The role of toxic compounds in bivalves in shaping the foraging behavior of the red knot in Banc d'Arguin

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Abstract. The red knot subspecies *Calidris canutus canutus* experiences a declining trend along their flyway from their breeding grounds in Siberia to their feeding grounds with high mortality in Banc d'Arguin, Mauritania. Here, knots face a trade-off between prey that limits intake rates due to shell processing rates in the gizzard, the digestive constraint and the less common toxic constraint found in the two prey bivalves, *Dosinia isocardia* and *Loripes lucinalis* (Loripes), respectively. Loripes contain sulphur that is partly metabolized by endosymbiotic chemoautotrophic bacteria located in their gills. The present study investigated whether it is the sulphur that limits maximum intake rates on Loripes by comparing intake rates on artificially detoxificated and fresh Loripes. Site differences in sulphur contents were additionally examined to see whether spatial differences in toxicity are strong enough to be displayed in intake rates. We found that intake rates on Loripes with lower sulphur contents (detoxification) were higher than on fresh collected Loripes. Sulphur, therefore, probably limits intake rates when offered ad libitum. Site differences also existed probably due to prey toxicity but its role compared to prey quality (flesh to shell ratio) in making a site more or less suitable remains unclear. However, reliable sulphur content data are still be needed to make final statements about the role of toxic compounds in shaping the foraging behaviour of the red knot in Banc d'Arguin.

Keywords: Red knot – Calidris canutus canutus – Toxic constraint – Intake rates – Loripes lucinalis – Banc d'Arguin
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Introduction.

The Arctic is warming up as twice as fast as the rest of the world due to anthropogenic climate change (Screen & Simmonds, 2010). Much evidence on the downstream effects of a warming world and the decline of population sizes has been implicated in afro-Siberian migratory birds (Vickery *et al.*, 2014; van Gils *et al.*, 2016). Hence, those birds are optimal indicator species to study environmental changes in the light of climate warming in their breeding, overwintering and stop-over grounds (Zöckler, 2011).

Birds face many physiological challenges during migration, especially in terms of feeding and digestion. In between those often highly distant flight events, migratory birds need to adjust the environmental conditions they find at all stopover sites, from the northern breeding to the southern feeding grounds, to fuel their storages. Here, they need to maximize average long-term intake rates to, in turn, maximize flight distances and to optimize other fitness related non-migratory factors such as reproduction and moult which all comes at a cost of the flights (Alerstam & Lindström 1990; McWilliams & Karasov, 2001; Van Gils *et al.,* 2005; Klassen *et al.*, 2012).

On an optimality feeding perspective (Charnov, 1976), migratory birds are assumed to optimize their foraging and, specifically, their diet choice, in order to maximize average long-term intake rates. What prey type will be accepted in the diet depends on the availability of harvestable prey, prey that is accessible, ingestible (Piersma, de Goeij, & Tulp, 1993; Klassen *et al*., 2012) and digestible in which energetic benefits differ per food type. Without limitations in prey accessibility, the rate of ingestion is often constraint through searching and handling times. Digestible constraints limit the rate of ingestion through digestive processes in the gizzard

and thus, long-term energy intake rates. Therefore, harvestable prey types that increase long-term energy intake rates, should be included in the diet (Van Gils *et al.,* 2003; Van Gils *et al.,* 2005).

Various constraints determine maximum energy intake rates that can be achieved in a certain area. This may govern foraging behaviour, diet and habitat selection and finally population dynamics (see example: Duffy, 2003; Piersma, 2012). Studying the underlying mechanisms that constrain intake rates of individual migratory birds is, therefore, crucial to predict future population trends and to develop conservation management practices to maintain critical populations in a changing world.

A special case is found in the red knot (*Calidris canutus canutus*) which faces a trade-off between the digestible and the less common toxin constraint (Oudman *et al.,* 2015; Oudman *et al.,* 2014; Van Gils *et al.*, 2013) within the few harvestable prey species at their overwintering sites where also highest mortality occurs over all stopover sites (Leyrer *et al.,* 2013).

This medium-sized migratory species overwinters in Banc d'Arguin West Africa where it commonly feeds on molluscs to

maximize fueling their storages (Van Gils *et al.,* 2003) for the upcoming migration to their breeding grounds in Taymyr, Siberia (Piersma *et al.* 1992). They mostly feed on hard-shelled bivalves that are ingested as a whole and crushed in the muscular gizzard (Piersma, Koolhaas & Dekinga, 1993). Intake rates of red knots in Banc d'Arguin are known to be determined by the digestive quality of the prey. Accordingly, intake rates are expected to be mainly restricted by its digestive capability in place of searching and handling times (Van Gils *et al.*, 2005). In other words, knots prefer minimizing shell masses to increase energy intake.

In Banc d' Arguin, the birds forage on seagrass beds, dominated by *Zostera noltii*, where the bivalves *Dosinia isocardia* (Dunker 1845; Dosinia) and *Loripes lucinalis* (Lamarck, 1818; Loripes) are the most abundant. Over the last years, abundances changes where Loripes dominated the benthic fauna while Doninia decreased in abundance (Hoonkoop *et al.,* 2008; Salem *et al.,* 2014). The birds depend on a mixed diet of both species to survive (Oudman *et al.*, 2014), Loripes thus may play a role of growing importance in the knots diet. They were also found to have thinner shells compared to Dosinia and therefore have higher flesh-to-shell ratios. In combination with its high abundance, Loripes should be

the preferred prey and should contribute the most to the knots diet (van Gils *et al.,* 2012). But they don't. It was shown that the fraction of Loripes found in their droppings is much lower than expected based on field abundances and its higher digestive quality compared to Dosinia (van Gils *et al*., 2012, 2013; Onrust *et al*., 2013). Oudman *et al.* (2014; 2015) experimentally demonstrated that the intake rates on Loripes were much lower to what was expected based on shell masses and gizzard sizes. During those experiments, knots that were on a Loripes diet only, suffered from diarrhoea which was probably caused by endosymbiotic chemoautotrophic bacteria that live inside the gills of Loripes. Those bacteria oxidize sulphide, which is absorbed from the ground, to toxic sulphur (Van der Heide *et al,*. 2012). This sulphur content may shape the toxin constraint on the intake rate of Loripes in Banc d'Arguin (van Gils *et al*., 2013; Oudman *et al*., 2015). But this hypothesis still needs to be validated.

