Trophic cascade induced by molluscivore predator alters pore-water biogeochemistry via competitive release of prey

Jan A. van Gils,[1] Matthijs van der Geest,[1,4] Erik J. Jansen,[1] Laura L. Govers,[2] Jimmy de Fouw,[1]
and Theunis Piersma[1,3]

[1]*Department of Marine Ecology, NIOZ Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg (Texel),
The Netherlands*
[2]*Department of Environmental Science, Institute for Water and Wetland Research, Radboud University Nijmegen, Faculty of Science,
P.O. Box 9010, 6500 GL Nijmegen, The Netherlands*
[3]*Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen,
P.O. Box 11103, 9700 CC Groningen, The Netherlands*

Abstract. Effects of predation may cascade down the food web. By alleviating interspecific competition among prey, predators may promote biodiversity, but the precise mechanisms of how predators alter competition have remained elusive. Here we report on a predator-exclosure experiment carried out in a tropical intertidal ecosystem, providing evidence for a three-level trophic cascade induced by predation by molluscivore Red Knots (*Calidris canutus*) that affects pore water biogeochemistry. In the exclosures the knots' favorite prey (*Dosinia isocardia*) became dominant and reduced the individual growth rate in an alternative prey (*Loripes lucinalis*). *Dosinia*, a suspension feeder, consumes suspended particulate organic matter (POM), whereas *Loripes* is a facultative mixotroph, partly living on metabolites produced by sulfur-oxidizing chemoautotrophic bacteria, but also consuming suspended POM. Reduced sulfide concentrations in the exclosures suggest that, without predation on *Dosinia*, stronger competition for suspended POM forces *Loripes* to rely on energy produced by endosymbiotic bacteria, thus leading to an enhanced uptake of sulfide from the surrounding pore water. As sulfide is toxic to most organisms, this competition-induced diet shift by *Loripes* may detoxify the environment, which in turn may facilitate other species. The inference that predators affect the toxicity of their environment via a multi-level trophic cascade is novel, but we believe it may be a general phenomenon in detritus-based ecosystems.

Key words: Banc d'Arguin, Mauritania; bivalves (Dosinia isocardia, Loripes lucinalis); *facilitation; growth rate; hydrogen sulfide; interspecific competition; predation; predator-exclosure experiment; Red Knot,* Calidris canutus canutus; *seagrass beds; top-down effect; toxicity.*

Introduction

In the current biodiversity crisis, predators have often been the ones disappearing first (Byrnes et al. 2007): they are lowest in number (Purvis et al. 2000) and most sensitive to habitat fragmentation (Srivastava et al. 2008), but are nevertheless overfished (Myers and Worm 2003) and overhunted (Johnson et al. 2007). As predators often play key roles in the structuring and organization of ecological communities (Chase et al. 2002), species loss may accelerate after the highest trophic levels in a food web have disappeared (Duffy 2003, Estes et al. 2011). Therefore, in order to predict future shifts in food webs, it is critical to get a better understanding of the cascading top-down role that predators play in ecosystems (Heithaus et al. 2008, Terborgh and Estes 2010).

Although predation is detrimental for those prey individuals being killed, predation may be beneficial for the surviving individuals. This is because by reducing the number of prey, predators may alleviate the competition for space and resources among those prey individuals that remain. Under some conditions, such predator-mediated competitive release may promote species coexistence (Paine 1966), but negative or no effects of predation on prey species coexistence have also been claimed (Chase et al. 2002). Much depends on whether the prey compete for the same resources, whether they are able to exploit alternative resources under stringent competition, and whether they are fed upon by generalist or specialist predators or by predators using an intermediate strategy.

The extinction of one of two competing prey species can best be prevented by predators that are neither full specialists nor full generalists, but rather exhibit some intermediate form of polyphagy (Vandermeer and Pascual 2006). This matches earlier conclusions that predators switching diet promote prey coexistence (Murdoch 1969). More generally, it can be stated that
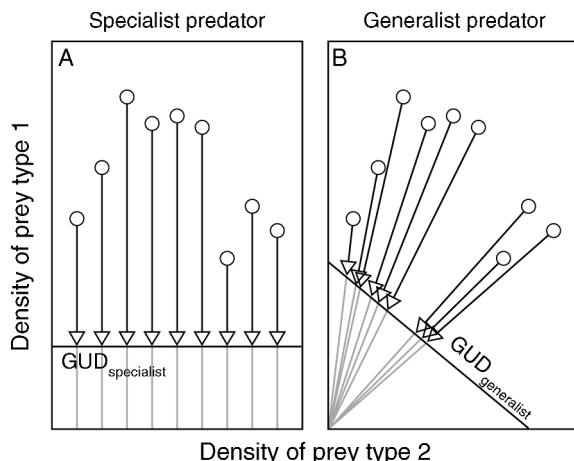
FIG. 1. (A) Specialist predators only feed on a single prey type and will deplete it down (arrows) to a fixed giving-up density (GUD; horizontal line). (B) By contrast, generalist predators feed on multiple prey types, in the present case two. Both prey types will be depleted down to a combination of GUDs (diagonal line), depending on the initial prey densities (open circles, which are equivalent to those in panel A). Depletion trajectories are straight whenever searching efficiencies are similar on both prey types. Gray lines are drawn to guide the eye. Note that in generalist predation, higher initial prey densities in one prey type will lead to lower GUDs in the other prey type—a short-term form of apparent competition (Holt and Kotler 1987).

adaptive behavior by predators enhances the stability in systems where otherwise one prey species outcompetes another (Fryxell and Lundberg 1997). By contrast, unless there are as many specialist predator species as there are competing prey species, fully specialized predators cannot maintain prey coexistence in a bistable competitive system (Schreiber 1997). On the opposite side of the spectrum, generalist predators that show no preference for one species over the other can under no condition stabilize a two-species bistable system (Hutson and Vickers 1983). This is because generalist predation leads to apparent competition between two prey species, meaning that the increase in one prey species enhances the predation pressure on the other prey by supporting a larger predator density, which eventually could lead to extinction of the latter prey species—even if prey do not compete for the same resources (Holt and Lawton 1994).

For a prey facing competitive exclusion there is one way out: it should switch to alternative resources. Thus, if prey coexistence is maintained by predation, and if those predators are removed from the system, we may expect the competitively weaker prey to switch resources (provided it has the machinery to do so). Though generalist–specialist competition has since long puzzled ecologists (see review by Abrams [2006]), the impact of predation on this form of competition has barely received empirical attention. Furthermore, and this holds in general for predator-mediated coexistence, rather little empirical work has been performed on the

actual mechanisms at the level of the *individual* prey (Gurevitch et al. 2000). There are some well-known examples of how predators affect the abundances of their prey *and* the prey's resources (Estes and Palmisano 1974, Ripple and Beschta 2004), but how such three-level trophic cascades feedback into the *performance* of individual prey remains to be investigated.

