Sulfur in lucinid bivalves inhibits intake rates of a molluscivore shorebird

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Authors' contributions: JvG and JP conceived the concept. JvG and TO designed methodology and collect the data. TO analysed the data and led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication. JvG provided funding and supervised the project.

ABSTRACT

A forager's energy intake rate is usually constrained by a combination of handling time, encounter rate and digestion rate. On top of that, food intake may be constrained when a forager can only process a maximum amount of certain toxic compounds. The latter constraint is well described for herbivores with a limited tolerance to plant secondary metabolites. In sulfidic marine ecosystems, many animals host chemoautotrophic endosymbionts, which store sulfur compounds as an energy resource, potentially making their hosts toxic to predators. The red knot *Calidris canutus canutus* is a molluscivore shorebird that winters on the mudflats of Banc d'Arguin, where the most abundant bivalve prey *Loripes orbiculatus* hosts sulfide-oxidizing bacteria. In this system, we studied the potential effect of sulfur on the red knots' intake rates, by offering *Loripes* with various sulfur content to captive birds. To manipulate toxicity, we starved *Loripes* for 10 days by removing them from their symbiont's energy source sulfide. As predicted, we found lower sulfur concentrations in starved *Loripes*. We also included natural variation in sulfur concentrations by offering *Loripes* collected at two different locations. In both cases lower sulfur levels in *Loripes* resulted in higher consumption rates in red knots. Over time the red knots increased their intake rates on *Loripes*, showing their ability to adjust to a higher intake of sulfur.

Keywords: digestive constraint, lucinid bivalve, red knot, sulfide, toxicity

INTRODUCTION

Constraints on a forager's intake rate are important aspects of its prey and patch choice (Stephens and Krebs 1986) and have been studied for a long time. For example, in his seminal work, Holling (1959) showed that prey density, searching efficiency and handling time constrain a forager's ingestion rate. Later he showed that satiation also limits a forager's energy intake rate (Holling 1966). This is a general constraint that occurs when rate of ingestion exceeds rate of digestion temporarily (Belovsky 1984; Charnov 1976; Jeschke et al. 2002).

Toxicity of food sets another 'internal' limitation to maximum intake rate. The toxin constraint is set by a maximum tolerance to a toxin by the consumers (Hirakawa 1995). Common toxins are the secondary metabolites present in plants, animals and microorganisms (Luckner 2013). In plants, such compounds function as chemical defense against herbivores (Freeland and Janzen 1974; Iason 2005; Singer et al. 2002), and in animals toxins can also lead to avoidance or reduction of predation (Berenbaum 1995; Bloxham et al. 2014; Lindquist and Hay 1995). In microorganisms, the synthesis of secondary metabolites occurs commonly (Berdy 2005; O'Brien and Wright 2011). Plants and animals that live in symbioses with microorganisms therefore often contain toxins, which can help defend them against herbivores and predators (Clay 2014; Flórez et al. 2015; White Jr and Torres 2009).

Symbioses between marine invertebrates have evolved multiple times in diverse animal and bacterial groups, and are found widespread in the oceans from shallow-water seagrass meadows to deep-sea hydrothermal vents and seeps (Dubilier et al. 2008). If the bacteria in these symbioses store elemental sulfur as an intermediate of hydrogen sulfide oxidation, the tissues of their hosts can contain 10 times more sulfur than animals that do not host sulfur-oxidizing symbionts (Vetter and Fry 1998). Thus, in contrast to the above examples, sulfur would not be considered a secondary metabolite, but an intermediate of the symbiont's core energy metabolism. Their role as nutritional symbionts has been relatively well studied (Felbeck and Somero 1982; Sogin et al. 2020), but so far, their potential role as defensive symbionts that protect the host against predation by storage of large amounts of sulfur has received far less attention. In the one study that has so far addressed this topic, Kicklighter et al. (2004) observed a limited intake by shallow-water generalist consumers, fishes and crabs, on tissues of

some animals from hydrocarbon seeps and hydrothermal vents. In that study, four out of the five unpalatable tissues were trophosome and gill tissues, which contain endosymbionts that use sulfide as an energy source and store elemental sulfur. In contrast, the gill tissues of mussels that contain endosymbionts that do not use sulfide, appeared to be palatable. This suggests that the accumulation of sulfur, particularly in the form of elemental sulfur, by sulfur-oxidizing bacteria might cause unpalatability of host tissues and therefore shape a toxin constraint for predators in sulfidic ecosystems.

In intertidal mudflats predators have access to animals living in sulfidic sediment layers (Jørgensen 1982). Especially sediments of seagrass beds are rich in sulfide because of the large amount of organic matter, which is decomposed by sulfate reducing bacteria under anaerobic conditions, with sulfide as an end product (Jørgensen 1982; Jørgensen et al. 2019; Marbà et al. 2007). In turn, sulfide is used as energy source by chemoautotrophic bacteria that are living in the sediment, but also live as endosymbionts in the gill tissue in lucinid bivalves (Jørgensen et al. 2019; Taylor and Glover 2000). Some species of the Lucinidae family are found in high densities in seagrass meadows where they live between the seagrass rhizomes in the sulfide-rich sediments (van der Heide et al. 2012). During low tide, shorebirds feed on these lucinids, despite the fact that ingestion of these prey induces diarrhea in the birds and that intake rates on these lucinids are lower than on other bivalve species (Oudman et al. 2015; Oudman et al. 2014; van Gils et al. 2013). This limited intake rate might be caused by the ingestion of sulfur, stored within the bacterial symbionts (Oudman et al. 2014).

