Investigating the foraging behaviour of *Calidris canutus*: can the toxic constraint be alleviated?

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Abstract

Red knots are omnivorous shorebirds, that winter in the Banc d'Arguin, Mauritania. In the Banc d'Arguin, the red knots mainly forage on molluscs, of which *Loripes lucinalis* is by far the most common. Despite its large abundance and favourable flesh-to-shell ratio, red knots prefer other species over *Loripes*. Through a symbiosis with gill bacteria, *Loripes* contain relatively large amounts of sulphur. This sulphur likely constraints the red knots in their intake of *Loripes*. However, starving the *Loripes* leads to decreased sulphur content, and possibly to higher intake rates by red knots. The intake rates of captive red knots on *Loripes* that were either fresh or starved for 6, 10 or 22 days were measured through an experiment. It was recorded that the *Loripes* dry weight intake rate of red knots increases linearly with starvation time, because the birds take less long breaks during foraging. Furthermore, it was recorded that while the experiment ran, the birds started to cope better with eating *Loripes*, leading to increased intake rates of *Loripes,* independent of treatment. This, the observation that birds drink less while foraging on starved *Loripes* and the indication that individual bird characteristics do not seem to influence intake rates help to better understand red knot ecology.

Introduction

Red knots (*Calidris canutus*) are migratory shorebirds that breed in the Arctic, spend a short period around the Dutch Wadden Sea, but are found in Western Africa for the largest part of their annual cycle (Leyrer, 2011). The world heritage site Banc d'Arguin, Mauritania, is the favourite wintering grounds of the red knots (Leyrer et al., 2006). The Banc d'Arguin has vast seagrass (*Zostera noltii*) overgrown mudflats, in which a variety of species live. Red knots are omnivorous birds, but at these wintering grounds its diet mainly consists of molluscs (Dekinga and Piersma, 1993; Piersma et al., 1993). The most frequently occurring mollusc in the Banc d'Arguin is the thin-shelled *Loripes lucinalis* (van der Geest et al., 2011; Salem et al., 2014), which plays an important role in its seagrass ecosystems (van der Heide et al., 2012).

As explained by the optimal foraging theory, foragers should select their diet based upon the profitability of the available prey, with profitability expressed as energy content of the prey per invested searching and handling time while consuming it (Stephens and Krebs, 1986). In the light of this theory, one would expect the frequently occurring and thin-shelled *Loripes* to be the preferred prey species of red knots, especially since the intake rates of bivalve prey by red knots is generally limited by shell-mass (van Gils et al., 2003). However, despite *Loripes* its large abundance and their favourable flesh-to-shell ratio, *Loripes* is not the most preyed upon mollusc by red knots in the Banc d'Arguin: among others the thick-shelled *Dosinia Isocardia* is preferred over *Loripes* (van Gils et al., 2012).

A possible explanation for this lack of foraging on *Loripes* by red knots is the toxic constraint. A toxic constraint occurs when the maximum tolerance of an element is lower than the intake of this element while foraging at the most efficient handling (e/h), leading to decreased intake rates (Hirakawa, 1995). *Loripes* are known to contain relatively high levels of sulphur (van der Heide et al., 2012), which is a result of a symbiotic relationship that the bivalves have with bacteria in their gills (Johnson and Fernandez, 2001). This bacteria filters sulphide out of the sediment, and stores this as sulphur (de Fouw, 2016). The high sulphur content of *Loripes* likely constrains the intake rates of red knots and causes the birds to prefer other prey (Oudman et al., 2015).

Elisabeth et al. (2014) found that for two different species of lucinid bivalves starvation leads

to decreased sulphur content, whereas Kemper (2015) recently discovered that this also occurs with starved *Loripes*. When *Loripes* are starved, the bivalves start to decompose the bacteria in their gills, and consequently the sulphur content decreases (Kemper, 2015). This opens up interesting experiment opportunities: will red knots consume starved *Loripes* at a higher rate than fresh individuals?