In this paper we try to contribute by testing for the effects of predation on bivalves by a molluscivore shorebird, the Red Knot (*Calidris canutus canutus*). We do so in a large-scale field experiment in a tropical intertidal ecosystem, Banc d'Arguin (Mauritania), which is the main wintering area for this subspecies of Red Knot (Piersma 2007). In this area, two species stand out as the most abundant and most suitable prey for Red Knot. These are *Dosinia isocardia* (Dunker, 1845), a specialist suspension-feeding venerid bivalve and *Loripes lucinalis* (Lamarck, 1818), a lucinid bivalve which is believed to suspension-feed, but which, to a large extent, obtains its nutrition through a symbiosis with chemo-autotrophic bacteria living inside its gills (Johnson et al. 1994). These bacteria obtain their energy by oxidizing sulfide (H_2S), which is produced by sulfate reducers during anaerobic degradation of organic matter. In seagrass beds, the dominant and preferred habitat for Red Knots in our study area (Altenburg et al. 1982), these two species together make up 72% of all mollusks, 79% of all bivalves and even 85% of all ingestible bivalves (Honkoop et al. 2008). Based on the total number of shorebirds wintering at Banc d'Arguin (Zwarts et al. 1998), their diets, and their energy requirements, Red Knots should be responsible for about 80% of all mollusk consumption by vertebrate predators in Banc d'Arguin. Over a period of a full year, we locally excluded knots from their prey using exclosures. Besides measuring the effects of predation on biomass densities of both prey, we quantified the effects on growth rate in *Loripes* and on changes in one of its resources, sulfide. The depletion trajectories of these two bivalve species can tell us whether Red Knots are generalist or specialist predators.

Are Red Knots specialist or generalist predators?

In general, plotting so-called "depletion trajectories" enables exploring the diet strategy applied by the predator (Brown and Mitchell 1989; Fig. 1). In a simple one-predator–two-prey system, a specialist predator will only feed on a single prey (type 1) and deplete its densities towards a critical, so-called "giving-up density" (GUD; horizontal line in Fig. 1A) (Brown 1988). By contrast, a generalist will feed on both prey species and will give up feeding at a certain combination of both prey densities (diagonal line in Fig. 1B; Holt and Kotler 1987). Intermediate strategies do exist—e.g., predators can switch from being specialist to becoming a generalist (a strategy termed "the expanding specialist" by Heller [1980])—but they are not considered here.
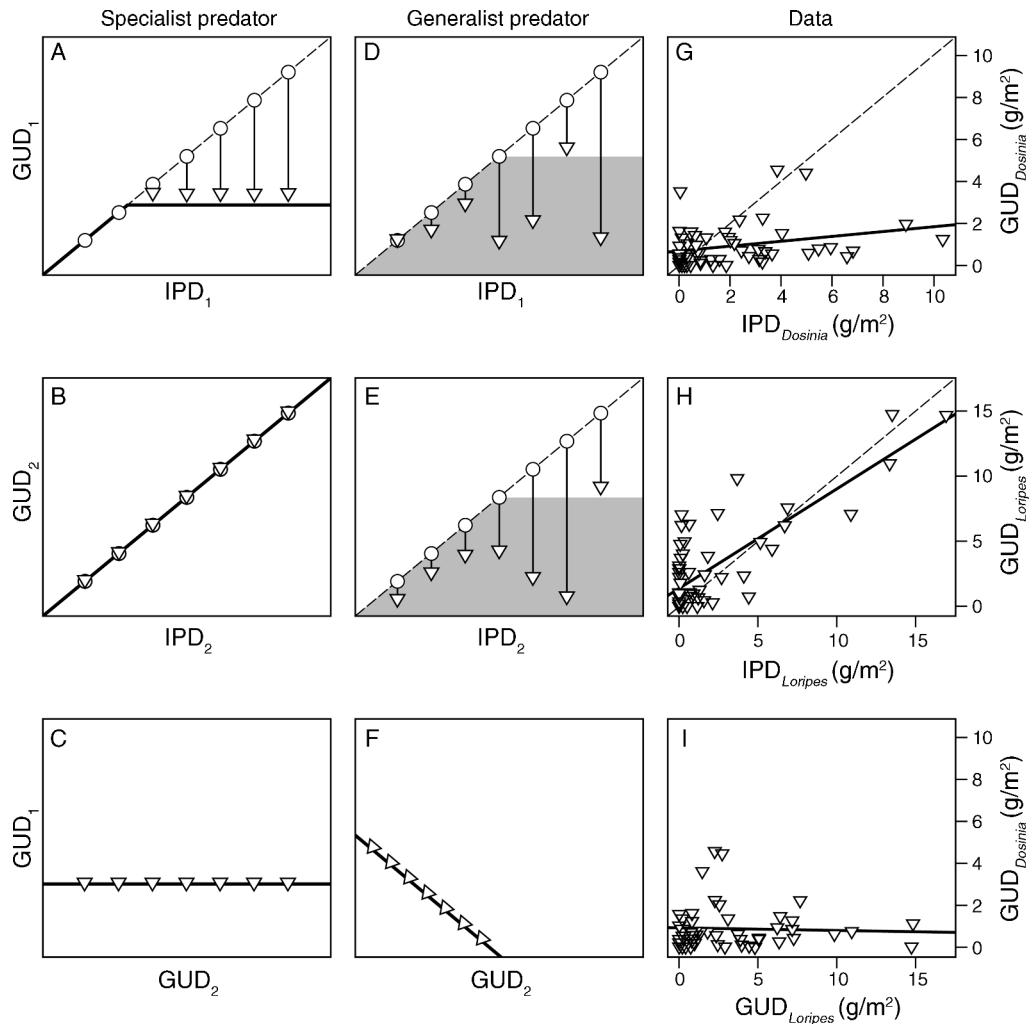
Fig. 2. Illustrations of different predator dietary strategies. (A) A specialist predator will deplete prey type 1 down to a fixed giving-up density, GUD_1, leaving patches unexploited when the initial prey density $IPD_1 < GUD_1$. (B) It will not feed upon prey type 2; hence $GUD_2 = IPD_2$. (C) Therefore, GUD_1 is constant and independent of GUD_2. (D) A generalist predator will feed on both types; hence GUD_1 is not constant but depends on the density of prey type 2. (E) Similarly, GUD_2 depends on the density of prey type 1. (F) Therefore, GUD_1 and GUD_2 co-vary negatively in the generalist predator. (G) GUDs on the mollusk *Dosinia* (biomass densities in controls) were low and constant relative to IPDs (biomass densities in exclosures); (H) GUDs on the mollusk *Loripes* were not different from IPDs; (I) GUDs on *Dosinia* densities were not correlated with GUDs on *Loripes*. The dashed lines in panels (G) and (H) are the 1:1 lines.

In this framework, regressions of GUD against initial prey density (IPD) should be diagnostic for the diet strategy applied by the predator (Fig. 2). Considering the specialist predator and the prey that it specializes on, GUD will be constant and independent of IPD above a certain IPD (Fig. 2A), whereas GUD and IPD will be similar in the prey type that it ignores (Fig. 2B). Hence, there will be no relation between the GUD on prey type 1 and the GUD on prey type 2 (Fig. 2C; comparable to Fig. 1A). By contrast, in the generalist predator there will be much variation in the GUD on prey type 1 (Fig. 2D) as well as on type 2 (Fig. 2E), variation that is unrelated to a prey type's IPD. GUDs on prey type 1 will relate negatively to the GUDs on prey 2 (Fig. 2F; comparable to Fig. 2B).