The present study experimentally investigated whether it is the sulphur that constrains intake rates on Loripes. Releasing the toxic effect of sulphur would enable the knots to increase long-term energy intake rates. In order to do so, Loripes were artificially starved. In this process, Loripes are expected to deplete their sulphur stores

through the digestion of the bacterial endosymbiont in the gills. We additionally included spatial differences in Loripes sulphur contents to investigate the importance of this sulphur constraint in the field which may lead to differences in bird segregation and individual survival rates.

Methods.

The present study was conducted in Mauritania at the Parc National du Banc d'Arguin (PNBA) field station (19°52.42'N, 16°18.50'W) near the village Iwik between 06 January and 10 February 2018 (Figure 1).

2018. All birds were ringed, weighed and their biometry was measured directly after the catching events. The average body mass of the flock was around 117 g within a range of 103 g to 127. Bill lengths ranged from 32,4 to 39,3 . The birds were kept together in an indoor cage of 150 × 100 × 50 cm provided with beach sand and fresh water for drinking. The cage was cleaned between the sessions, regularly. We weighted the birds each day to assess their health status during the whole captive period until they were released on 9 February 2018 in a good condition. The birds received food every evening to keep a healthy state (body

Figure 1: Mudflat system of Banc d'Arguin (left) and zoom into study area (right).

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Experimental Animals

Red Knot (*Calidris canutus canutus*). 12 red knots were caught with mist nets near a roosting area at Albegh Eiznaya (Figure 2) in three capture events on 12, 13, 14 January

masses around 100 g) which consisted of the West African bloody cockle *Anadara senilis.*

***Loripes lucinalis*.** The mudflats are dominated by the seagrass species *Zostera*

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noltii where Bacteria in the ground reduce sulphate to sulphide during the decomposition of it. Loripes absorb the sulphide, which is then transported to their gills were endosymbiotic chemoautotrophic bacteria live. Those bacteria oxidize the sulphide into sulphate. The fixed carbon in this process contributes to the growth of both, the bivalve and the bacteria. The excess of the absorbed sulphide is converted and stored as sulphur in the gills (Van der Heide *et al.,* 2012). This sulphur is expected to shape the toxin constraint which is studied in the present project.

We collected the Loripes by sieving the top layer (~20cm) of the mudflats. Size classes between 9mm-11mm were held as experimental Loripes based on a pre-analysis of the length distribution in the field and on preferred prey sizes of the knots.

We needed a method to loosen the toxic constraint. Therefore, we let the experimental Loripes starve for 10 days which was based on a previous study by van Gils & Oortwijn (2016, Unpublished; Methods by Anna Kemper & Jill Petersen, unpublished data) who found that Loripes after 10 days of starvation contained less sulphur than fresh Loripes. Starvation was induced by hanging the Loripes in the sea in small nets. Loripes are then forced to deplete their sulphur stores, as they cannot obtain sulphide from the sediment. This depletion is correlated to the degradation of the bacterial endosymbiont in the gills, which is probably digested under starved conditions (König *et al.,* 2015). Each day, we let one collected set of Loripes starved by using described method. The experiments started after the first set of Loripes was starved for 10 days. We adittionally collected fresh Loripes at each experimental day to investigate whether it is the sulphur that limits intake rates on Loripes by comparing intake rates on fresh and starved ones.

Spatial variations were additionally included in the present feeding experiments to study how strong natural variations in sulphur contents are displayed by different intake rates between sites. Sampling took place each day at Baie d'Aouatif

(19°52.486'N, 16°17.261'W) and Albegh Eiznaya (19°53.554'N, 16°18.838'W), alternately (Figure 2). The sites are known to differ in various environmental conditions, especially in Loripes toxicity. De Fouw (unpublished Data, 2010) showed that Loripes from Albegh Eiznaya contain more sulphur than Loripes from the Baie
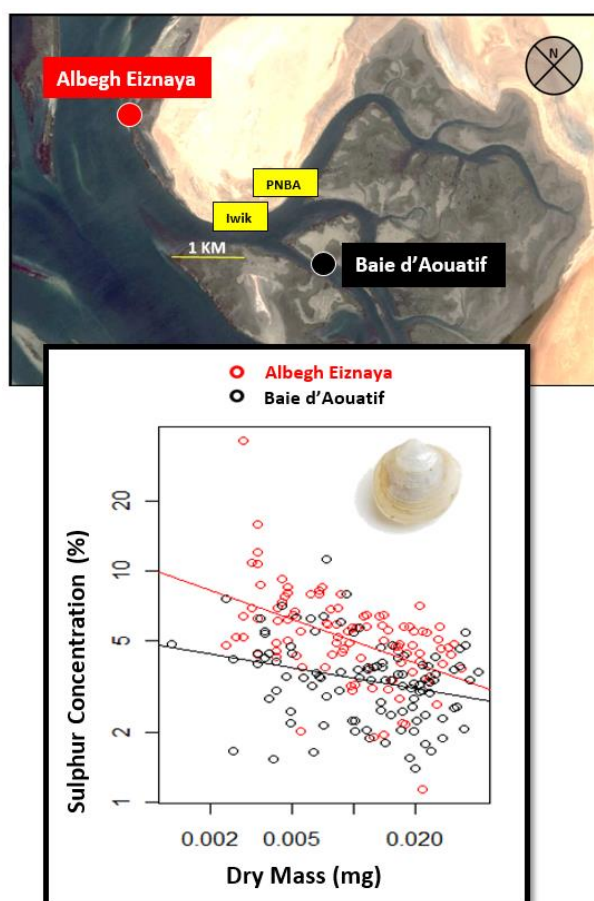
Figure 2: Collection sites and Loripes sulphur content differences. The figure above shows the two sites, Albegh Eiznaya and Baie d'Aouatif, as well as the PNBA field station near the village Iwik. The below figure present the sulphur concentration (y-as) in Loripes from those two sites, against their dry masses (mg).

d'Aouatif. We expected that the intake rates on Loripes from two different sites will display the differences in sulphur contents (Figure 2).

The feeding experiment

Setup. Experiments were conducted each day after the first collected Loripes were starved for 10 days. Loripes of the four treatments (Figure 3 a), containing the two sites, fresh and starved Loripes, were offered ad libitum to the experimental birds. At one day, we offered Loripes from one of the both sites. At each experimental day, we had two sessions with Loripes under different starvation treatments, 10 days of starvation and fresh Loripes (Treatment 10 and Treatment 0). The two treatments were randomly assigned to Session 1, around 16:00, and Session 2, around 21:00. The birds did not eat before Session 1. Each day, we alternate between Baie d'Aouatif and Albegh Eiznaya (Figure 3 b).