In this paper, it is hypothesised that red knots have increased intake rates on *Loripes* that have been starved, because the starvation time would cause the *Loripes* to contain less sulphur, leading to an alleviated toxic constraint. Furthermore, a development in the ability of red knots to cope with *Loripes* during the time the experiment would run was expected, independent of *Loripes* starvation. Accordingly, it is hypothesised that when the red knots follow a *Loripes* based diet, they will develop specific gut flora that help to achieve increased intake rates on *Loripes*. Finally, an effect of individual characteristics of each red knot, such as life stage and size, on the intake rates of starved *Loripes* and the development of intake rates over time is expected. To test these hypotheses, a large-scale experiment was conducted to achieve a better and more broad insight on the factors that affect the intake rates of red knots, and the possible ecosystem level effects hereof.

Materials and methods

Study area

The experiment was conducted in December of 2015 and January and February of 2017, during expeditions to the research station near Iwik, Banc d'Arguin National Park, Mauritania. In order to obtain a satisfactory sample size, two expeditions were needed.

Birds

Red knots were captured with mist nets during night time at high-tide and new moon, at the location with the highest abundance of red knots near the research station (Leyrer et al., 2012). After ringing, most birds were released the morning after, but in 2015 three birds were kept to settle in aviaries (50 x 50 x 50 cm), whereas six birds were retained for the experiment in 2017.

The birds that were used for the experiment were selected on life stage and beak length, to be able to find indications of the effect of these characteristics on *Loripes* feeding behaviour. The birds remained in their individual aviary for the most time, but every morning they were put together in a larger aviary for about an hour in which they could socialise and wash themselves. Overnight they were offered a combination of *Loripes* and flesh of the large bivalve *Senilia senilis*. The amount of overnight food was limited, to keep the birds at a relatively low but healthy body weight, to achieve maximum intake rates during the feeding sessions. The birds had access to fresh water at all times, including during the intake sessions, to maximise their intake rates (Oudman et al., 2014).

The experiment started after the birds were used to their aviaries and to eating just *Loripes* from the trays, which was seven to ten days after catching. In both years, the birds were released simultaneously and in good health when the experiment was finished.

Bivalves

Large amounts of *Loripes* were collected daily on the mudflats near the research station, at a site with known high densities (van der Geest et al., 2014), by sieving the top layer of mud. Back at the station, the *Loripes* were measured and divided into groups of 'small' (7.5-9.5 mm) and 'large' (9.5-11.5 mm) individuals, since their sulphur concentrations might be size-dependent (Rossi et al., 2013). These ranges were based on abundance and feasibility for the birds to swallow them.

The collected *Loripes* were exposed to four different treatments: no treatment (fresh), starved for 6 days, starved for 10 days and starved for 22 days. The number of starvation days of the different treatments were chosen by aiming for the longest feasible starvation time, while still accomplishing a large sample size. To starve the *Loripes*, they were hung in water-permeable bags in the gully near the research station. Here, the bivalves could survive, but were not able to feed of the sediment. The theory is that the *Loripes* will start to decompose their sulphur content, reducing their toxicity over time.

Table 1 The distribution of the 309 trials that took place, per treatment and bird. N1YYNP, N1YNRP, N1YNGP, N1RRPP, N1GYPY, N2PRYY, N2PNRY, N2PRNP and N2PNRR are the individual birds.

| Treatment | | 2015 | | | 2017 | | | | | | Total |
Size class	Starvation (days)	N1YYNP	N1YNRP	N1YNGP	N1RRPP	N1GYPY	N2PRYY	N2PNRY	N2PRNP	N2PNRR	309
Small	0	6	6	6	6	6		6	6		42
	6	6	5	5	5	5		4	5		35
	10	1	2	1	7	6		6	6		29
	22				4	4		3	4		15
Large	0	6	6	6	8	8	10	8	8	10	70
	6	4	5	5	5	5	4	5	5	4	42
	10	4	3	3	7	7	8	7	6	8	53
	22				4	4	4	4	4	3	23