On the basis of functional-response parameters and quitting-harvest rates (QHR) we can predict GUDs for both an imaginary specialist knot and a generalist knot. Red Knots obey Holling's type II functional response (Piersma et al. 1995). By rearranging this well-known equation, we arrive at the GUD (no./m²) on the prey that the specialist knot feeds on:

$$GUD = \frac{QHR}{ea - QHRah} \qquad (1)$$

where e is the average energy contents per available prey, a is searching efficiency, and h is handling time.

Rearranging Holling's type II on two prey types (Brown and Mitchell 1989), we see that in the generalist knot the GUD on *Dosinia* (GUD_{Dos}) depends on the GUD on *Loripes* (GUD_{Lor}) (and vice versa):

$$GUD_{Dos} = \frac{QHR}{e_{Dos}a - QHRah} - \frac{GUD_{Lor}(e_{Lor}a - QHRah)}{e_{Dos}a - QHRah} \quad (2)$$

with e_{Dos} and e_{Lor} representing the average energy contents per available prey of *Dosinia* and *Loripes*, respectively, and assuming a and h to be similar in both species. On the basis of direct measurements on metabolic rates of actively foraging Red Knots (Piersma et al. 2003) and daily foraging times (van Gils et al. 2007), it has been estimated that knots feeding in Banc d'Arguin require a minimum intake rate (meat, not shells) of 0.2 mg ash-free dry mass ($AFDM_{meat}$) per second in order to maintain a balanced energy budget (van Gils et al. 2009), which we will take as QHR. Functional-response parameters for Red Knots feeding in seagrass habitat have recently been quantified experimentally (J. de Fouw and J. A. van Gils, *unpublished data*): $a = 4$ cm^2/s, $h = 1$ s. Estimates for e_{Dos} and e_{Lor} were derived from benthic sampling results presented below (see *Materials and methods: Prey density*): 2.57 and 7.28 mg $AFDM_{meat}$, respectively. As these are GUDs on the *available* part of the food supply, we need to correct for the fraction available (0.73 in *Dosinia* and 0.70 in *Loripes*; derived from benthic sampling results presented below in *Materials and methods: Prey density* and *Fecal analysis*) to get to *total* GUDs. Next, in order to express the total *numerical* GUDs as total *biomass* GUDs we need to take into account the average $AFDM_{meat}$ per prey (equivalent to e_{Lor} in *Loripes* and 2.95 mg in *Dosinia*, which is slightly larger than e_{Dos} as some size classes of *Dosinia* are too large to be ingested).

MATERIALS AND METHODS

Exclosures

In October 2009 we placed 112 exclosures, equally divided over seven tidal flats in the vicinity of the scientific station of Parc National du Banc d'Arguin (PNBA) at Iwik (19°53.0′ N; 16°17.7′ W). Each exclosure consisted of eight PVC poles (0.5 m long), which were inserted vertically in the sediment (to a depth of 0.4 m) and aligned in a 1-m^2 square. A nylon rope was pulled through a hole in the top of each pole and acted as a 10-cm-high fence. Such a simple construction has proved effective in the past in keeping out shorebirds from small-scale plots (van Gils et al. 2003). About half of the exclosures ($N = 57$) did not survive the whole year until the end of the experiment. Sometimes exclosure poles were washed out by the tide, or crabs had made burrow structures around the poles resulting in a large puddle inside the exclosure. These exclosures (and their paired controls) were removed from further analyses.

Prey density

In October 2010 we sampled bivalve densities in order to study the effects of predation on prey density. One sample was taken in the middle of each exclosure, and one paired sample (the control) was taken outside each exclosure, 2.5 m away from the exclosure sample (random direction). Within the framework of depletion trajectories presented above (Fig. 2), we consider exploited prey densities in the controls as giving-up densities (GUDs) and unexploited prey densities in the exclosures as "initial" prey densities (IPDs), even though the latter cannot be considered as *true* initial densities at the time exclosures were placed. Dispersal, recruitment, and non-predatory mortality may have changed the densities within the exclosures throughout the year. However, assuming similar changes have also taken place in the controls (i.e., assuming those changes to be density-independent), our approach to study effects of predation by comparing exclosures with their paired controls seems valid.

Each benthic sample constituted a sediment core (diameter, 15 cm), taken to a depth of 20 cm and sieved over a 1-mm mesh. The upper 4 cm was sieved separately from the rest of the core in order to distinguish accessible from inaccessible prey (Red Knots have bills ~3.5 cm long). Top and bottom samples were also used to collect *Loripes* individuals that were calcein-stained to estimate growth rate (details given below in *Prey growth rate*). In the laboratory we measured lengths (to the nearest 0.1 mm) of all individuals and $AFDM_{meat}$ of a subset of individuals. The latter was done by separating flesh from shell and drying it for three days at 60°C. Next, that dried meat was weighed (to the nearest 0.1 mg) and incinerated for 5 h at 550°C; subsequently, ash mass was determined (to the nearest 0.1 mg). The resulting $AFDM_{meat}$ (in grams)-to-length (L, in millimeters) relationships (for *Dosinia*, $AFDM_{meat} = 10^{-5.05}L^{3.07}$, $N = 166$ specimens, $R^2 = 0.96$, $P < 0.001$; for *Loripes*, $AFDM_{meat} = 10^{-4.73}L^{2.96}$, $N = 191$ specimens, $R^2 = 0.95$, $P < 0.001$) were used to predict $AFDM_{meat}$ for the remaining individuals that were not incinerated, which enabled us to express species-specific total biomass densities (i.e., top and bottom layers pooled).

Prey growth rate

We used the technique of calcein staining to determine bivalve growth rates (van der Geest et al. 2011). Calcein is a fluorescent marker that bivalves incorporate into their shells upon ingestion and can be made visual by illuminating the shell with UV light under a fluorescence microscope. Growth can then be determined as the maximum growth axis between the calcein mark at the exterior of the shell (i.e., the calcareous layer deposited when calcein was administered) and the ventral margin (i.e., the latest calcareous layer, deposited just before collecting the bivalve). The technique was validated for *Loripes lucinalis* (van der Geest et al. 2011), to which we

refer for further details (note that in this paper *L. lucinalis* is called *L. lacteus*; recent taxonomic insights have led to this nomenclature change; R. von Cosel, *personal communication*).

Calcein was administered in October 2009, at the moment exclosures were placed. During low tide, a PVC ring (diameter, 30 cm; height, 15 cm) was pushed 10 cm into the sediment in each exclosure and its control. This "basin" was then filled up by 0.5 L of calcein solution (containing 0.1 g calcein). As the next high tide would flush the solution, we again filled up the basin the next day with a similar solution in order to make sure that the bivalves were exposed long enough to the marker. Next day PVC rings were removed. One year later, in October 2010, samples of the calcein-stained individuals were collected by taking one core inside and one core outside the exclosure, exactly at the spot where calcein was administered the year before (the middle point between two short PVC sticks placed 1.5 m apart marked the control spot). These samples were also used to estimate prey densities (explained above in *Prey density*).