If sulfur ingestion is the cause for this limited intake rate on lucinid bivalves, this can be tested by offering consumers prey with different amounts of sulfur. Consumers would then be expected to reach higher intake rates when sulfur levels are lower. An opportunity to possibly manipulate sulfur concentrations in lucinid bivalves is by ‘starving’ them (Elisabeth et al. 2014; Lechaire et al. 2008). In this procedure, bivalves are taken out of the sulfidic sediment, and without sulfide inflow, the chemoautotrophic bacteria most likely will oxidize their stored elemental sulfur to dissolved sulfate, which is subsequently excreted. Another possibility to obtain lucinid bivalves with varying sulfur levels is by collecting them from different locations as sulfur levels inside lucinid bivalves might vary

spatially, as a result of varying sulfide levels in the sediment (Rossi et al. 2013). Sulfur levels inside lucinids would be expected to be higher in dense seagrass beds, likely with a high sulfide production (Larkum et al. 2006), compared to lucinids from locations with lower seagrass cover.

In this study we present a feeding experiment with captive individuals of a molluscivore specialist, in which we measured the intake rates on lucinid bivalves with varying toxin levels, induced by a starvation treatment, but also by natural (i.e. spatial) variation.

MATERIALS AND METHODS

Study system

The study was carried out in January and February 2018 in Parc National du Banc d'Arguin in Mauritania (20°14'N, 16°06'W), where red knots *Calidris canutus canutus* (Linnaeus, 1758) feed amongst others on *Loripes orbiculatus* (Poli, 1795) (Oudman 2017; van Gils et al. 2012). Red knots are midsized shorebirds (average weight in winter in Mauritania 124 g, ten Horn et al. unpub. data), that breed in Arctic Siberia and winter along the coast of West Africa, notably Banc d'Arguin (Leyrer et al. 2006; Piersma 2007). There, they forage on seagrass-covered (*Zostera noltii*, Hornem.) intertidal mudflats (Wolff and Smit 1990). *Loripes orbiculatus*, the most abundant mollusk species in this ecosystem (Ahmedou Salem et al. 2014; Honkoop et al. 2008), is thin-shelled and hosts sulfide-oxidizing bacteria in their gills (Herry et al. 1989; Petersen et al. 2016). They live in between the rhizomes of the seagrass at a depth of 3.5 cm on average, available to red knots which are probing the wet sediment with their bill of 3.5 cm on average (van Gils et al. 2016).

Birds

The red knots for the experiment were caught with mist nets during the night at a high-tide roost, called Abelgh Eiznaya, close to the PNBA research station at Iwik (Leyrer et al., 2012). They were brought to the research station, where they were individually marked with color rings and their body size measures were taken. From then on, they were housed in small individual cages (0.5 m x 0.5 m x 0.5 m) and every morning they were put together in a larger cage in which they could socialize and

wash themselves for about an hour. The individual cages contained a fresh water tray and a food tray (both round plastic cups, height 3 cm, diameter 10 cm, without sediment). Overnight they were offered staple food which was a mixture of *Loripes* and flesh of the large bivalve *Senilia senilis* (Linnaeus, 1758). We limited the amount of overnight food to keep the birds at a relatively low but healthy body weight (range 92-129 g), aiming for maximum intake rates during the experimental trials (van Gils et al. 2005). Freshwater was always available for the birds. The experiment started when all birds were observed to have eaten *Loripes* from their individual food trays, which was 5-7 days after catching.

Bivalves

Loripes subjects were collected at two different locations to exploit a potential source of natural variation in sulfur content: location A, with a relatively dense seagrass cover of 94% (Abelgh Eiznaya, 19°53’33.24’’N, 16°18’50.28’’W) and location B, with relatively low seagrass cover of 44% (Twimitirt, 19°52’29.16’’N, 16°17’15.66’’W) (S. Yahya Cheikhna Lemrabott et al., unpub. data). Preliminary data indicated that *Loripes* from the two sites differed in sulfur concentration (J. de Fouw, unpub. data). *Loripes* subjects were collected daily from either one of the two locations by sieving the top layer of mud (2 mm mesh). Half of the collected *Loripes* was used in the experiment the same day as control subjects, and the other half went into a starvation treatment, in which the specimens were placed in water-permeable bags in the sea nearby the research station for 10 days. The aim of the starvation treatment was a reduction in sulfur concentration in *Loripes* subjects (Elisabeth et al., 2014).

Experiment

We measured intake rate of red knots feeding on *Loripes* during half-hour feeding trails. We used a 2 X 2 experimental design in which we offered red knot subjects *Loripes* that varied in sulfur concentration (Starved versus Control) and the location from which they were collected (Locations A and B). In total, we conducted 480 trials, using 12 birds over 20 consecutive days. Each day, every bird was subject to two trials: one trial with Starved *Loripes* and one trial with Control *Loripes*, both from the same location, alternating the locations each successive day. Bird subjects were prevented from feeding for at least two hours before a trial started to ensure maximal intake rates (Oudman et al. 2015). To prevent interference of size-specific characteristics and preferences, we only used *Loripes*

with a length ranging from 9.0 mm to 11.0 mm (red knots have strong size-preferences (Dekinga and Piersma 1993; Onrust et al. 2013)). This range was selected based on abundance and feasibility for the birds to swallow them. Given this narrow size range, we assume that the captive red knots select their prey randomly from the feeding tray, but if a certain size would be preferred, we expect this to be equal across treatments.