Experimental design

For the experiment, 90 sessions were run, in which the *Loripes* intake rates of the birds, the response variable, was measured. Per session, the birds were given a sufficient amount of *Loripes* from one of the treatments (0, 6, 10 or 22 days starved; large or small) and had a set time period to consume as many as they could (two hours in 2015, one hour in 2017). As each session entailed one to six birds, a total of 309 trials were carried out (80 in 2015 and 229 in 2017, see Table 1), using a total of nine captive birds (three in 2015 and six in 2017). Each bird was usually involved in three trials per day, in which the combination of treatments (starvation time, size) was randomised as much as logistically possible.

Prior to each session, the red knots had no access to food for at least two hours, in order to make the assumption plausible that the measured intake rates were the maximum intake rates. For each trial, the amount of offered *Loripes* in the beginning (ranged from 77 to 300) and leftover *Loripes* at the end (ranged from 9 to 207) were counted, and herewith the consumption was calculated.

Around half of the trials were successfully recorded with video cameras (four birds, all in 2017). Furthermore, for every session ten *Loripes* were randomly selected to be opened and dried in the field station, to be analysed on dry weight and sulphur content back in the Netherlands. Additionally, for each bird faeces were collected once after each treatment, to be analysed in the Netherlands on leftover energy content. Finally, in 2017 cloacal swabs of the birds were taken every other day, and brought back to the Netherlands for analysis on gut flora.

Data processing and analysis

Back in the Netherlands, the dry weight samples and videos were analysed. The faeces samples, sulphur samples and cloacal swabs are still being worked on. 152 one hour long video recordings were watched and behaviour was scored in Noldus the Observer, in which the foraging and drinking behaviour of the four recorded red knots were scored. The dry *Loripes* samples that were taken, were dried further in a 60°C stove for three days, and could then be used to calculate the dry weight consumption of the birds in flesh and shell per session. Hereafter, they were taken to Bremen, to be analysed there on sulphur content of *Loripes* for each session.

For the intake rates of the experiment sessions, the intake per session per bird in dry weight *Loripes* per second was calculated with the dried *Loripes* samples, length of the session and the calculated consumptions. Days were used as a continuous variable in the analysis, as it is expected that *Loripes* have a constant metabolism and thus a stable decrease of sulphur over time. Subsequently, a set of candidate linear mixed effects models, including all combinations of explanatory variables, was ranked according to the likelihood of each model. Rank was determined by calculating the Akaike weight of the model. The model that explained the variation in dry flesh *Loripes* intakes best contained experiment day and starvation time of the *Loripes*, with bird as random effect (Table 2, AICc=-887.980).

The behaviour scores output from Noldus the Observer were analysed with generalized linear models. All tests were done in R, version 3.4.1 (R Development Core Team 2017).

Table 2 Models relating intake rate of dry flesh *Loripes* by captive red knots to experiment day (D), starvation time of *Loripes* (ST), size class of *Loripes* (Sz), bill length (Bl), age of red knot (Ag) and year (Yr). Analysis included 309 trials, with three and six birds divided over two winters. Models are sorted by AICc, with the most parsimonious model in bold (i.e., model having the fewest parameters K among models which ΔAICc<2).