Previously we showed that ~35% of all *Loripes* individuals treated with a similar concentration of calcein as we used had a clear, measurable calcein mark when re-collected three months after marking (van der Geest et al. 2011). Unfortunately, and for yet unknown reasons, no clear measurable calcein marks could be detected in *Dosinia* shells. Hence, we have no estimates of growth rates for this species.

We fitted Von Bertalanffy's growth function to our data, a commonly used equation when modeling indeterminate bivalve growth. In this function, growth rate dH_t/dt declines with an increase in size H_t (the shell height at the onset of the experiment in 2009) in the following way:

$$\frac{dH_t}{dt} = k(H_\infty - H_t) \qquad (3)$$

where H_∞ is the mean maximum size and k is the growth constant. For each individual *Loripes* we estimated k by defining dH_t/dt as the difference in shell height between 2010 and 2009, H_t as shell height in 2009 and H_∞ as 11 mm (M. van der Geest and J. A. van Gils, *unpublished data*). As there is some individual variation around H_∞ we performed a sensitivity analysis with respect to the value of H_∞. To deal with pseudoreplication (due to having multiple *Loripes* per exclosure) we used a linear mixed-effect model with random intercepts for each exclosure-control pair using the *nlme* package (Pinheiro et al. 2009) in *R* (R Development Core Team 2011). We selected only those pairs for which we had growth estimates from both the exclosure and its control ($N = 10$ pairs).

Sulfide

At the end of the experiment, October 2010, we determined pore-water sulfide concentrations in the exclosures and their controls. We collected pore water samples at three different depths (0–4 cm, 4–8 cm, 8–12 cm), using 60-mL vacuum syringes connected to ceramic moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Total pore-water sulfide concentrations (4 mL) were measured immediately, after returning at the field station, with a mixture of 50% sample and 50% sulfide anti-oxidation buffer (SAOB), using an ion-specific silver-sulfide electrode (following Lamers et al. 1998). Sulfide measurements were only carried out on a random subset of exclosures and their paired controls ($N = 21$ pairs; we could only import a limited amount of SAOB into Mauritania).

Fecal analysis

In order to confirm the degree of diet specialization by Red Knots we collected samples of their fecal droppings. This was done at the onset of the exclosure experiment, i.e., October 2009. In total, we collected 17 samples of droppings (more or less equally divided over the seven tidal flats), each consisting of 40 ($\pm$ 3) droppings on average, which were stored in the freezer. Back in The Netherlands, samples were dried for three days at 60°C; subsequently we determined dry mass (DM_{drop}) of those fragments that were retained on a 300–μm sieve, separately for both species (Dekinga and Piersma 1993). Next, heights of all intact hinges were measured in order to reconstruct species-specific consumed size distributions, enabling us to calculate the species-specific average dry shell mass $\overline{DM_{shl}}$ of a consumed prey (Dekinga and Piersma 1993). For this purpose we used regression coefficients of DM_{shl} (in grams)–length (in millimeters) relationships that were determined on specimens collected in Banc d'Arguin in January 2011 (for *Dosinia*, $DM_{shl} = 10^{-3.43}L^{2.63}$, $N = 124$ specimens, $R^2 = 0.96$, $P < 0.001$; for *Loripes*, $DM_{shl} = 10^{-4.19}L^{3.25}$, $N = 119$ specimens, $R^2 = 0.96$, $P < 0.001$; J. Onrust, J. de Fouw, T. Oudman, M. van der Geest, and J. A. van Gils, *unpublished data*). In a recent calibration study on Red Knots it was shown that ~65% of the ingested DM_{shl} is found back as DM_{drop}, both for *Dosinia* as well as for *Loripes* (J. Onrust, J. de Fouw, T. Oudman, M. van der Geest, and J. A. van Gils, *unpublished data*). Therefore, N_{diet}, the number of prey items per species per sample is given by $DM_{drop}/(0.65\overline{DM_{shl}})$.

At the same time, in order to relate diet composition resulting from the fecal analysis to available food stocks at that time, we collected benthic samples. In total, we collected 224 samples, of which half were taken just next to each exclosure (at a distance of 1 m), while the other half were taken at a distance 25–100 m away from each exclosure. We used the same methodology as described in *Prey density*, above. We used only prey items that were both accessible and ingestible to calculate N_{avbl}, the average number of available prey items per species per sample per tidal flat (all *Loripes* size classes are ingestible, while only *Dosinia* <13.2 mm can be ingested (based on Zwarts and Blomert [1992]).
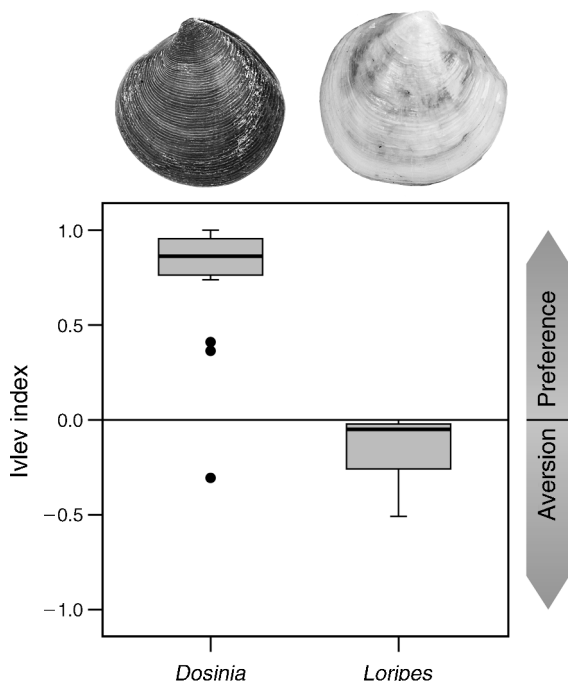
FIG. 3. Diet selectivity at the onset of the exclosure experiment, October 2009, by means of analyses of Red Knot feces. Red Knots (*Calidris canuta*) had a clear preference for *Dosinia* (Ivlev electivity index >0) and a clear aversion of *Loripes* (Ivlev index <0). Box and whisker plots give the median (horizontal line inside the box), interquartile range (box), and outliers (small black dots).

We applied Ivlev's electivity index (*I*) to express prey preference (Jacobs 1974). For a given prey species, the index compares its relative fraction in the diet F_{diet} with its relative fraction in the available food supply F_{avbl} in the following manner: $I = (F_{diet} - F_{avbl})/(F_{diet} + F_{avbl})$. Hence, *I* ranges from −1 to 1, with $I > 0$ indicating preference and $I < 0$ indicating aversion. Taking *Dosinia* (D) as an example, its contribution to the diet $F_{diet,D}$ relative to *Loripes* (L) is given by: $N_{diet,D}/(N_{diet,D} + N_{diet,L})$. Similarly, $F_{avbl,D}$, the relative contribution of *Dosinia* to the available food supply, equals $N_{avbl,D}/(N_{avbl,D} + N_{avbl,L})$. Diet and availability data were linked at the level of tidal flats.