Response variables

Before every session we randomly selected 5 and 10 *Loripes* specimens, for sulfur determination and dry mass measurements, respectively. The specimens for sulfur determination were preserved in formaldehyde, dried in the laboratory, ground to fine powder in a ball mill and analyzed for total sulfur content (% dry weight) on an elemental analyzer (Thermo Scientific). The specimens meant for dry weight determination were opened up and dried in the field station and later brought back to the Netherlands, where they were further dried at 60°C. Afterwards, flesh and shell was weighted separately.

To determine the birds' intake rate, the number of consumed *Loripes* specimen were determined by subtracting the number of *Loripes* leftover at end of a trial (range 6-60) from the number of *Loripes* offered at the onset of a trial (range 50-70, but mostly 60 – this number was chosen such that the birds would always have enough to eat in the trials).

Gizzard height and width of the birds were measured at Day 2, 6 and 9 of the experiment, using ultrasonography (Dietz et al. 1999). With these measurements gizzard masses were estimated (Dietz et al. 1999), which consequently were used to calculate potential shell processing rates (van Gils et al. 2003).

Statistical analysis

To analyze the variation of sulfur percentage (*S*), flesh dry mass (*DM*) and shell mass (*SM*) between the sampled *Loripes*, several linear mixed-effects models were created for all response variables separately, using *lme4* in R (Bates et al. 2014), with all possible combinations of Treatment (*T*),

Location (*L*) and Day (*D*) as fixed effects and *Session* as a random effect. An intercept-only model was also included. The best approximating model, i.e. the model with the fewest parameters within 2 ΔAICc of the top model, was selected based on Akaike's Information Criterium, adjusted for small sample size (AICc) (Burnham and Anderson 2002), using the AICcmodavg package in R (Mazerolle 2017). The variation in intake rates (*I*, the number of *Loripes* eaten per trial), was modeled the same way, with Treatment, Location and Day as fixed effects and both Session and individual Bird (*ID*) as random effects.

To understand how much sulfur the red knots consumed in their trials, the average amount of sulfur (in mg) per individual *Loripes* was calculated per treatment, by multiplying the average flesh dry mass by the average sulfur percentage, both per treatment. For visualization, standard errors were calculated with the variance of these products (Goodman 1960) and the minimum sample size (which was for the sulfur measurements, rather than the dry weight measurements).

RESULTS

Both the location and the starvation treatment caused variation in total sulfur content of *Loripes,* as aimed for. The best approximating model to explain variation in *Loripes* total sulfur content (n=195, Table 1) showed that starvation resulted in a lower percentage of total sulfur in an individual *Loripes* (estimate=-0.684 percentage point, df=40.92, t=-3.925) and that *Loripes* from location B contained a lower percentage of total sulfur than *Loripes* from location A (estimate=-0.378 percentage point, df=40.90, t=-2.169) (Fig. 1a, Table S1).

The starvation treatment did not affect the mass of the *Loripes*. The best approximating model for dry flesh mass of *Loripes* included location only (Table S2), in which the dry mass in location B was 1.16 mg lower than in location A (df=39.94, t=-2.125, Table S1). Shell mass was not affected by any of the variables, the best approximating model was the intercept-only model (Table S2).

For the variation in intake rates, i.e. the number of *Loripes* consumed by a red knot per trial (n=480), the best approximating model included starvation treatment, experiment day and location (Table 2).

This model showed that the intake rate was higher on starved *Loripes* (estimate=1.34, df=39.06, t=2.574) and on *Loripes* from location B (estimate=1.57, df=39.06, t=3.005) (Fig. 1b, Table S1). Additionally, it showed an increased intake rate of 1.01 per day, independent of treatment (df=39.06, t=22.164) (Fig. 2, Table S1), resulting in a doubling of the intake rate throughout the experiment.

To calculate shell mass processing rates, we used the mean gizzard masses (g ± s.e.m.) observed on experiment day 2, 6 and 9: 6.7±0.15, 6.6±0.20, 6.7±0.69.

DISCUSSION

The intake rate of red knots was higher on *Loripes* with lower total sulfur contents (Fig. 1), which is consistent with our expectation. The starvation treatment, in which *Loripes* was kept in seawater for ten days without contact with the sediment, led to lower concentrations of sulfur (Fig. 1a). Sulfur levels in *Loripes* also varied spatially, with higher contents in *Loripes* collected in a dense seagrass field than in *Loripes* from a mudflat with lower seagrass cover (Fig. 1a).

Taking the results one step further, and trying to understand how the quantitative differences shape a consumption constraint, proves a little harder. One would expect that the sulfur constraint works such that there is a maximum amount of sulfur that can be processed per time unit (as holds for the shell material processing constraint found earlier in red knots by van Gils et al. (2003); and as modelled theoretically by Hirakawa (1995)). Hence, multiplying the sulfur contents (in mg, by multiplication of sulfur percentage by dry flesh mass) per *Loripes* with the number of *Loripes* ingested by a red knot per trial should form a constant across treatments (i.e. total amount of sulfur ingested per trial). It does not. With a reduction in the amount of sulfur per *Loripes*, intake rate goes up less steeply than expected (Fig. 3: blue arrows vs. grey lines). In other words, highest sulfur uptakes (~18 mg per trial) occur in the treatment where *Loripes* contains the highest sulfur concentration (unstarved *Loripes* from location A).