Procedure. The twelve birds were separated into pre-cleaned 0.5 x 0.5 m cages equipped with fresh water before the experimental sessions. For each Session, a cup of Loripes (Treatment 0 or 10) from one of the two sites. 60 Loripes were offered to each bird per session (=trial) for 30 minutes which was based on the maximal intake rates from the two previous years. The leftovers were counted after each session to calculate the Loripes consumption per trial. In total 480 trials were performed during the experimental period.

Samples

Loripes Dry & Ash weights. 10 Loripes were taken out of each session (total: 400) at random to calculate consumption rates for the four different treatments (A0, A10, B0, B10; see Figure 3 a).
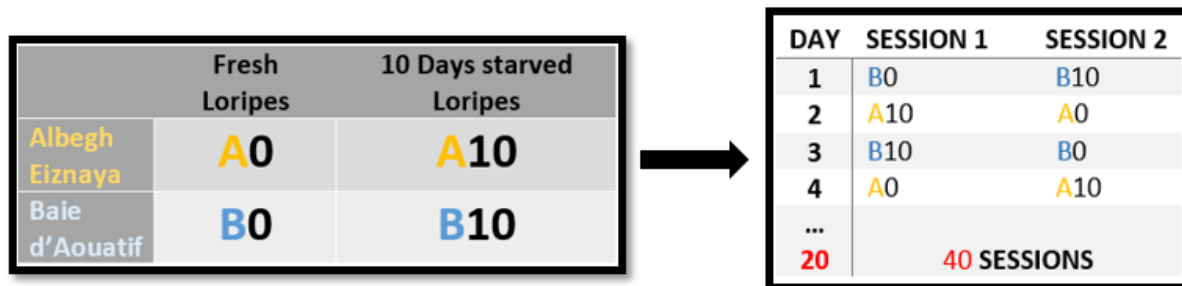
Figure 3: a) The four Loripes treatments for the feeding experiment concept. We collected Loripes from the two sites Albegh Eiznaya and Baie d'Aouatif and within the sites, we have had fresh and starved Loripes. *b) Experimental scheme.* The experiments were conducted on 20 days alternating between Albegh and Baie. Within the days there were two sessions, one with starved and one with fresh Loripes which makes 40 Sessions in total

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The flesh was separated from the shell and dried for 1 day at 60°C. Back at NIOZ, the pre-dried, Loripes shells were measured in length (mm) with 0.01 mm precision. The separated flesh and shells were then dried for another 2 days at 60° C. After drying, dry shell and flesh weights were measured. The dried flesh was additionally burnt at 500 °C overnight to get the Ash Free Dry Mass (AFDM), only organic weight, which is a reliable measurement for comparisons for intake rates between the treatments.

Ethics

The permission for animal feeding experimentation was given by the authorities of the Parc National du Banc d'Arguin. Official guidelines to animal experimentation do not exist in Mauritania. We made all effort to reduce the impacts on the animal's health, physiologically and psychologically. Each day, the captive knots were measured and inspected by eye to take care of that. The experimental birds were released in a healthy condition on 09 February 2018. The experiments strictly followed Dutch guidelines on animal experimentation even no official guidelines are available in Mauritania.

Statistical Analysis

All analytical statistics were performed in R version 3.4.4 (R Core Team, 2018).

Intake Rates. Based on the Ash Free Dry Mass (AFDM) and the leftover counts from the experiments, the AFDM consumption in mg per second for each trial (referred to intake rates) was calculated and used as response variable in a linear mixed model. Maximum intake rates in this study are based on the maximum flesh intake rates because sulphur, the expected toxin constraint resides inside the flesh. The toxin constraint will, therefore, be referred in

terms of ash-free-dry masses (AFDM) instead of shell dry masses (ShD).

Model selection took place in a three-step analysis (Zuur *et al.*, 2009). First, we started with a full model where the fixed component part included all explanatory variables. The fixed part consisted of starvation treatments, sites, day number and bill length, including an interaction between starvation treatment, site and day number (see Formula 1). The best random structure was then examined by using restricted maximum likelihood estimation (REML) for all full linear mixed models (function "lmer" in packages "lme4" and "lmerTest"; Bates *et al.,* 2015) which included different combinations of random effect terms, bird-ID and day number (intercept only; slope only; slope and intercept correlated and de-correlated). The best-fitting random part model was identified by choosing the model with the lowest value of the Akaike's information criterion (AICc) for small sample sizes (suggested by Burnham & Anderson, 2004) and the fewest parameters among models which ΔAICc < 2 (Burnham & Anderson, 2002). The optimal fixed effect structure was determined by comparing models with all possible combinations of the fixed effect structure using ordinary likelihood estimations by sharing the best random part

structure investigated in the step before. The optimal model was again chosen based on the lowest AICc with the fewest parameters (ΔAICc < 2). The final model is presented using REML estimations. The same method was adopted to find the best model that explains the number of Loripes consumed during 30 minutes.

> *Formula 1: Overview of the full model.* For response variables: AFDM mg/s^{-1} ~ and Number consumed Loripes~
>
> *~Starvation Treatment*Sites*Day number +*
>
> *Bill length+ **random structure***

Model assumptions. The final model was checked for normality, linearity and *homoscedasticity* (homogeneity of variances) using model residual plots.

Who eats what on its first two days? A simple t-test was performed to test whether the mean bill length (mm) differs between individuals that feed on Loripes (Eater) or not (Non-Eater) in the first two days. We used data from the feeding experiments in the years 2015, 2017 and 2018, for higher sample sizes.

Loripes condition. I used a t-test to investigate possible site differences in Loripes' shell length (mm) and AFDM weights (mg) (Length and weights from section: *Loripes Dry & Ash weights*).

RESULTS.

We performed in total 480 trials within 40 experimental sessions. Each of the four treatments was run 10 times for each of the experimental birds.