RESULTS

Depletion trajectories

There was a weak but significant relationship between the total *Dosinia* densities in the controls (i.e., the GUDs [giving-up densities of the bivalve prey *Dosinia*]) and the total *Dosinia* densities in the exclosures (i.e., the IPDs [initial prey densities]; Fig. 2G, $y = 0.66 + 0.12x$, $F_{1,53} = 4.71$, $P < 0.05$). However, in addition to good feeding sites this analysis included poor feeding sites containing few prey attractive to Red Knots. After excluding sites at which exclosure densities were below the predicted specialist GUD of 0.85 g/m^2, the correlation between

GUD and IPD disappeared ($y = 0.80 + 0.09x$, $F_{1,27} = 1.06$, $P = 0.31$). Leaving out the nonsignificant slope from this latter model yields an intercept that is larger than 0 (estimate ± SE = 1.13 ± 0.21, $P < 0.001$), which suggests that *Dosinia* densities were depleted to a constant, non-zero GUD (cf. Fig. 2A). This intercept does not differ from the predicted specialist GUD ($t = 1.37$, $P = 0.18$).

There was a strong and significant correlation between the total *Loripes* densities in the controls (i.e., the GUDs of the bivalve prey *Loripes*) and the total *Loripes* densities in the exclosures (i.e., the IPDs; Fig. 2H; $y = 1.42 + 0.76x$, $F_{1,53} = 89.59$, $P < 0.001$). The slope of this relationship was significantly lower than 1 ($F_{1,53} = 9.10$, $P < 0.005$), but not when forcing it to go through the origin ($F_{1,54} = 1.06$, $P = 0.31$). The correlation remained significant, even when selecting only data for which densities in the exclosure exceeded the predicted specialist GUD of 0.73 g/m^2 ($y = 0.51 + 0.85x$, $F_{1,20} = 62.38$, $P < 0.001$). This result suggests that predation on *Loripes* was marginal (cf. Fig. 2B).

The slope of the regression between the total densities in the controls (GUDs) of *Dosinia* and those of *Loripes* did not differ from 0 (Fig. 2I; $y = 0.93 - 0.01x$, $F_{1,53} = 0.07$, $P = 0.79$), which corroborates the prediction for a specialist predator on *Dosinia* (Fig. 2C) and refutes the prediction for a generalist predator (Fig. 2F).

Fecal analysis

Comparing the relative proportions of *Dosinia* and *Loripes* in the diet with those available in the field yielded a clear result (Fig. 3). *Dosinia* was much preferred over *Loripes* ($t = 6.5$, df = 15, $P < 0.001$), with the Ivlev index for *Dosinia* being larger than 0 ($t = 7.0$, df = 15, $P < 0.001$), indicating a significant preference and an Ivlev index being smaller than 0 for *Loripes* ($t = -3.4$, df = 16, $P < 0.005$), indicating a significant aversion. Note, however, that the Ivlev index for *Dosinia* is smaller than 1 ($t = -3.6$, df = 15, $P < 0.005$), while it is larger than −1 for *Loripes* ($t = 18.0$, df = 16, $P < 0.001$); this indicates that *Loripes* was not entirely ignored by Red Knots during the experiment.

Prey growth rate

Loripes grew 12% faster in the controls than in the exclosures (Fig. 4; $k_{cont} = 0.66$, $k_{excl} = 0.58$, $t = 2.89$, $P < 0.005$, $N = 10$ pairs, $N = 105$ *Loripes*). The significance of this outcome was insensitive to the assumed mean maximum value of shell height H_∞ across a wide range of values for H_∞ (8.7–30.2 mm), reaching much beyond the natural range of H_∞ (10–12 mm; M. van der Geest and J. A. van Gils, *unpublished data*).

Sulfide

Sulfide concentrations of the pore water were lower in the exclosures than in the controls, but only so in the deepest layer of 8–12 cm. Here, concentrations were reduced by 70% (Fig. 5; $N = 21$ pairs). A linear mixed-
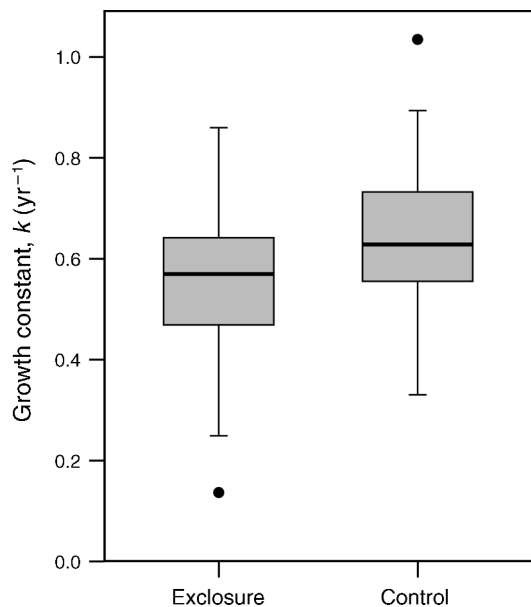
FIG. 4. *Loripes* grew faster in the controls than in the exclosures, as shown here by boxplots of individual Von Bertalanffy's growth constants k. Box and whisker plots give the median (horizontal line inside the box), interquartile range (box), and outliers (small black dots).

effect model with random intercepts showed the following depth-dependent estimates for $\log_{10}$-transformed H_2S_{excl}/H_2S_{cont} ratios: 0–4 cm, 0.01 ($t = 0.04$, $P = 0.97$); 4–8 cm, 0.14 ($t = 0.58$, $P = 0.57$); 8–12 cm, -0.51 ($t = -2.11$, $P < 0.05$). It is the deepest layer that had the highest natural sulfide concentrations (288.8 μmol/L vs. 98.4 μmol/L in the 4–8 cm layer and 17.6 μmol/L in the 0–4 cm layer; estimated by random-intercept mixed-effect model on controls only).

DISCUSSION

In our study, Red Knots (*Calidris canutus*, a molluscivore shorebird) showed a strong preference for the bivalve *Dosinia* over the bivalve *Loripes* (Figs. 2 and 3). In fact, on the basis of the exclosure results alone, we may conclude that knots behaved as specialists, largely ignoring *Loripes* and depleting *Dosinia* to a constant giving-up density (GUD). The fact that the observed GUD on *Dosinia* matched with the predicted GUD when feeding on *Dosinia* only (0.85 g $AFDM_{meat}/m^2$), strongly supports the idea that Red Knots almost fully relied on *Dosinia* as their food source. Only at the beginning of our experiment, in October 2009, it seems that *Loripes* featured more in the diet of knots. This suggestion is based on the results of the analyses of fecal droppings, in which the Ivlev electivity index on *Loripes* is significantly above -1 (Fig. 3). A value of -1 means full ignorance; the observed mean of -0.16 (SE = 0.05) suggests aversion but not full ignorance. Most likely, knots had to include *Loripes* in their diet in 2009 as *Dosinia* was then much less abundant, occurring in

densities below the minimal GUD (mean ± SE = 0.5 ± 0.1 g $AFDM_{meat}/m^2$, $N = 112$ benthic samples, which represents only those sites that were resampled in 2010), than one year later, in October 2010 (1.2 ± 0.1 g $AFDM_{meat}/m^2$, $N = 112$ benthic samples). Overall, by taking also the dropping analyses into account, we may conclude that knots behave as so-called "expanding specialists" (Heller 1980), meaning that that they do accept alternative prey such as *Loripes* in times of scarcity of their favorite prey *Dosinia* (J. A. van Gils, M. van der Geest, J. Leyrer, T. Oudman, J. Onrust, J. de Fouw, T. vander Heide, P. J. van den Hout, B. Spaans, A. Dekinga, M. Brugge, and T. Piersma, *unpublished manuscript*).