So, although our results clearly link sulfur content of the bivalves to palatability, based on the available data, we could not determine a mechanism of sulfur toxicity. This might be because the total sulfur measured in *Loripes* consist of several compounds, including intermediates of bacterial sulfur

oxidation such as thiosulfate and sulfite, in addition to hydrogen sulfide and elemental sulfur (Cary et al. 1989; Dando et al. 1986; Lebata 2000). These sulfur compounds differ in toxicity and the measured amounts might therefore not translate directly into the 'degree' of toxicity. Thiosulfate and sulfite are non-toxic, but hydrogen sulfide is a well-known toxin that inhibits mitochondrial cytochrome oxidase (Cooper and Brown 2008). Elemental sulfur was shown to have toxic effects in ruminants if 'excessive' quantities above 0.4% of total feed intake was ingested (Kandylis 1984). This toxic effect of elemental sulfur is thought to be due to its reduction to hydrogen sulfide under anoxic conditions in the digestive tract. Elemental sulfur may be directly toxic to the red knots, or the toxic effects may be indirect due to transformation of elemental sulfur to hydrogen sulfide in the digestive tract. Regardless of the exact mechanism and sulfur compound involved, our results are consistent with the hypothesis that sulfur storage in the animal tissues due to the metabolic activity of the symbionts causes toxic effects in predators.

Despite not knowing the mechanism of sulfur toxicity, we propose two additional reasons for the 'mismatch' of the results with our quantitative expectations. First, red knots might run into their 'normal' shell mass processing constraint (van Gils et al. 2006; van Gils et al. 2003), when feeding on less toxic *Loripes* (i.e. *Loripes* with a lower sulfur concentration). We measured gizzard size, which sets the shell mass processing capacity (van Gils et al. 2003), three times during the first 9 days of the experiment. In the first week of the experiment, intake rates fell below the maximum intake rate set by the gizzard size (Fig. 2: blue bars), showing that gizzard size is not a limiting factor. After this, intake rates and therewith required gizzard sizes increased, exceeding the gizzard sizes measured in the first days (Fig. 2). Potentially, gizzard size started playing a role after this point, but in that case the effect of sulfur on the intake rate would be reduced, which would have improved the model that contained interactions of both the location and starvation effect with day. However, the best approximating model did not contain these interactions, indicating that the effects were stable throughout the experiment. So, although not measured, gizzard sizes probably increased in the second half of the experiment and did therefore not limit intake rates.

Second, digestive efficiency may decrease with an increase in *Loripes* sulfur concentration and that the amount of actually *assimilated* sulfur is the true constraint and remained constant across treatments and locations. In fact, it has been shown earlier that digestive efficiency did go down with an increased consumption of (untreated) *Loripes* (V. Hin & T. Oudman, unpubl. data; Oudman 2017), most likely associated with the diarrhea effect that comes when eating *Loripes* (see Oudman et al. 2014).

Increased consumption over time

Another surprising quantitative result that warrants discussion, is the steady increase in the *Loripes* intake rate throughout the entire experiment, with an intake twice as high in the end as in the beginning of the experiment (Fig. 2). This would only be possible if the gizzards had grown, to enable higher shell processing rates (see right vertical axis of Fig. 2). But this also means a doubled amount of sulfur intake, raising the question how it is possible that the red knots were able to process sulfur at such higher rates. One possibility is that the red knots gradually adjusted their gut microbiome. Gut bacteria can degrade toxins (Ceja-Navarro et al. 2015; Kikuchi et al. 2012; Kohl et al. 2014; Ping et al. 2007) and the microbiome shifts quickly after a diet switch (Turnbaugh et al. 2009; Zhang et al. 2012). During the first two days prior to the start of the experiments, when the birds were offered *Loripes*, but not all of them would immediately accept this mildly toxic diet, we found an effect of bill length on whether the birds would accept eating *Loripes*. It turned out that the birds that accepted *Loripes* as their diet had longer bills than birds that initially refused to eat *Loripes*, a result that was also found in two pilot experiments (Fig. 4, t-test=3.86, df=32, $p<0.001$). This is remarkable, because a longer bill is not necessary to obtain food from the feeding trays, and other staple food was also eaten from there. We know that in the wild, birds with longer bills consume more *Loripes* (Fig. 3A in van Gils et al. 2016; up to 40% of their diet in the birds with the longest bills), most likely because they can probe deeper and thus have access to a larger proportion of the burrowed *Loripes* population. Potentially, these birds have already ‘gardened’ a gut microbiome that is better suited to deal with the sulfur uptake that comes with consuming *Loripes*. Analysis of red knot gut microbiome samples might provide answers in the future.

An intriguing question is why red knots in the wild are not adapted to eating *Loripes* at the high rates found in the last days of the experiment. In the mudflats of Banc d'Arguin, *Loripes* is the most abundant bivalve species (Ahmedou Salem et al. 2014) and with a high flesh to shell ratio, it has a high digestive quality (Oudman et al. 2014; van Gils et al. 2005; Verlinden and Wiley 1989). It would therefore be very beneficial to be adapted to cope with sulfur, enabling high intakes rate on *Loripes*. However, consuming *Loripes*, and thus ingesting sulfur, comes with negative side-effects, like diarrhea (Oudman et al. 2014). The diarrhea probably causes an osmoregulatory problem, because in marine environments there is no fresh water to compensate for this water loss. Living in saline environments and ingesting bivalves whole is already challenging for the red knots' osmoregulatory system, because of the high salt intake (Gutiérrez 2014; Verboven and Piersma 1995). Red knots are adapted to that by having relatively large salt glands, which are capable of excreting high salt concentrations (Blakey et al. 2006; Gutiérrez et al. 2012; Staaland 1967; Verboven and Piersma 1995). However, salt excretion costs energy (Gutiérrez 2014) and having to compensate for water loss by drinking seawater raises these costs. On top of that, Gutiérrez et al. (2015) showed that red knots in an experimental setting with high salinity and high environmental temperatures reduced their food intake, which negatively affected several physiological and condition-related traits. In our experiment we offered fresh water *ad libitum*, which enabled compensation for water loss and therewith higher intake rates. This is in line with the experiment of Oudman et al. (2014), who found higher *Loripes* intake rates when offering fresh water, compared to offering seawater or no water. Nevertheless, throughout the full period of our experiment, birds continued to suffer from diarrhea, indicating that adaptation to *Loripes* consumption did not eliminate its negative consequences While it is possible that the diarrhetic water loss per *Loripes* consumed declined as the experiment progressed, we did not measure that.