Number of Loripes consumed. The total number of trials for each treatment was 120 (Table 1). The best-fitted model that describes the number of Loripes eaten within 30 minutes, is shown in model table Appendix A1. Here, the starvation treatment resulted in a higher consumption of 1.342 starved Loripes ± 0.356 (standard errors (se)) than on fresh Loripes (t= 3.772, Nobs=480, Ngr=12, p= 0.000184). The between-site variation is displayed by a higher consumption of Loripes from the Baie d'Aouatif (t= 4.403, Nobs=480, Ngr=12, p= 0.0000133) with 1.5723 Loripes ± 0.3571 (se). This is in accordance with our expectations that Loripes from Baie d'Aouatif are less toxic due to lower sulphur contents (see Figure 2). The number of Loripes consumed during the whole

Table 2: AFDM intake rates model summary. The obtained data on AFDM intake rates were the best describe by an interaction between the predictors Day number, starvation treatment and sites. The random part consists on a correlated structure between bird (intercepts) and day number (slopes). Estimates, standard errors and Confident intervals plus its significance are shown for the fixed part. Residues (o^2; $\tau 00_{Bird}$) and slope intercept interaction (p01) are shown for the random part. The r square is presented in marginal (m) and conditional (c).

| Coefficients	Response Variable		
	AFDM consumption in mg/s^{-1}		
	Estimate	St. Error (±)	Conf. Int. (significance)
Fixed Parts			
(intercept)	0.1781	0.0213	0.1350 – 0.2212 (***)
Starvation Treatment 10 (T)	0.0231	0.0094	0.0047 – 0.0415 (*)
Site Baie d'Aouatif (S)	0.0111	0.0091	-0.0067 – 0.0289
Day number	0.0088	0.0015	0.0058 – 0.0118 (***)
T : S	-0.0173	0.0129	-0.0424 – 0.0079
T : Day number	0.0008	0.0008	-0.0006 – 0.0023
S : Day number	-0.0014	0.0008	-0.0029 – 0.00004 (.)
T : S : Day number	0.0006	0.0011	-0.0015 – 0.0027
Random Parts			
σ^2		0.034	
$\tau 00_{Bird}$		0.070	
$\rho 01$		-0.755	
N_Bird		12	
Observations		480	
R^2m/ R^2c		0.41 / 0.83	
Signif. codes:	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1		

experimental period increased with 1.0057 ± 0.1653 (se) (t=6.085, Nobs=480, Ngr=12, p=0.0000788).

Intake rates. AFDM intake rates are expected to give insight on the sulphur constraint and are the best described by a linear mixed model with random intercepts and slopes shown in bold in APPENDIX A2. There was no violation with linear mixed model assumptions.

The 10-day starvation treatment affected AFDM intake rates on Loripes compared to fresh Loripes (t=2.452, Nobs=480, Ngr=12, p=0.0146), by increasing it by about 0.02 mg/s^{-1} (Table 1). Figure 4 shows that the starvation had an increasing effect on intake rates within both sites. The between-site variation differed unexpectedly from the results presented in *Number of Loripes consumed*, whereby AFDM intake rates on Loripes from Baie d'Aouatif, in interaction with the day number, were lower than on Loripes from Albegh Eiznaya (t= -1.887, Nobs=480, Ngr=12, p= 0.0598) with -0.0014 mg/s^{-1} (Table 1). -

The within-boxplot variations in Figure 4, for each Treatment, can be explained by a factor that we did not expect would be that strong. The factor of **time**. The consumption of Loripes increased over the 20 experimental days from average 27 to 41
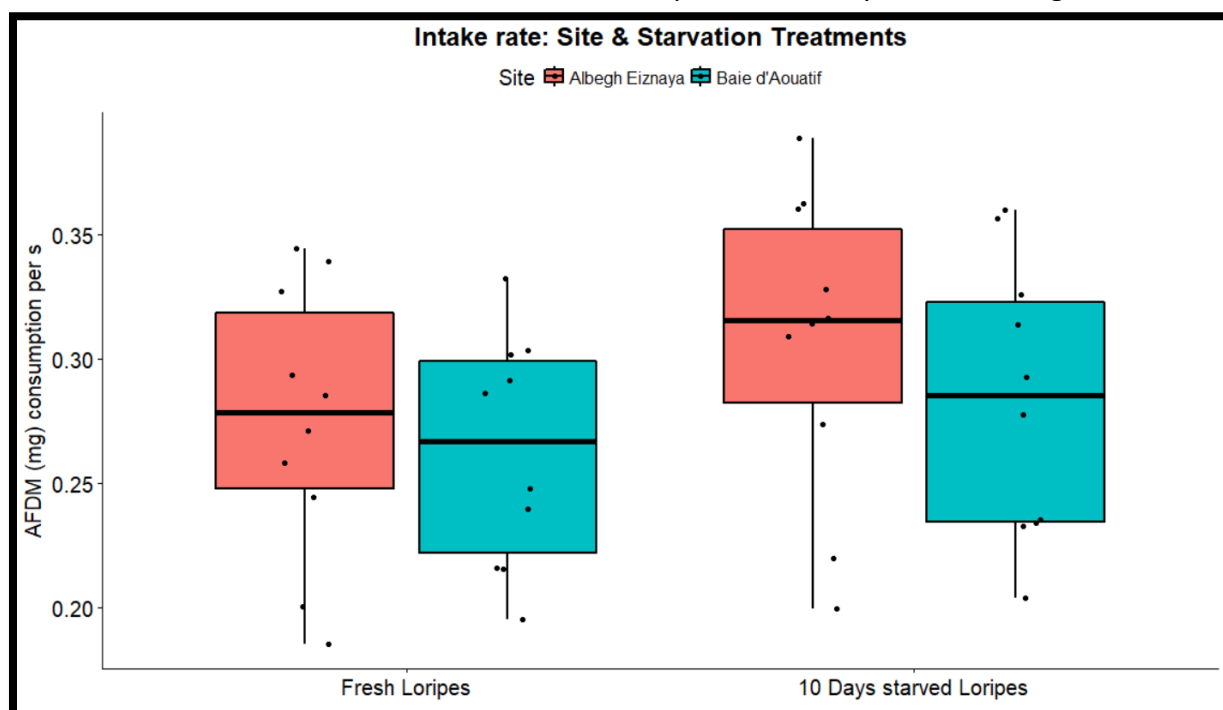
Figure 4: AFDM intake rates and Loripes Treatments. *The x-as shows the two starvation treatments "fresh Loripes" and "10 days staved Loripes" which are again separated into the sites Albegh Eiznaya (red/orange) and Baie d'Aouatif (green/blue). The black line within the boxplots presents the median.*

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Loripes. This increase in number led to a constant increase of ~ 0.0088 mg/s^{-1} (Table 1) AFDM per day (Figure 5; t=5.888, Nobs=480, Ngr=12, p= 0.00005). The trend on the interaction between site and day number, which affected AFDM intake rates as already mentioned above, can be seen in Figure 5. It seems that the differences between the starvation treatments diverged over time within Baie d'Aouatif the differences between the starvation treatments within Albegh Eiznaya remained constant.