Model	K	AIC$_c$	ΔAIC$_c$	AICcWt	Cum.Wt	LL
~D+ST+ST^2	6	-889.874	0.00	0.28	0.28	451.08
~D+ST+Sz	6	-888.087	1.79	0.11	0.39	450.18
~D+ST	**5**	**-887.980**	**1.89**	**0.11**	**0.50**	**449.09**
~D*Bl+ST	7	-887.779	2.09	0.10	0.60	451.08
~D+ST+Ag*Bl	8	-887.448	2.43	0.08	0.68	451.96
~D+ST+Sz+Ag*Bl	9	-887.446	2.43	0.08	0.76	453.02
~D+ST+Sz+Ag	7	-887.097	2.78	0.07	0.83	450.73
~D*ST	6	-886.287	3.59	0.05	0.88	449.28
~D*Ag+ST	7	-885.458	4.42	0.03	0.91	449.92
~D+ST*Bl	7	-885.323	4.55	0.03	0.94	449.85
~D+ST+Sz+Ag+Bl	8	-885.010	4.86	0.02	0.96	450.74
~D+ST+Sz*Bl	8	-884.188	5.69	0.02	0.98	450.33
~D+ST+Sz+Ag+Bl+Yr	9	-883.574	6.30	0.01	0.99	451.09
~D+ST+Sz*Ag*Bl	12	-882.812	7.06	0.01	1.00	453.93
~D+ST^2	5	-880.066	9.81	0.00	1.00	445.13
~D	4	-872.668	17.21	0.00	1.00	440.40
~D+Sz	5	-872.188	17.69	0.00	1.00	441.19
~ST+Sz	5	-736.773	153.10	0.00	1.00	373.49
~ST	4	-730.112	159.76	0.00	1.00	369.12
~Sz	4	-691.226	198.65	0.00	1.00	349.68
~Ag	4	-684.013	205.86	0.00	1.00	346.07
~Bl	4	-683.835	206.04	0.00	1.00	345.98
~Yr	4	-683.670	206.20	0.00	1.00	345.90

Results

Consumption

The red knots were recorded to have linearly higher consumption of dry flesh *Loripes* per starvation time (see Fig. 1, Treatment: t = 4.209, P < 0.001, df = 298). Furthermore, over time, the red knots had linearly increasing intake rates of dry flesh *Loripes*, independent of treatment (see Fig. 1, Day: t = 14.456, P < 0.001, df = 298).

Size of the *Loripes* did not affect the dry weight consumption, and had no effect on the intake rates. The life stage (juvenile/adult) of the birds did not affect the relationship between intake rates and starvation, and neither did beak length (see Table 2).
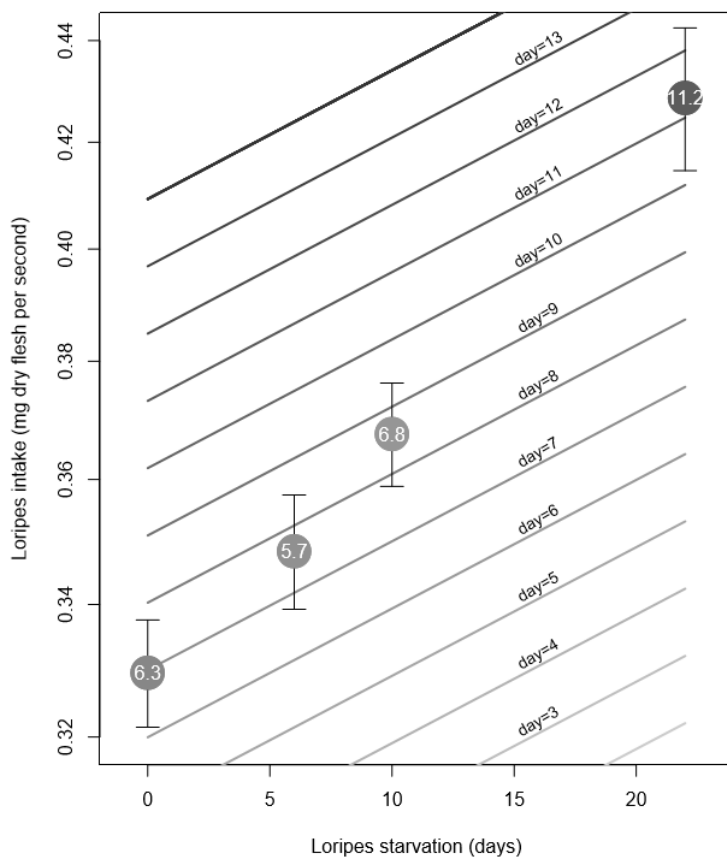
Fig. 1 Intake rate of red knots on *Loripes* (mg dry weight per second) against the starvation time of *Loripes* (days). The four dots show the average ± standard errors of the intake rates of nine birds in the four starvation treatments (0, 6, 10 or 22 days starved, N=309). The lines represent the predicted intake per day, based on the most parsimonious model: Intake rate ~ Starvation time + Experiment day in which both factors are positively related to the intake rate. Each line shows the predicted intake rate at one experiment day. Numbers in the dots show the mean day of the experiment in which this treatment was fed to the birds.