Natural variation of toxicity

With sulfur levels in *Loripes* varying between and within mudflats, red knots can potentially lower their sulfur intake by accepting *Loripes* to their diet at spots where their sulfur content is low. This might explain foraging patterns and diet choices at certain locations (Oudman et al. 2018; van Gils et

al. 2015). We collected *Loripes* at two locations and found a difference in sulfur content and thus toxin constraint, resulting in higher intake rates on *Loripes* from the less toxic location. However, these specimens also had lower body masses and may therefore not be more beneficial to forage upon (mean DM intake in control trials: location A: 0.589 g, less toxic location B: 0.564 g). This difference in body mass reflects a difference in body condition, which is higher in a dense seagrass meadow with higher sulfide levels in the sediment (van der Geest et al. 2020; van der Heide et al. 2012). The endosymbionts in their gills presumably thrive better under high sulfide conditions, resulting in higher sulfur levels per *Loripes* (van der Geest et al. 2020). The difference we found between locations is probably too small to affect foraging patterns, but it would be interesting to study how sulfur content and body mass of *Loripes* varies spatially. There might be spots where these characteristics are more beneficial, i.e. low sulfur content and high body mass, than in the locations we collected them (Rossi et al. 2013). Subsequently, it would be interesting to see if foraging red knots include more *Loripes* in their diet in those places. Toxicity of *Loripes* might also be size dependent (Roques et al. 2020; Rossi et al. 2013). We selected only part of the suitable sized *Loripes* for this experiment, but individuals outside this range might be more of less toxic and this could also be related to their depth (sulfide levels increase with sediment depth). Seasonality might also affect the sulfur content in *Loripes* and the subsequent toxin constraint (Cardini et al. 2019; Roques et al. 2020). Van der Geest et al. (2014) showed that the contribution of the endosymbionts to the diet of *Loripes* is lowest in autumn and highest in spring, potentially limiting intake rates on *Loripes* in spring the most. This might shape a problem for red knots fueling up for spring migration, especially since non-toxic bivalve species are depleted in spring (Ahmedou Salem et al. 2014). Piersma et al. (2005) indeed found fueling rates of red knots in Banc d'Arguin to be relatively low.

Concluding remarks

Sulfur inhibits intake rates for red knots foraging on *Loripes*. Intake rates are higher on starved *Loripes*, that contain less sulfur, and on *Loripes* from a mudflat where their toxic load is lower. Intake rates on *Loripes* increased during the experiment, but in the wild this might not be possible, without access to freshwater to compensate for water loss, caused by diarrhea. From the perspective of the

Loripes, the sulfur-containing endosymbionts not only provide them with nutrition, it also limits predation on them by red knots and likely other consumers. This might be one of the key factors in the successful life of *Loripes* (reaching densities of up to 4000 individuals per m^2 (van der Geest et al. 2011)) and therewith healthy seagrass meadows (van der Heide et al. 2012).

ACKNOWLEDGEMENTS

We thank Jones Quartey, Michelle Jewell, Saskia Kühn, Susanne van Donk, Sil Piek and Sarah Zauner for helping with the hard work, collecting Loripes every day and taking care of the birds. We thank Anne Dekinga and Job ten Horn for catching the birds and taking their measurements. We thank Paul van der Ven of the General Instrumentation facility at Radboud University Nijmegen for sulfur elemental analysis. We also thank Parc National du Banc d'Arguin, especially Lemhaba Ould Yarba, for facilitating the expeditions. Lastly, we thank Matthijs van der Geest and Theunis Piersma, handling editor Chris Whelan and two anonymous reviewers for their comments on the manuscript, and Dick Visser for preparing the figures.

DECLARATIONS

Funding: The work was supported by structural NIOZ funding to J.A.v.G., J.d.F. was supported by NWO Open Competition #ALWOP.203.

Conflicts of interest: No competing interests declared.

Ethics approval: Ethics approval was not required for this study according to local legislation [law of Mauritania]

Consent to participate: Not applicable.

Consent to publication: Not applicable.

Availability of data and material: Data will be deposited in the Dryad Digital Repository.

Code availability: Not applicable.

Authors' contributions: JvG and JP conceived the concept. JvG and TO designed methodology and collect the data. TO analysed the data and led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication. JvG provided funding and supervised the project.

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Table 1. Linear mixed-effect models relating sulfur percentage (*S*) of individual *Loripes* to starvation treatment (*T*), location of *Loripes* collection (*L*) and sampling day (*D*). Analysis includes 195 individual *Loripes*. Session is included as random effect. Models are sorted by AIC_c. Only models with $AIC_cWt > 0.01$ are shown, so the intercept-only model dropped out.