Individual Differences. An increase in intake rates can be seen in all experimental birds but this differed between individuals (APPENDIX B). Bill length did not explain intake rates in the best fitting model that describes intake rates. However, the model gave a negative correlation between intercepts and slopes (Table 1) meaning bird with higher intake rates at the start tend to decrease or slowly increase intake rates over time compared to birds with low intake rates that tend to increase intake rates faster.
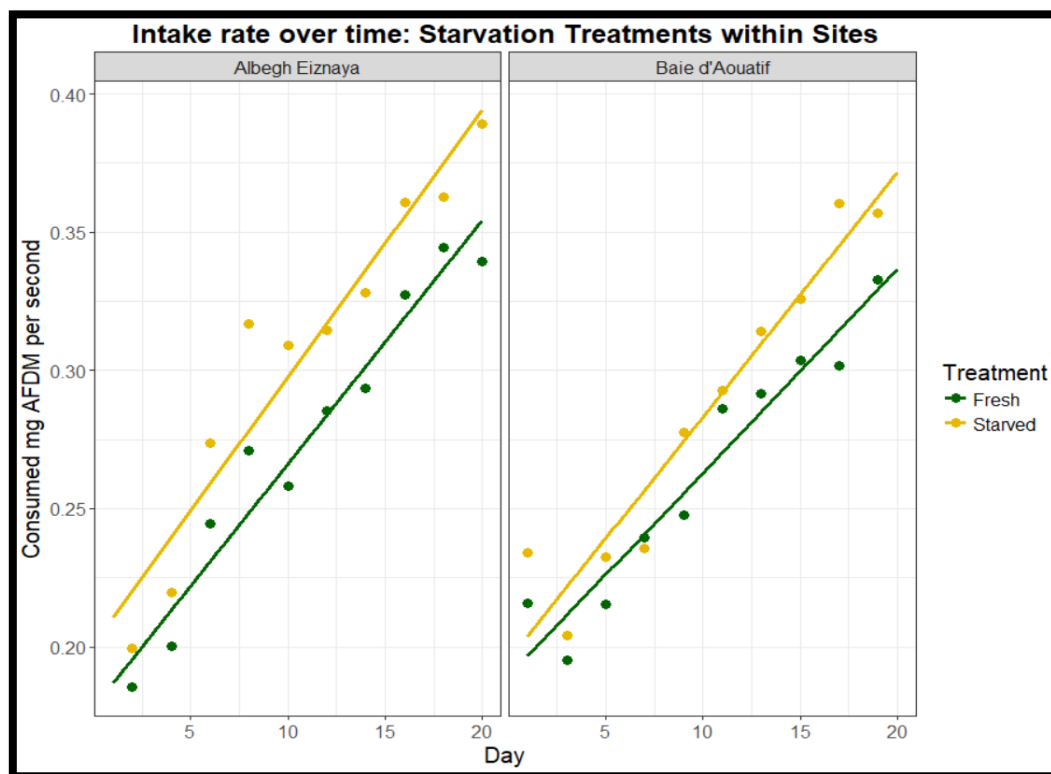

Figure 5: Intake rates on the four Loripes treatments over time. *The x-as shows the day number from day 1 to day 20 where Loripes from one site were offered at one day which alternated each day between Baie d'Aouatif and Albegh Eiznaya. Plots were split into the two different sites and intake rates were separated into fresh Loripes (darkgreen) and starved Loripes (yellow). The dots are the mean AFDM intake rates per session over which a linear smoothing line was integrated.*

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Bill length may thus play an important role in terms of feeding rates on Loripes. We found that individuals with longer bills do eat on Loripes in the first two days in captivity whereas Knots with shorter bills did not (Figure 6; t=-3.8886, Nobs= 34, p= 0.0004785).

Loripes Condition. The number of Loripes eaten is not the same as the AFDM consumption between the two sites, as presented in the previous analysis. Thus, differences between the number of Loripes eaten and AFDM intake rates are probably due to differences in prey quality. Loripes from Albegh were significantly shorter by 1.4 % compared to Loripes from the Baie (t= -3.4309, Nobs=880 p= 0.0006298). Yet, AFDM to shell ratio was higher in Albegh Eiznaya than in Baie d'Aouatif (t=2.0457, Nobs=396, p=0.04146) so that AFDM intake rates exceeds the number of Loripes eaten. In Figure 7 can be seen that shell mass intake rates at Baie d'Aouatif were constantly higher compared to shell mass intake rates on Loripes from Albegh Eiznaya while AFDM consumption is equal. Hence, digestive constraints on intake rates due to shell mass progressing rates may become important, beside toxicity, at a certain point when discussing spatial differences.
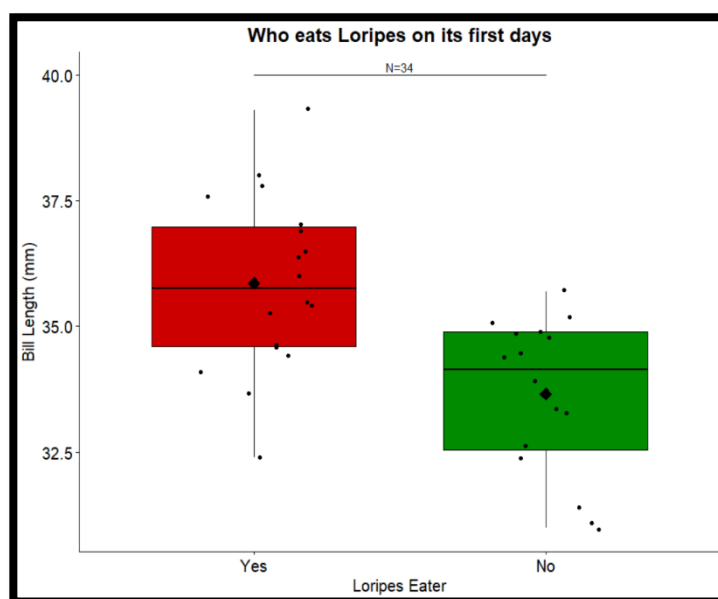

Figure 6: Who eats Loripes in the first two days? The x-as presents the groups Loripes Eater "Yes" and Non-Eater "No" against the birds bill lengths (mm) on the y-as. Black dots display the individuals. Black lines presenting the median and the black big dot, within the boxplots, the mean.