Video analysis

The videos that were analysed showed that on average, the birds took 43 sips of water when ingesting fresh *Loripes*, and for every day their prey was starved, the birds took 0.8 less sips (see Fig. 2, GLM: F = 15.563; P < 0.0001).

Furthermore, there was no significant relation recorded between average number of short breaks (>30 seconds) and starvation of prey (see Fig. 3a, GLM: F = 5.3789; P = 0.217), with a break defined as the period of time between two ingested *Loripes*. However, when considering the number of long breaks (>180 seconds), a linear relation was recorded. On average, the birds took 4.29 long breaks per hour when foraging on fresh *Loripes*, while the birds took 0.06 less long breaks per starvation day of *Loripes* (see Fig. 3b, GLM: F = 13.129; P = 0.0017)

Dosinia

When the *Loripes* intake rates by red knots are combined with data from earlier years, it shows that the red knots seem to achieve higher intake rates than before. This corresponds with the expected intake of *Dosinia*, calculated with the recorded densities in the field (see Fig. 4).

Discussion

The captive red knots were recorded to consume more *Loripes* dry weight when the *Loripes* had been starved, and thus that part of the hypothesis was confirmed. Unfortunately, at the time of finishing this paper, the sulphur samples have not been completely analysed yet. However, the fact that the red knots eat more *Loripes* when the prey is starved, while the flesh content remained stable, indicates that the sulphur content did decrease. The video analysis showed that the higher intake rates on starved *Loripes* are a result of the birds taking less long breaks.

Moreover, the red knots achieved increased intake rates over the time the experiment ran, independently of treatment. The reason behind this most likely lies in the fact that the birds over time get more comfortable with eating from trays and living in aviaries, and that the *Loripes* based diet causes the birds to develop intestine flora that helps them cope with the high sulphur content of *Loripes*. The cloacal
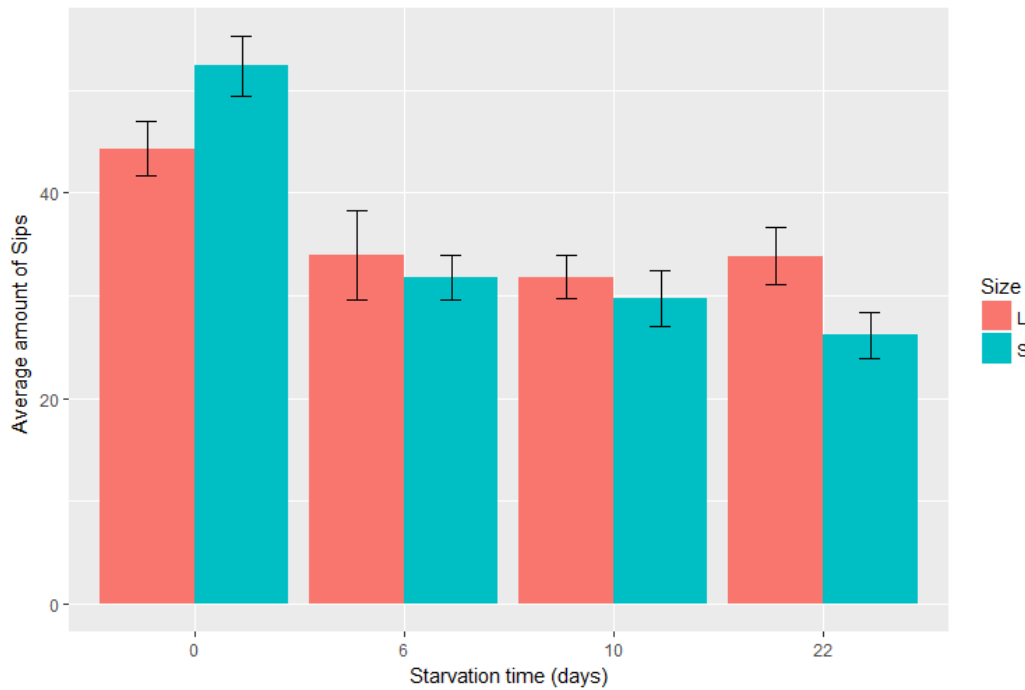
Fig. 2 The average number of sips that the four birds that were recorded on video took, per treatment (0, 6, 10 or 22 days starved, small or large, N=152). Error bars indicate the standard errors.