Model	K	AIC_c	ΔAIC_c	AIC_cWt	Cum.Wt	LL
~*T*+*L*+(1|*Session*)	5	602.88	0	0.37	0.37	-296.28
~*T***L*+(1|*Session*)	6	604.53	1.65	0.16	0.53	-296.04
~*T*+*D*+*L*+(1|*Session*)	6	604.99	2.11	0.13	0.66	-296.27
~*T*+(1|*Session*)	4	605.22	2.35	0.12	0.78	-298.51
~*T***L*+*D*+(1|*Session*)	7	606.66	3.78	0.06	0.84	-296.03
~*T***D*+*L*+(1|*Session*)	7	606.79	3.91	0.05	0.89	-296.1
~*T*+*D***L*+(1|*Session*)	7	607.01	4.13	0.05	0.94	-296.2
~*T*+*D*+(1| *Session*)	5	607.23	4.35	0.04	0.98	-298.46
~*T***D*+(1|*Session*)	6	609.02	6.14	0.02	0.99	-298.29

Table 2. Linear mixed-effect models relating the intake rate (*I*) of individual *Loripes* by captive red knots to starvation treatment of *Loripes* (*T*), experiment day (*D*) and location of *Loripes* collection (*L*). Analysis includes 480 trials, with 12 birds on 20 subsequent days. Session and individual bird are included as random effect. Models are sorted by AIC_c. Only models with AIC_cWt > 0.01 are shown, so the intercept-only model dropped out.

Model	K	AIC_c	ΔAIC_c	AIC_cWt	Cum.Wt	LL
~*T*+*D*+*L*+(1|*Session*)+(1|*ID*)	7	2964.33	0	0.36	0.36	-1475.04
~*T***D*+*L*+(1|*Session*)+(1|*ID*)	8	2965.53	1.20	0.20	0.56	-1474.61
~*T*+*D***L*+(1|*Session*)+(1|*ID*)	8	2966.08	1.76	0.15	0.70	-1474.89
~*T***L*+*D*+(1|*Session*)+(1|*ID*)	8	2966.35	2.02	0.13	0.84	-1475.02
~*T***L*+*D***L*+(1|*Session*)+(1|*ID*)	9	2968.11	3.78	0.05	0.89	-1474.86
~*D*+*L*+(1|*Session*)+(1|*ID*)	6	2968.39	4.06	0.05	0.94	-1478.10
~*T***L*+*T***D*+*D***L*+(1|*Session*)+(1|*ID*)	10	2969.35	5.03	0.03	0.97	-1474.44
~*T*+*D*+(1|*Session*)+(1|*ID*)	6	2970.39	6.06	0.02	0.98	-1479.11