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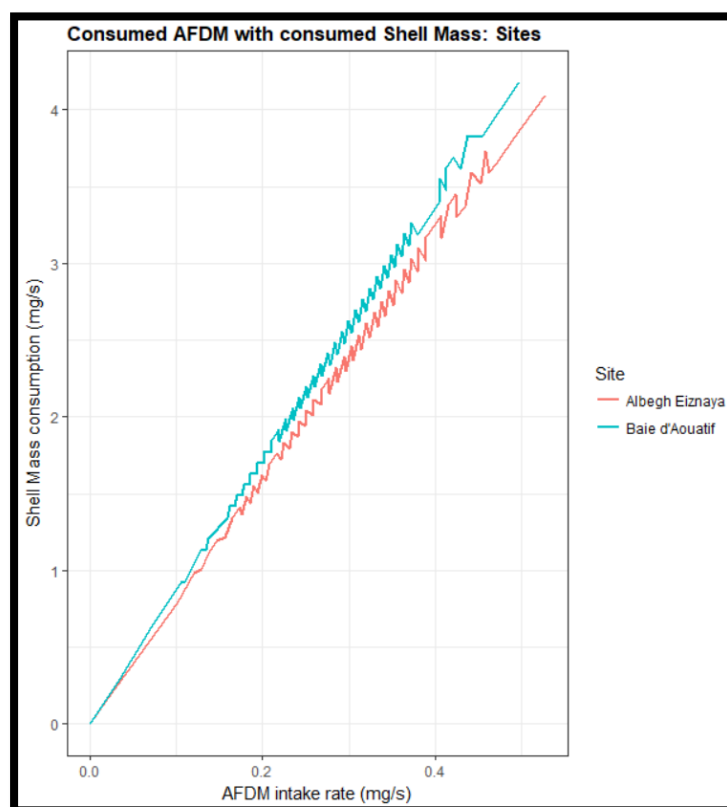
Figure 7: AFDM vs. Schell mass intake rates. Shell mass intake rates were based on shell dry weights in mg. The lines are the mean intake rates per session separated into Albegh Eiznaya (red/orange) and Baie D'Aouatif (blue/green).

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Discussion.

We found increased intake rates of about 9% on starved Loripes, Loripes that contain less sulphur (Figure 8a; t= -2.954, Nobs=269, p= 0.00342), than on fresh Loripes. In line with this, sulphur probably shapes the toxin constraint that limits the red knot increasing its maximum intake rates when eating on *Loripes lucinalis* in Banc d'Arguin.
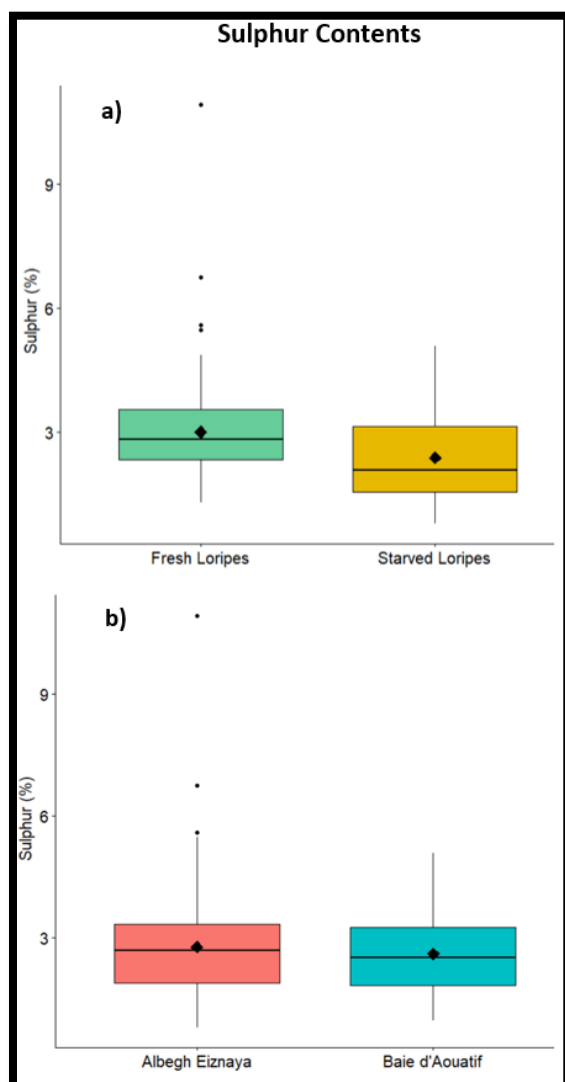
***Figure 8: Loripes Sulphur content percentages**. The y-as shows sulphur percentage of the Loripes samples for a) the starvation treatments b) the two sites. Within block plots, black lines present the median and the black square the mean.*

However, sulphur content data in Figure 8 (Data & Methods see APPENDIX C2) may only present a trend as percentages were roughly estimated. More reliable data are needed to validate this hypothesis.

Natural site variations in sulphur contents would make one site more suitable than the other by enabling the knots to maximise intake rates or to minimise foraging times in the field. The present study additionally investigated this by comparing intake rates on Loripes from two different sites, Albegh Eiznaya and Baie d'Aouatif (Figure 2). The consumption was higher on Loripes from Baie than from Albegh, when only looking at the number of Loripes consumed during the trials. Yet, AFDM intakes rates were significantly higher on Loripes from Albegh than from Baie (Figure 4). This is the other way around than expected based on previous years sulphur content data (de Fouw, October 2010, unpublished; Figure 2) but the sulphur content between the sites did not differ (t=-0.574, Nobs=269, p= 0.56679) in the present study. We found many Loripes eggs in the field during the expedition and Loripes are expected to digest the bacteria during periods of spawning (Johnson & Fernandez, 2001) which may be one reason why we did not found significant differences in sulphur contents between the sites.

We found that Loripes from Albegh have a higher flesh to shell ratio than the Loripes from the Baie, meaning that more Loripes need to be eaten in Baie d'Aouatif to achieve same AFDM intake rates as in Albegh Eiznaya. Thus, shell mass intake rates are higher in the Baie compared to Albegh. Therefore, Albegh would be a more suitable habitat based on AFDM intake rates only. This prey quality hypothesis may explain why intake rates in Albegh were higher if sulphur contents do not differ between sites in reality (trend shown in Figure 8).