Initially, the preference of *Dosinia* over *Loripes* came as quite a surprise to us. Relative to their shell mass *Dosinia* contains 2–3 times less meat than *Loripes* (using regression equations given earlier for $AFDM_{meat}$ and DM_{shl}), and it is well established that Red Knots prefer prey with high meat/shell ratios (van Gils et al. 2005). Only recently have we been able to grasp the knot's aversion for this energy-rich prey. Feeding trials showed that captive Red Knots offered a mono-specific diet of *Loripes* developed diarrhea and were less eager to continue eating (T. Oudman, J. Onrust, J. de Fouw, B. Spaans, J. A. van Gils, *unpublished manuscript*). Due to the sulfur-based metabolism in *Loripes* it is very likely that the diarrhea is due to a sulfide release in the knot's digestive tract once *Loripes* meat is being digested. It has been shown that pigs *Sus domesticus* develop diarrhea and lose weight when on a sulfide-rich diet (Wetterau et al. 1964). Furthermore, shallow-water fishes and crabs were deterred from feeding when offered prey that were collected in sulfide-rich deep-sea hydrothermal vents,
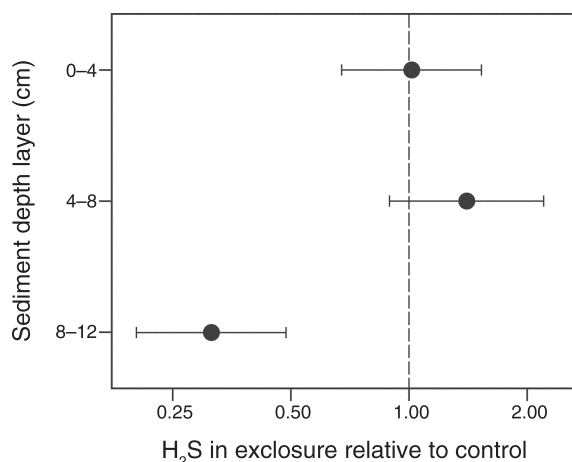

FIG. 5. Sulfide concentrations were lower in the exclosures than in the controls, but only so in the sulfide-richest deepest layer (8–12 cm). Plotted are means ± SE of the ratio between sulfide in the exclosure and in the paired control, with the dashed vertical line representing the prediction of no difference between exclosure and control.
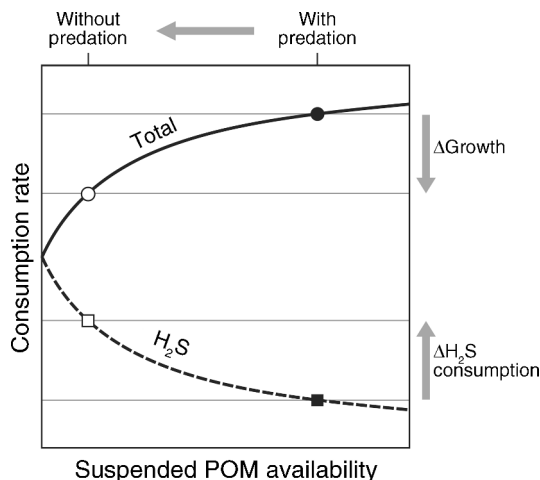
FIG. 6. Schematic depiction of a possible mechanism showing how *Loripes* may experience hampered growth in the absence of predation on *Dosinia*. Without predation, *Dosinia* densities will be higher, and hence suspended POM (particulate organic matter) availability will be lower. Assuming that in *Loripes* the consumption of suspended POM is mutually exclusive with the utilization of sulfide, the absolute use of sulfide will increase when suspended POM availability decreases, although the total consumption rate (suspended POM and sulfide), and hence the growth rate, will decline.

presumably due to the high sulfide concentrations inside these prey (Kicklighter et al. 2004).

In spite of relatively low predation pressure on *Loripes*, we did find an effect of predation on this species: *Loripes* experiencing predation grew faster than those without predation (Fig. 4). Possibly, *Loripes* benefited from the depletion of *Dosinia* stocks, which would imply some sort of competition between *Loripes* and *Dosinia*. Even though *Loripes*' principle source of energy stems from the oxidation of sulfide by endosymbiotic bacteria living inside its gills, mixotrophy has been observed in *L. lucinalis* (Johnson et al. 1994) and in other members of the Lucinidae family (Duplessis et al. 2004, Dufour and Felbeck 2006), especially during periods of gonad development (Le Pennec et al. 1995). In general, lucinids do have a functional, though reduced, digestive system (Allen 1958) in which particles of phytoplanktonic origin have been found (Le Pennec et al. 1988), suggesting the ability for this family to be mixotrophic. Possibly, *Loripes* relies on diatoms and suspended particulate organic matter (POM) when porewater sulfide concentrations are low; it is the other way around when suspended POM availability declines: then *Loripes* needs to rely more and more on sulfide. We propose that the latter mechanism explains our results: by excluding knots, *Dosinia* was able to flourish, increase in numbers and use much of the available suspended POM. Hence, *Loripes* experienced reduced food intake, leading to retarded growth that it could partly compensate for by increasing its use of sulfide; hence the observed decline in pore-water sulfide

concentrations inside the exclosures (Fig. 5). This proposed mechanism can be captured in a simple Holling type II functional response model on two resources, as exemplified in Fig. 6. There is ample evidence for interspecific competition among suspension-feeding bivalves (e.g., Peterson and Black 1987, Jonsson et al. 2005). Especially when flow velocities of the water column are low, suspension-feeders can deplete their own and their neighbors' resources on the small scale of centimeters (Herman et al. 1999). Dense seagrass meadows in particular are able to strongly attenuate currents and waves (Larkum et al. 2006), which makes the competition for suspended POM at the seagrass-covered tidal flats of Banc d'Arguin (Mauritania) very probable. Consistent with this point of view, a recent analysis of eight consecutive years of benthos sampling in our study area showed a suggestive negative correlation between densities of *Loripes* and *Dosinia* (Pearson's $r = -0.79$, $P < 0.05$, $N = 8$ years; (J. A. van Gils, M. van der Geest, J. Leyrer, T. Oudman, J. Onrust, J. de Fouw, T. vander Heide, P. J. van den Hout, B. Spaans, A. Dekinga, M. Brugge, and T. Piersma, *unpublished manuscript*).

Alternatively, the lower sulfide concentration inside the exclosures may be enhanced by the bioturbation caused by high *Dosinia* densities. It is known that suspension feeding and deposit feeding leads to bioturbation, such as has recently been shown in a closely related species, *Dosinia discus* (Gingras et al. 2008). However, if this was the mechanism behind the sulfide decline, then we would have expected this decline to be strongest in the top-4-cm layer in which most suspension-feeding *Dosinia* live (70–80%; this study). In contrast, we only saw changes in the deepest, sulfide-richest layer (Fig. 5), an observation that matches well with the mechanism proposed in the previous paragraph.