552 **FIGURE LEGENDS**

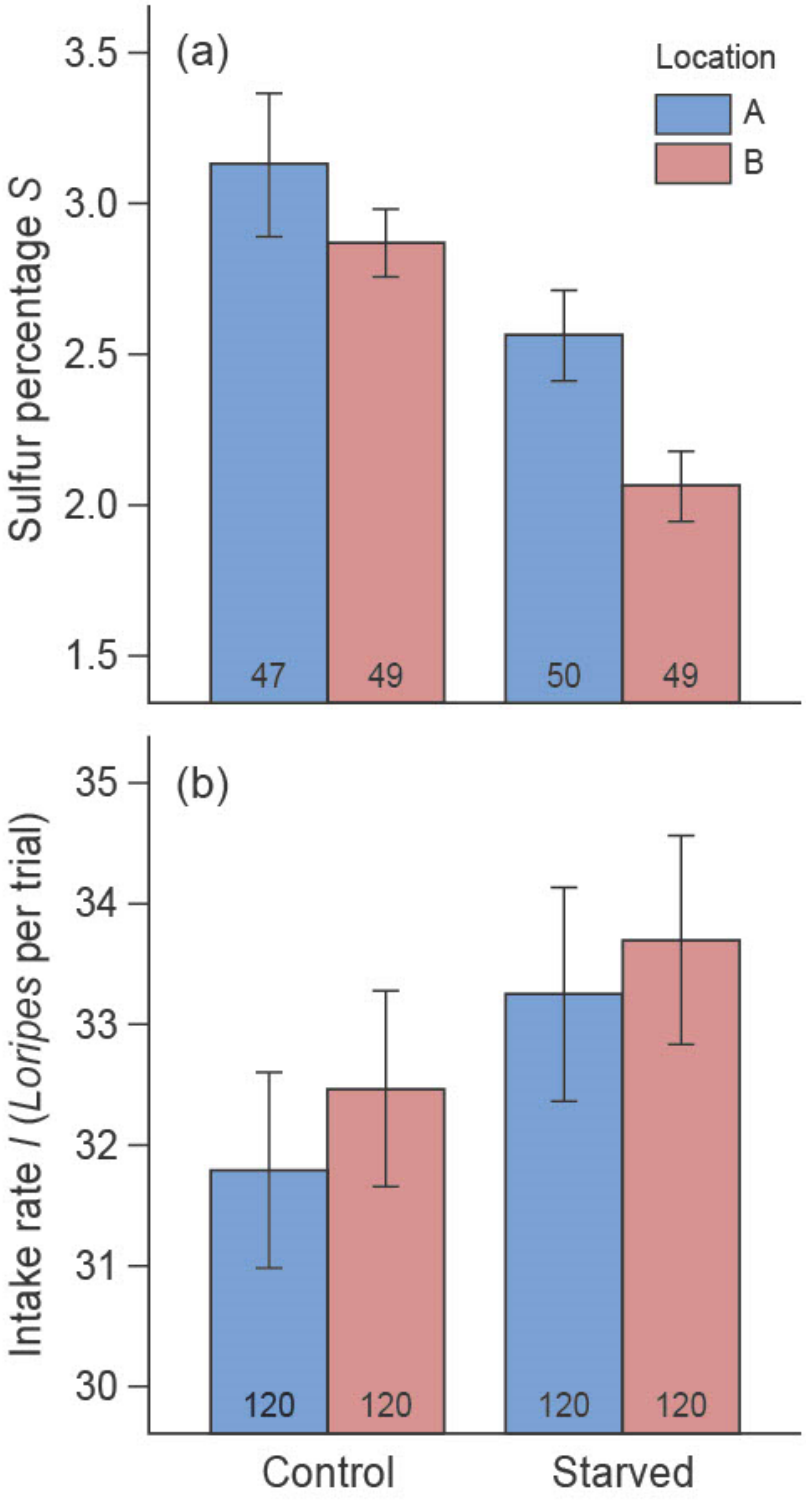
553

554 **Figure 1. Starvation of *Loripes* lowers sulfur content, leading to higher intake rates of red knots.**
555 a) Mean ± s.e.m. total sulfur percentage in individual *Loripes*. b) Mean ± s.e.m. number of *Loripes*
556 consumed per trial by red knots. Sample sizes are indicated within bars.

557 **Figure 2. Intake rates of red knots on *Loripes* increased throughout the experiment.** Dots show
558 intake rates averaged ± s.e.m. per session (40 sessions, with 12 birds (N=480)). The y-axis on the
559 right, shows the required gizzard mass per dot. Green lines show calculated gizzard mass, based on
560 gizzard measurements and therewith predicted maximum intake rates.

561 **Figure 3. Numerical intake rate of red knots in relation to *Loripes* sulfur content.** Dots show
562 intake rates averaged ± s.e.m. for each location and treatment, against sulfur mass per *Loripes*
563 averaged ± s.e.m.. Arrows show effect on intake rate and sulfur contents of starvation treatment within
564 each location. Gray lines in the background show lines of equal sulfur intake (mg per trial).

565 **Figure 4. Long-billed birds accepted *Loripes* sooner than short-billed birds.** Boxes show the
566 distribution of the bill lengths of individual red knots, that consumed (Yes) or rejected (No) *Loripes* in
567 their second trial before the start of the experiment.



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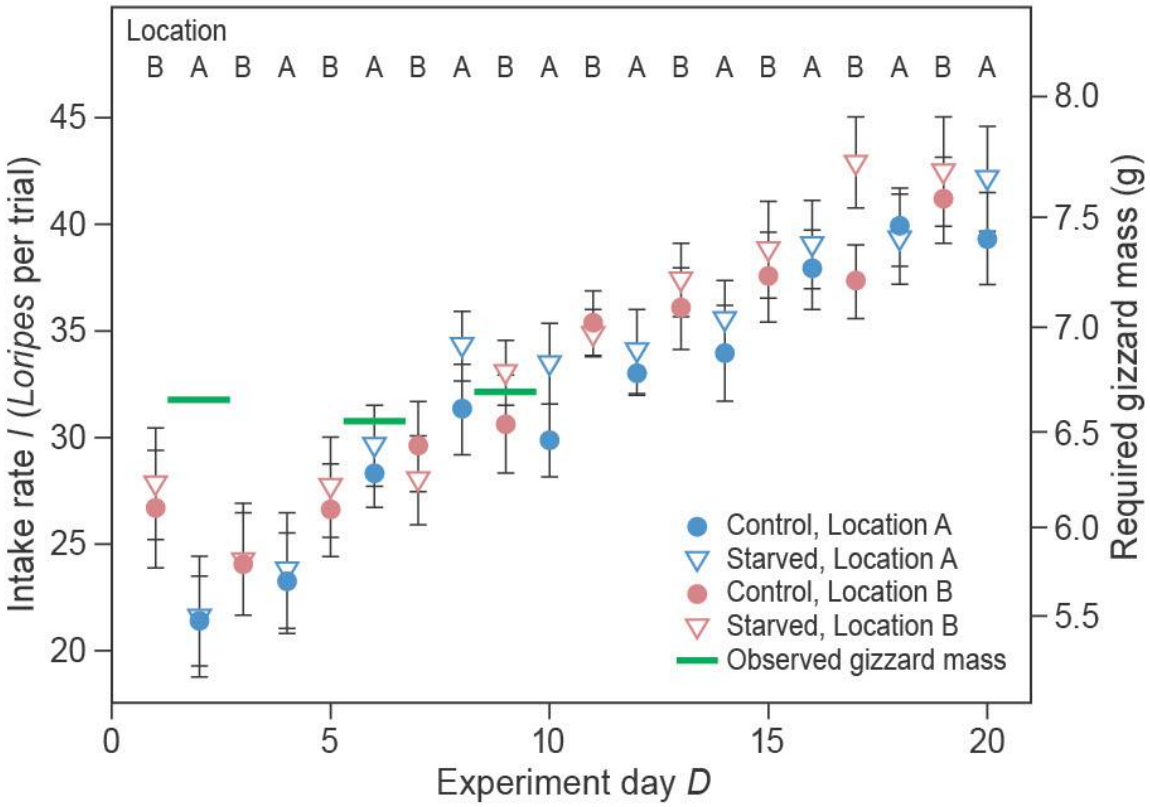
569 **Figure 1**