Red knots at Banc d'Arguin segregate into two subpopulations divided in the two roosting and feeding areas Albegh Eiznaya and Baie d'Aouatif. It was shown that a higher proportion of birds that arrive at Banc d'Arguin chose to stay at Albegh and show high site fidelity. Furthermore, survival rates in Albegh were found to be higher compared to the Baie indicating that habitat differences are important (Buehler *et al.,* 2009). However, reliable sulphur content data on Loripes from the two different sites are needed for further analysis on the importance of the toxin constraint versus prey quality in the field.

Knots may overcome the toxic effects of sulphur to increase overall intake rates if they are flexible enough to increase sulphur

tolerances by adapting detoxification pathways (Oudman *et al.*, 2015). The experimental birds were caught at Albegh Eiznaya meaning that the birds may be adapted towards the Loripes' sulphur contents found there. Still, these inner mechanisms are unknown for the red knot. During the 20 experimental days, intake rates increased by 62% over time which is more than 6 and 10 times higher than the effect of the starvation treatment and the site differences, respectively. These findings indicate that the birds are able to adjust their physiology to the toxicity when offered ad libitum, which is in contrast to Oudman *et al.* (2015) who hypothesized that sulphur tolerance couldn't be adjusted which he based on a consistent low fraction of Loripes in the knots field diet. Because the effects of sites and treatments were constant from the start to the end of the experiments, they may have major effects on short-time events, like habitat selection at arrival.

Our feeding experiment presents differences in feeding rates between individual birds with a negative correlation of intercepts and slopes (Table 1). Birds with a higher consumption at the start had a less strong positive increase in intake rates compared to birds with low starting consumption rates that displayed stronger

increases over time. This strong increase suggests that birds are able to increase intake rates on Loripes by probably adjusting detoxification pathways in a short time period, even with low *"proportions"* (assumed based on low starting consumption rates) of Loripes in the diet in the beginning. Nevertheless, how do those differences arise in the field?

Bill lengths may explain differences in intercepts and slopes, even this variable did not fit the data, obtained during the feeding experiments, the best (APPENDIX A2). Shorter billed individuals are not able to reach Loripes that are buried at a certain depth and are therefore forced to feed on alternative prey species. Longer billed individuals do have access to a larger proportion of Loripes that are buried in the ground. Therefore, fractions of Loripes in the diet increase with the bill length (van Gils *et al.,* 2016). We indeed observed that longer billed individuals do eat on Loripes in the first two days (Figure 6) while shorter billed birds did not. This may be because longer billed individuals become inured towards feeding on toxic Loripes because they do have access to it. This may be due to inner mechanisms that probably also explain the overall increase over time on ad libitum offered Loripes (Figure 5).

The inner mechanisms are still unknown as already mentioned before. The microbiota evidently affects the physiology and, in turn, the health of bird which may steer the birds foraging behaviour and habitat selection. For instance, different vulture species are known to feed on decaying animal carcasses that release toxic components. The vultures are exposed to bacterial toxins but they are able to tolerate it in order to exploit this niche without much competition. It was found that different vulture species that were inured to carrion tissues, contained similar gut flora assemblages (Waite & Taylor*,* 2015). This suggests that the microbiota might have a major function in controlling intake rates on toxic prey. We thus tried to make a step forward in understanding the inner mechanisms in determining intake rates on Loripes. We sampled cloacal swaps throughout the whole experimental period, starting on catching days (Data & Methods see APPENDIX C3). DNA and RNA sequencing will then give us important insights on the bacterial compositions and if it differed between the individuals at start and whether it changed over time. Those samples are still in progress but their results may give us an answer on what underlying mechanisms determine maximum intake rates on *Loripes lucinalis*.

Concluding Remark.

Sulphur probably shapes the toxin constraint that limits intake rates on Loripes of the red knot (*Calidris canutus canutus*) at Banc D'Arguin. The toxic effect of sulphur probably limits overall maximum intake rates. Natural variations in prey quality and toxicity could explain differences in intake rates, bird segregation and survival. But assumed differences in toxicity may not be displayed in intake rates as strong as differences in prey quality. This should further be investigated with specific sulphur content data. The knots were able to increase intake rates on Loripes in a very short time period when offered ad libitum. Having access to the bivalves, which may come along with the adjustment of the gut microbiota, plays a major role in shaping individual intake rates on Loripes in the field. These factors could be used to understand individual survival rates and to predict future population trends. Based on this, conservation management plans on critical knot populations could be integrated at the overwintering grounds in Banc d'Arguin.

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APPENDIX

A. Model selection based on AICc

A1 Consumed Loripes in Numbers

Best fitting Fixed Effects

Loripes Starvation Treatments (T) and Sites (S) . Day(D) and Bill Length (B) were correlated slope and intercept random terms for all models. Models are sorted by AICc, with the most parsimonious model given in bold (i.e., model having the fewest parameters DF among models which ΔAICc < 2). Additional presented are the lokLik = ordinary likelihood; DF = number of parameters + 1 for the estimate of the error variance; Weight. All models are shown with ΔAICc < 10.

| Model	Df	LogLik	AICc	ΔAICc	Weight
~T+S+D	**8**	**-1378.08**	**2772.46**	**0**	**0.46**
~T+S+D+ B	9	-1377.75	2773.89	1.43	0.22
~T*S+D	9	-1378.022	2774.43	1.97	0.17
~T*S+D+ B	10	-1377.70	2775.87	3.41	0.08
~T*S*D	12	-1376.36	2777.39	4.93	0.04
~T*S*D+B	13	-1376.04	2778.86	6.40	0.02

A2 AFDM intake rates

Best fitting Fixed Effects

Loripes Starvation Treatments (T) and Sites (S) . Day(D) and Bill Length (B) were correlated slope and intercept random terms for all models. Models are sorted by AICc, with the best-fitted model given in bold (i.e., model having the fewest parameters DF among models which ΔAICc < 2). Additional presented are the lokLik = ordinary likelihood; DF = number of parameters + 1 for the estimate of the error variance; Weight. All models are shown with ΔAICc < 10.