However, with *Loripes* being the most likely reason for the changes in the deepest layer, one would expect *Loripes* in the exclosures to have moved to this sulfide-richest layer. This was not the case (percentage of individuals that lived in top-4-cm layer: 70% in controls vs. 73% in exclosures, $t = 0.50$, $P > 0.6$, based on the 48 out of 55 exclosure–control pairs that contained *Loripes*). However, lucinids and closely related thyasirids are able to "mine" sulfide from deep anaerobic sulfide-rich sediment layers using their superextensile foot (up to 30 times the length of their shell; Dufour and Felbeck 2003). In this way, *Loripes* is able to exploit sufficient sulfide while at the same time remaining relatively close to the sediment surface, which makes it easier to take up enough oxygen and compete with *Dosinia* for the remaining suspended POM.

Whatever the precise mechanism, the exclusion of molluscivore predators seems to cascade down to the level of pore-water biogeochemistry by reducing sulfide concentrations. Three-level trophic cascades have been found before in coastal marine ecosystems, starting with the seminal paper by Estes and Palmisano (1974),

then named "cascades" by Paine (1980), and recently reviewed by Terborgh and Estes (2010) and Estes et al. (2011), the latter including top-down effects on biogeochemical cycles. The finding that predators affect the biogeochemical cycle of sulfide appears novel. Yet, it may be a general phenomenon in detritus-based ecosystems where sulfide is produced by the anaerobic decomposition of organic material. Though the exclusion of knots works out negatively for *Loripes*, as reflected by reduced rates of shell growth, it may be beneficial to other organisms since high sulfide concentrations are known to be toxic to both plants and animals (Bagarinao 1992). However, this short-term effect of excluding predation by shorebirds may be in contrast with long-term effects. In the absence of predation, the lower growth rates of *Loripes* may lead to hampered reproduction in *Loripes*, eventually leading to a *Loripes* population decline and extinction—a form of competitive exclusion due to an overall increase in predation-free *Dosinia*. This will likely have the opposite effect on pore-water biogeochemistry, leading to increased levels of sulfide since there would be no *Loripes* present to keep sulfide concentrations low. This would hamper most organisms living in the seagrass-covered tidal flats, including the seagrass itself. For example, a recent indoor experiment showed that seagrass grew better in the presence of *Loripes* due to *Loripes* reducing sulfide concentrations (T. van der Heide, L. L. Govers, J. de Fouw, J. Olff, M. van der Geest, M. M. van Katwijk, T. Piersma, J. van de Koppel, B. R. Silliman, A. J. P. Smolders, and J. A. van Gils, *unpublished manuscript*). Molluscivore shorebird populations are in steep decline worldwide (Piersma 2007, Delany et al. 2009). Often this is due to habitat loss at their temperate-zone stopovers, and not so much due to situations at their southern wintering grounds (van Gils et al. 2009, Kraan et al. 2010). Thus, by affecting shorebird numbers in the temperate zone, we may affect the ecosystem state in relatively pristine and untouched tropical wintering grounds. We realize this scenario is hypothetical and we hope that it remains this way.

LITERATURE CITED

Abrams, P. A. 2006. The prerequisites for and likelihood of generalist–specialist coexistence. American Naturalist 167:329–342.

Allen, J. A. 1958. On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). Philosophical Transactions of the Royal Society B 241:421–484.

Altenburg, W., M. Engelmoer, R. Mes, and T. Piersma. 1982. Wintering waders on the Banc d'Arguin, Mauritania. Report of the Netherlands Ornithological Expedition 1980. Stichting Veth tot steun aan Waddenonderzoek, Leiden, The Netherlands.

Bagarinao, T. 1992. Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24:21–62.

Brown, J. S. 1988. Patch use as an indicator of habitat preference, predation risk, and competition. Behavioral Ecology and Sociobiology 22:37–48.

Brown, J. S., and W. A. Mitchell. 1989. Diet selection on depletable resources. Oikos 54:33–43.

Byrnes, J. E., P. L. Reynolds, and J. J. Stachowicz. 2007. Invasions and extinctions reshape coastal marine food webs. PLoS ONE 2:e295.

Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A. Richards, R. M. Nisbet, and T. J. Case. 2002. The interaction between predation and competition: a review and synthesis. Ecology Letters 5:302–315.

Dekinga, A., and T. Piersma. 1993. Reconstructing diet composition on the basis of faeces in a mollusc-eating wader, the knot *Calidris canutus*. Bird Study 40:144–156.

Delany, S., D. Scott, T. Dodman, and D. Stroud. 2009. An atlas of wader populations in Africa and Western Eurasia. Wetlands International, Wageningen, The Netherlands.

Duffy, J. E. 2003. Biodiversity loss, trophic skew and ecosystem functioning. Ecology Letters 6:680–687.

Dufour, S. C., and H. Felbeck. 2003. Sulphide mining by the superextensile foot of symbiotic thyasirid bivalves. Nature 426:65–67.

Dufour, S. C., and H. Felbeck. 2006. Symbiont abundance in thyasirids (Bivalvia) is related to particulate food and sulphide availability. Marine Ecology-Progress Series 320:185–194.

Duplessis, M. R., S. C. Dufour, L. E. Blankenship, H. Felbeck, and A. A. Yayanos. 2004. Anatomical and experimental evidence for particulate feeding in *Lucinoma aequizonata* and *Parvilucina tenuisculpta* (Bivalvia: Lucinidae) from the Santa Barbara Basin. Marine Biology 145:551–561.

Estes, J. A., and J. F. Palmisano. 1974. Sea otters: their role in structuring nearshore communities. Science 185:1058–1060.

Estes, J. A., et al. 2011. Trophic downgrading of planet Earth. Science 333:301–306.

Fryxell, J. M., and P. Lundberg. 1997. Individual behavior and community dynamics. Chapman and Hall, London, UK.

Gingras, M. K., S. G. Pemberton, S. Dashtgard, and L. Dafoe. 2008. How fast do marine invertebrates burrow? Palaeogeography, Palaeoclimatology, Palaeoecology 270:280–286.

Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: a meta-analysis of field experiments. American Naturalist 155:435–453.

Heithaus, M. R., A. Frid, A. J. Wirsing, and B. Worm. 2008. Predicting ecological consequences of marine top predator declines. Trends in Ecology and Evolution 23:202–210.

Heller, R. 1980. On optimal diet in a patchy environment. Theoretical Population Biology 17:201–214.

Herman, P. M. J., J. J. Middelburg, J. van de Koppel, and C. H. R. Heip. 1999. Ecology of estuarine macrobenthos. Advances in Ecological Research 29:195–240.

Holt, R. D., and B. P. Kotler. 1987. Short-term apparent competition. American Naturalist 130:412–430.

Holt, R. D., and J. H. Lawton. 1994. The ecological consequences of shared natural enemies. Annual Review of Ecology and Systematics 25:495–520.

Honkoop, P. J. C., E. M. Berghuis, S. Holthuijsen, M. S. S. Lavaleye, and T. Piersma. 2008. Molluscan assemblages of

seagrass-covered and bare intertidal flats on the Banc d'Arguin, Mauritania, in relation to characteristics of sediment and organic matter. Journal of Sea Research 60:255–263.