swabs that were taken will help to map the gut flora development of the birds during the experiment, and are currently being analysed. The finding that the maximum intake of *Loripes* in captivity correlates with the amount of *Dosinia* in the field, suggests that the red knots already eat more *Loripes* in the field and have better gut flora to digest *Loripes* than in previous years.

Another interesting finding was that age and beak length did not have an effect on the intake rates. One would expect that birds with long beaks already eat more *Loripes* in the field, since it is a deep burrowing species (van Gils et al., 2016), but no effect was recorded. It is also interesting that juveniles and adults seem to adjust to the *Loripes* diet in a similar way, both have increased intake rates over time and have increased intake rates with starved *Loripes* (both are limited by sulphur). Possibly the sample size within the variating characteristics was too small to find any results. Due to global warming, the species red knots experience body shrinkage: young birds get smaller body sizes and beaks, and are found to have reduced survival rates in the Banc d'Arguin (van Gils et al., 2016). The slighter chance of survival of the birds with smaller beaks mainly lies in them not being able to reach the deeply buried bivalves (van Gils et al., 2016). However, in the experiment carried out in this paper, the smaller beaked birds had similar intake rates on the offered *Loripes*. One would expect that
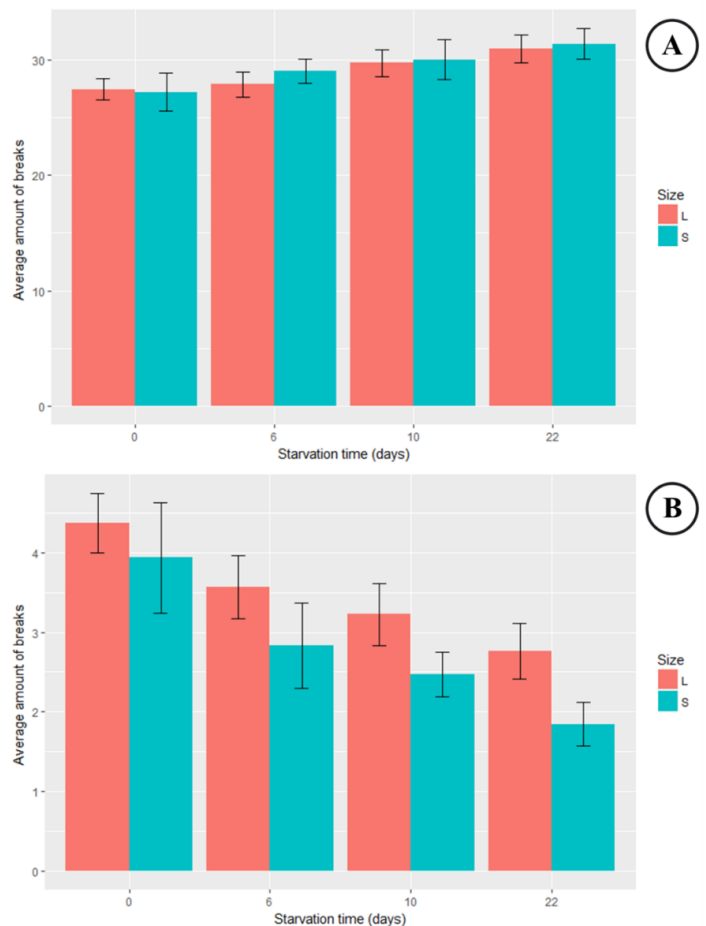
Fig. 3 The average number of breaks that the four birds that were recorded on video took, per treatment (0, 6, 10 or 22 days starved, small or large, N=152). **A:** number of breaks that were at least 30 seconds long. **B:** number of breaks that were at least 180 seconds long. Error bars indicate the standard errors.

