digestive constraint. The diarrhetic effect might explain why birds eating *Loripes* drank more water than birds on a *Dosinia* diet. It is plausible that water and toxic *Loripes* are partially complementary resources (sensu Rapport 1971; Tilman 1982). However, increased water intake could not free the experimental birds from the toxin constraint, as their food intake remained far below the digestive constraint. In the field, *Loripes* intake might thus be increased to a limited extent by increasing the excretion capacity of the salt glands (Gutiérrez et al. 2011*a*, 2011*b*).

Adding water as a variable in the presented diet selection model would cause both c and q to change with increasing water intake. This, in turn, would influence the ratio between r_d^* and r_l^* (eq. [4]), suggesting that temporal changes or spatial gradients in salinity, or changes in salt excretion capacity will influence the composition of the preferred diet (Hochman and Kotler 2006; Shrader et al. 2008). Another factor that we did not take into account is decreased energy assimilation efficiency, which would occur if diarrhea decreases retention time in the gut. Extending the model to take this into account would lead to a lower predicted share of *Loripes* in the diet (T. Oudman and V. Hin, unpublished manuscript).

Explaining Diet Choice in the Field

This experimental study shows that energy-maximizing red knots face multiple intake constraints and consequently prefer a mixed diet. In the field, however, red knots may have other objectives than energy intake maximization and might not encounter ad libitum food conditions either. Nonetheless, the feeding limitation by the toxin constraint and its relation to other constraints as outlined in this article should apply in the field. To test the consequences of the toxin constraint for free-living red knots, van Gils et al. (2013) constructed a more complex model that includes search and handling times, digestive and toxin constraints, and yearly mean abundances of both *Dosinia* and *Loripes* (coined the toxin-digestive rate model, TDRM). The TDRM predicted that in 6 out of 8 years in which prey abundances were measured, the toxin constraint would limit the intake of *Loripes*. In contrast to a similar model without a toxin constraint, the predictions of the TDRM were in accordance with the actual diet choice of free-living red knots as determined by fecal analysis. It was concluded that red knots in Banc d'Arguin are

dependent on the presence of both *Dosinia* and *Loripes* for their survival.

Many predators face conflicting constraints when foraging, including the choice between toxic but energy-rich prey versus nontoxic but energy-poor prey. The foraging preferences of red knots in Banc d'Arguin confirm that multiple foraging constraints are a likely cause for mixed diets.

Acknowledgments

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