Hutson, V., and G. T. Vickers. 1983. A criterion for permanent coexistence of species, with an application to a two-prey one-predator system. Mathematical Biosciences 63:253–269.

Jacobs, J. 1974. Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's electivity index. Oecologia 14:413–417.

Johnson, C. N., J. L. Isaac, and D. O. Fisher. 2007. Rarity of a top predator triggers continent-wide collapse of mammal prey: dingoes and marsupials in Australia. Proceedings of the Royal Society B 274:341–346.

Johnson, M., M. Diouris, and M. Le Pennec. 1994. Endosymbiotic bacterial contribution in the carbon nutrition of *Loripes lucinalis* (Mollusca: Bivalvia). Symbiosis 17:1–13.

Jonsson, P. R., J. K. Petersen, O. Karlsson, L. O. Loo, and S. F. Nilsson. 2005. Particle depletion above experimental bivalve beds: in situ measurements and numerical modeling of bivalve filtration in the boundary layer. Limnology and Oceanography 50:1989–1998.

Kicklighter, C. E., C. R. Fisher, and M. E. Hay. 2004. Chemical defense of hydrothermal vent and hydrocarbon seep organisms: a preliminary assessment using shallow-water consumers. Marine Ecology-Progress Series 275:11–19.

Kraan, C., J. A. van Gils, B. Spaans, A. Dekinga, and T. Piersma. 2010. Why Afro-Siberian Red Knots *Calidris canutus canutus* have stopped staging in the western Dutch Wadden Sea during southward migration. Ardea 98:155–160.

Lamers, L. P. M., H. B. M. Tomassen, and J. G. M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environmental Science and Technology 32:199–205.

Larkum, A. W. D., R. J. Orth, and C. M. Duarte, editors. 2006. Seagrasses: biology, ecology and conservation. Springer, Dordrecht, The Netherlands.

Le Pennec, M., A. Herry, M. Diouris, D. Moraga, and A. Donval. 1988. Chimioautotrophie et nitrition chez les Lucinacea, bivalves littoraux de milieux réducteurs. II. Charactéristiques morphologique des bactéries symbiotiques et modifications structurales adaptatives des branchies de l'hôte. Haliotis 18:207–217.

Le Pennec, M., A. Herry, M. Johnson, and P. Beninger. 1995. Nutrition–gametogenesis relationship in the endosymbiont host-bivalve *Loripes lucinalis* (Lucinidae) from reducing coastal habitats. Pages 139–142 in A. Eleftheriou, A. D. Ansell, and C. J. Smith, editors. Biology and ecology of shallow coastal waters. Proceedings of the 28th European Marine Biological Symposium, Crete, Greece, 23–28 September 1993. Olsen & Olsen, Copenhagen, Denmark.

Murdoch, W. W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecological Monographs 39:335–354.

Myers, R. A., and B. Worm. 2003. Rapid worldwide depletion of predatory fish communities. Nature 423:280–283.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist 100:65–75.

Paine, R. T. 1980. Food webs: linkage, interaction strength and community infrastructure. Journal of Animal Ecology 49:667–685.

Peterson, C. H., and R. Black. 1987. Resource depletion by active suspension feeders on tidal flats: influence of local density and tidal elevation. Limnology and Oceanography 32:143–166.

Piersma, T. 2007. Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. Journal of Ornithology 148 (Supplement 1):S45–S59.

Piersma, T., A. Dekinga, J. A. van Gils, B. Achterkamp, and G. H. Visser. 2003. Cost–benefit analysis of mollusc-eating in a shorebird. I. Foraging and processing costs estimated by the doubly labelled water method. Journal of Experimental Biology 206:3361–3368.

Piersma, T., J. van Gils, P. de Goeij, and J. van der Meer. 1995. Holling's functional response model as a tool to link the food-finding mechanism of a probing shorebird with its spatial distribution. Journal of Animal Ecology 64:493–504.

Pinheiro, J. C., D. M. Bates, S. DebRoy, D. Sarkar, and the R Development Core Team. 2009. NMLE: linear and nonlinear mixed-effects models. R package version 3.1-96. http://cran.r-project.org/web/packages/nlme/index.html

Purvis, A., J. L. Gittleman, G. Cowlishaw, and G. M. Mace. 2000. Predicting extinction risk in declining species. Proceedings of the Royal Society B 267:1947–1952.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ripple, W. J., and R. L. Beschta. 2004. Wolves and the ecology of fear: can predation risk structure ecosystems? BioScience 54:755–766.

Schreiber, S. J. 1997. Generalist and specialist predators that mediate permanence in ecological communities. Journal of Mathematical Biology 36:133–148.

Srivastava, D. S., M. K. Trzcinski, B. A. Richardson, and B. Gilbert. 2008. Why are predators more sensitive to habitat size than their prey? Insights from bromeliad insect food webs. Amercian Naturalist 172:761–771.

Terborgh, J., and J. A. Estes. 2010. Trophic cascades: predators, prey, and the changing dynamics of nature. Island Press, Washington, D.C., USA.

van der Geest, M., J. A. van Gils, J. van der Meer, H. Olff, and T. Piersma. 2011. Suitability of calcein as an *in situ* growth marker in burrowing bivalves. Journal of Experimental Marine Biology and Ecology 399:1–7.

Vandermeer, J., and M. Pascual. 2006. Competitive coexistence through intermediate polyphagy. Ecological Complexity 3:37–43.

van Gils, J. A., A. Dekinga, P. J. van den Hout, B. Spaans, and T. Piersma. 2007. Digestive organ size and behavior of Red Knots (*Calidris canutus*) indicate the quality of their benthic food stocks. Israel Journal of Ecology & Evolution 53:329–346.

van Gils, J. A., S. R. de Rooij, J. van Belle, J. van der Meer, A. Dekinga, T. Piersma, and R. Drent. 2005. Digestive bottleneck affects foraging decisions in Red Knots *Calidris canutus*. I. Prey choice. Journal of Animal Ecology 74:105–119.

van Gils, J. A., I. W. Schenk, O. Bos, and T. Piersma. 2003. Incompletely informed shorebirds that face a digestive constraint maximize net energy gain when exploiting patches. American Naturalist 161:777–793.

van Gils, J. A., M. van der Geest, C. Kraan, E. O. Folmer, E. J. Jansen, and T. Piersma. 2009. How the carrying capacity of the Wadden Sea regulates the number of wintering waders at Banc d'Arguin. Limosa 82:134–140.

Wetterau, H., W. Oerkert, and W. G. Knape. 1964. Tests for the application of dried green fodder with higher H_2S content (experiments with poultry and fattened pigs). Fetterung 5:383–393.

Zwarts, L., and A.-M. Blomert. 1992. Why knots *Calidris canutus* take medium-sized *Macoma balthica* when six prey species are available. Marine Ecology - Progress Series 83:113–128.

Zwarts, L., J. van der Kamp, O. Overdijk, T. van Spanje, R. Veldkamp, R. West, and M. Wright. 1998. Wader counts of the Banc d'Arguin, Mauritania, in January/February 1997. Wader Study Group Bulletin 86:53–69.