the longer billed birds used in the experiment have more experience with eating *Loripes* and are more capable of doing so, especially in the beginning of the experiment. The question remains what this implies. Do the birds eat the same amount of *Loripes* in the field (possibly none at all)? More research on this subject is needed.

Lastly, the video analysis showed that the red knots take less sips of water while foraging on *Loripes* that have been starved. The reason behind this drinking behaviour probably lies in the diarrhea the birds get when foraging on *Loripes*, and the loss of water that accompanies this (Oudman et al., 2014; personal observation). The amount of energy that is leftover in the faeces is an indication of how severe the diarrhea was, and it would be interesting to see if the energy in the collected faeces differs per starvation treatment of the prey. The faeces samples are yet to be analysed. If the eventual results correspond with the decreasing water intake observed in the videos, this would indicate that sulphur is the reason of diarrhea. In this experiment, the birds had access to fresh water, since it was attempted to maximise the intake rates and red knots are known to ingest more when they have access to fresh water (Oudman et al., 2014). However, in the Banc d'Arguin there is no fresh water available for the birds to drink from, only salt water. So, if the red knots need the water to achieve high intake rates on *Loripes*, this could lead to high salt intakes in the field. This could lead to another constraint for the red knots when feeding on *Loripes*: a salt constraint. This, and the other findings of this study help to better understand the red knot ecology and their behavioural choices, and opens up opportunities for interesting future research.
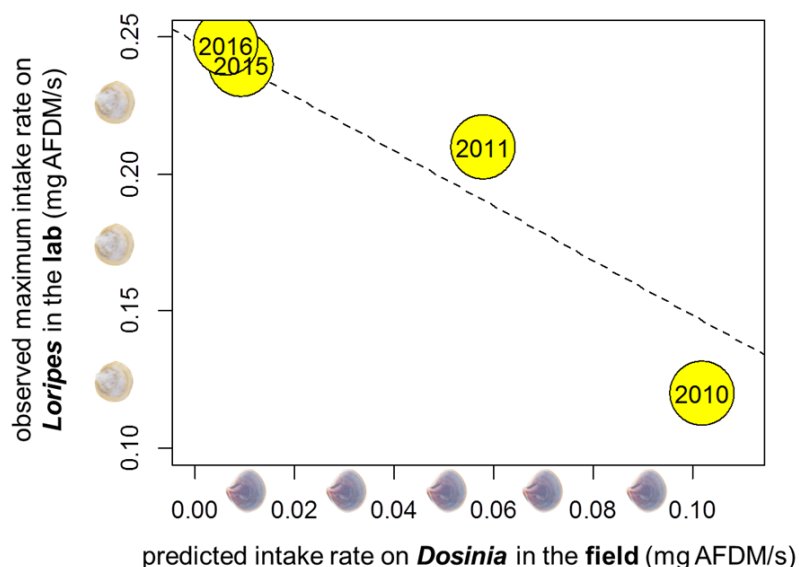
Fig. 4 Maximum intake rate of captive red knots on *Loripes* against the predicted intake rate on *Dosinia* in four winters. The intake rate on *Dosinia* is derived from the densities measured in the mudflats around Iwik in the Banc d'Arguin. The maximum intake rate on *Loripes* is measured during the intake experiments with captive red knots.

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