Model	Df	LogLik	AICc	ΔAICc	Weight
~T*S*D	**12**	**902.48**	**-1780.29**	**0**	**0.51**
~T*S*D+B	13	902.82	-1778.86	1.43	0.25
~T*S+D	9	897.82	-1777.26	3.04	0.11
~T*S+D+ B	10	898.16	-1775.85	4.45	0.06
~T+S+D	8	895.97	-1775.63	4.66	0.05
~T+S+D	9	896.31	-1774.23	6.06	0.02

B. Individual Differences
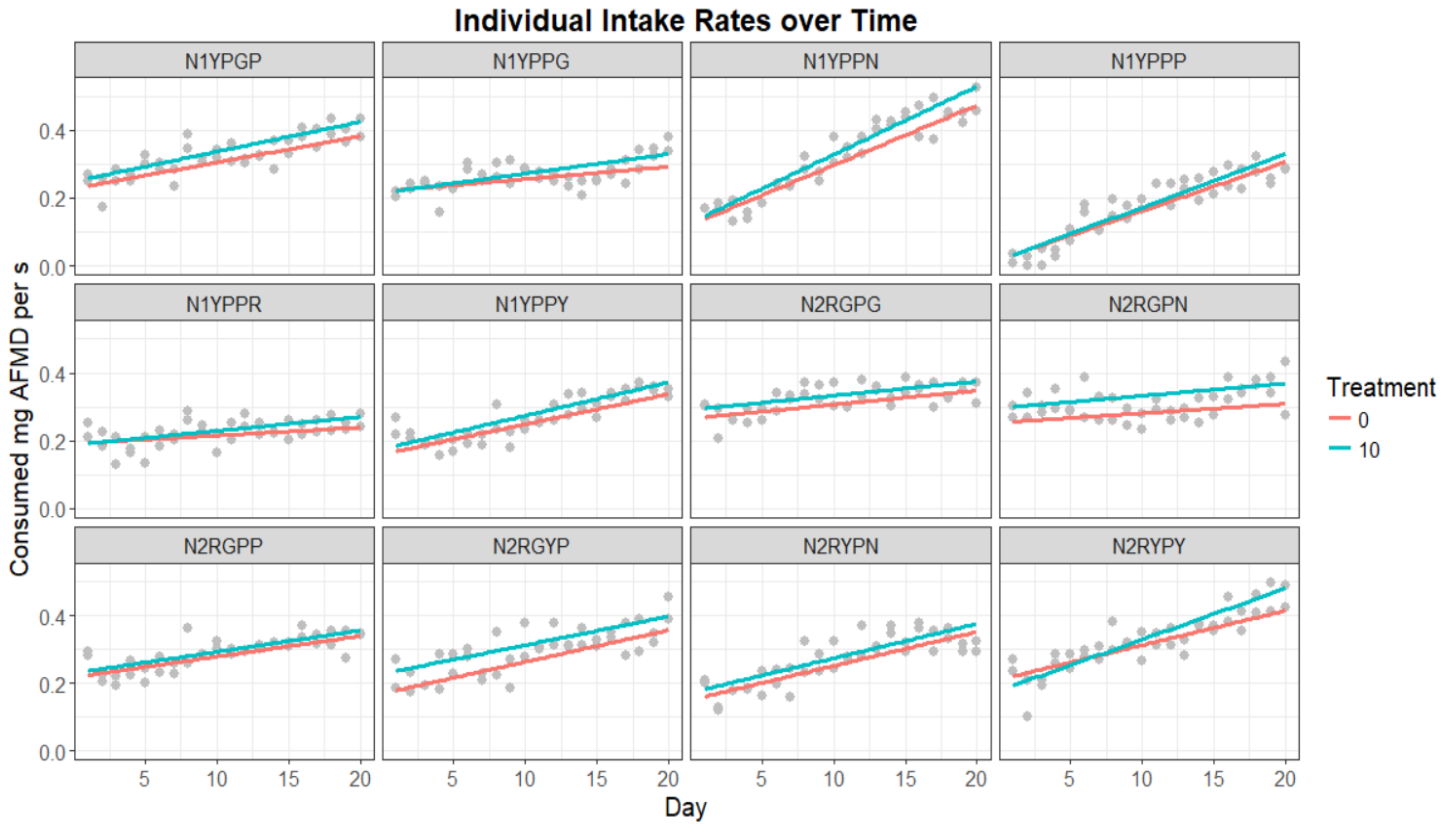
Figure: Individual Differences. *Birds (ID:Colorring) were separated into individual grids. The x-as presents the day number (1-20) and the y-as the AFDM intake rate in mg per second. Lines were additionally separated into fresh Loripes (red-orange) and 10 days starved Loripes (blue-greenish).*

C. Additional Data

C1 **Loripes Sulphide content.** 5 Loripes per Session (total: 200) were sampled at random and stored in 0.5 ml menthanol (70 %). Sulfur measurements were done at the Max-Plank Institute in Bremen.

C2 **Loripes Isotope Analysis**. We took 5 Loripes, at random, out of each session (total:200) and stored them in formaldehyde. In addition, 20 Loripes from four sessions (each Treatment; A0, A10, B0, B10) were stored in the freezer to compare storing methods. At NIOZ, the shell length (mm) of each Loripes, in total 280, was measured. The flesh was removed and stored separately in 2 ml Eppendorf cups, in preparation to freeze drying. The samples were freeze dried for three days and grinded afterwards. The pre-in weighed samples (3,5-4 mg) enriched with vanadium pentoxide (4 – 5 mg) in 30 mg tin cups, were analysed in Nijmegen.

C3 **Cloacal Swaps**. Cloacal swaps were taken from each bird on its catching day and afterwards each 3-4 days in the morning before the experiments. Cloacal swabbing is a method for collecting samples for microbiome analysis from the cloacal area. Samples were collected by gently inserting the appropriate swab into the cloaca. Then the swap was gently rotated for several times to maximize the sample output. All cloacal swaps, 10 per experimental bird (10x12=120), were stored in 1 ml RNA Later in the freezer. Samples that were stored in nitrogen, in a dry shipper, were taken additionally on four dates (4x12=48) to compare storing methods. Six test samples stored in both, RNA later and Nitrogen, were taken to test working procedures at NIOZ.

The extraction of RNA and DNA and preparation for the 16S rRNA gene sequencing took place at the NIOZ. We decided to include RNA for our analysis because quantifying RNA transcriptome from the bacteria enables us to define gene expression and which bacteria are active under what circumstances (Bashiardes *et al*., 2016) beside only looking at what DNA is there. This allows us to analyse changes in microbiotic composition and activity more precisely.