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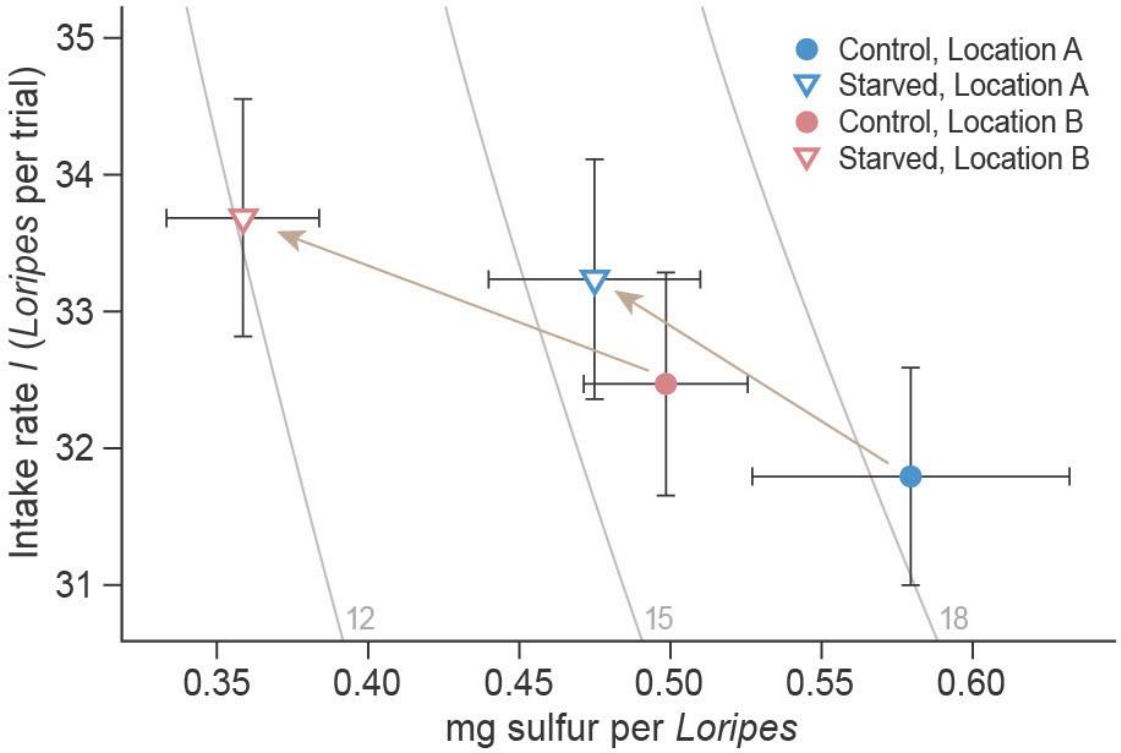
571 **Figure 2**



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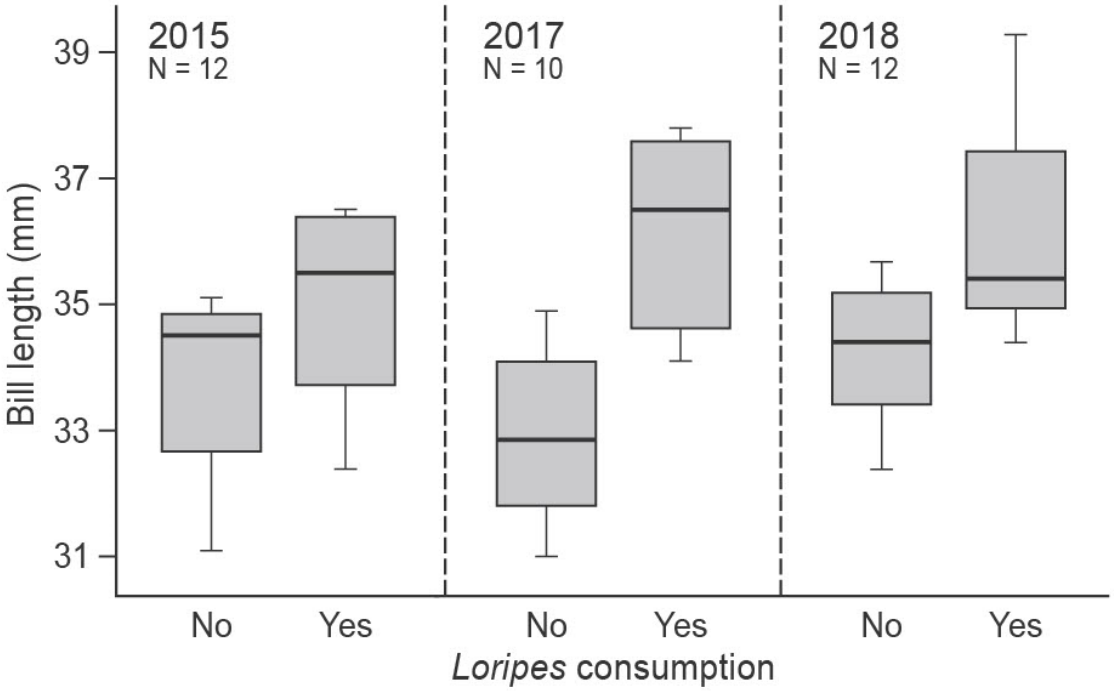
573 **Figure 3**



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575 **Figure 4**